

444

Federation Proceedings

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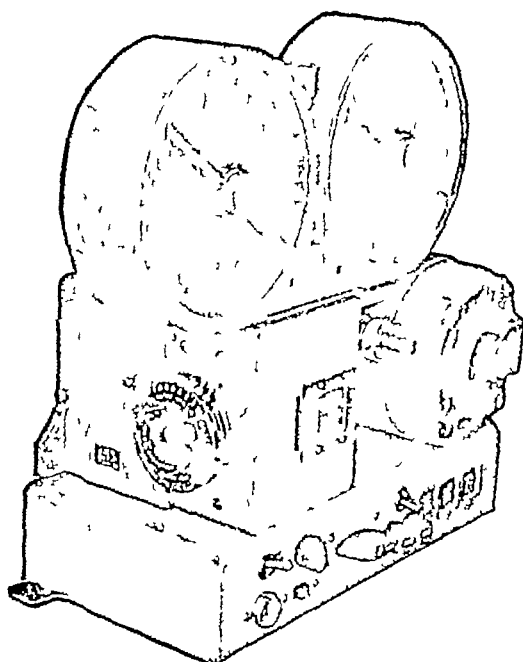
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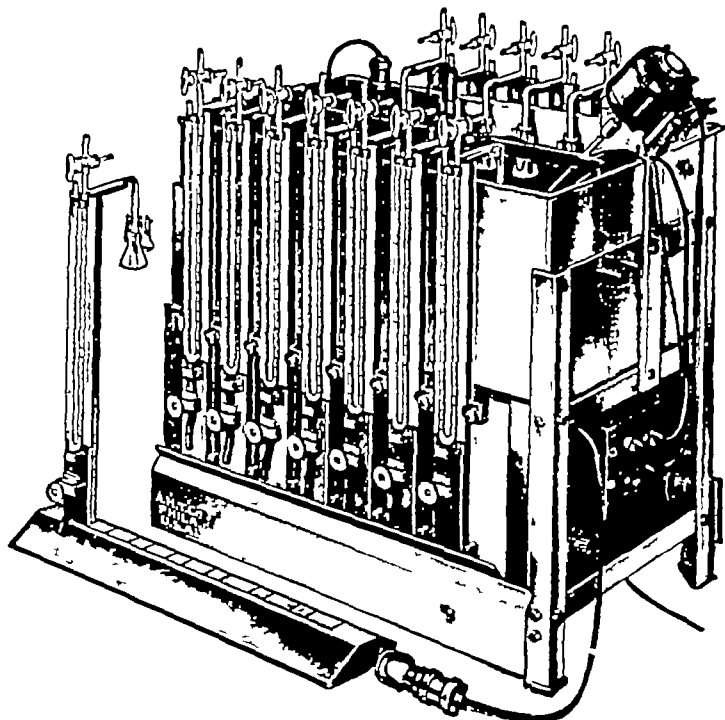
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FEDERATION PROCEEDINGS is published quarterly by the Federation of American Societies for Experimental Biology. The *March* issue consists of the Program (Part II) of the Annual Meeting of the Federation, and the Abstracts (Part I) of the papers presented at the scientific sessions. Both parts include an author index. The abstracts are arranged alphabetically according to the first author and segregated as to Societies. The *June* and *September* issues contain symposia and other special papers presented at Federation meetings as selected by the Editorial Board. The *December* issue contains the membership list and other matters pertinent to the Constituent Societies of the Federation.

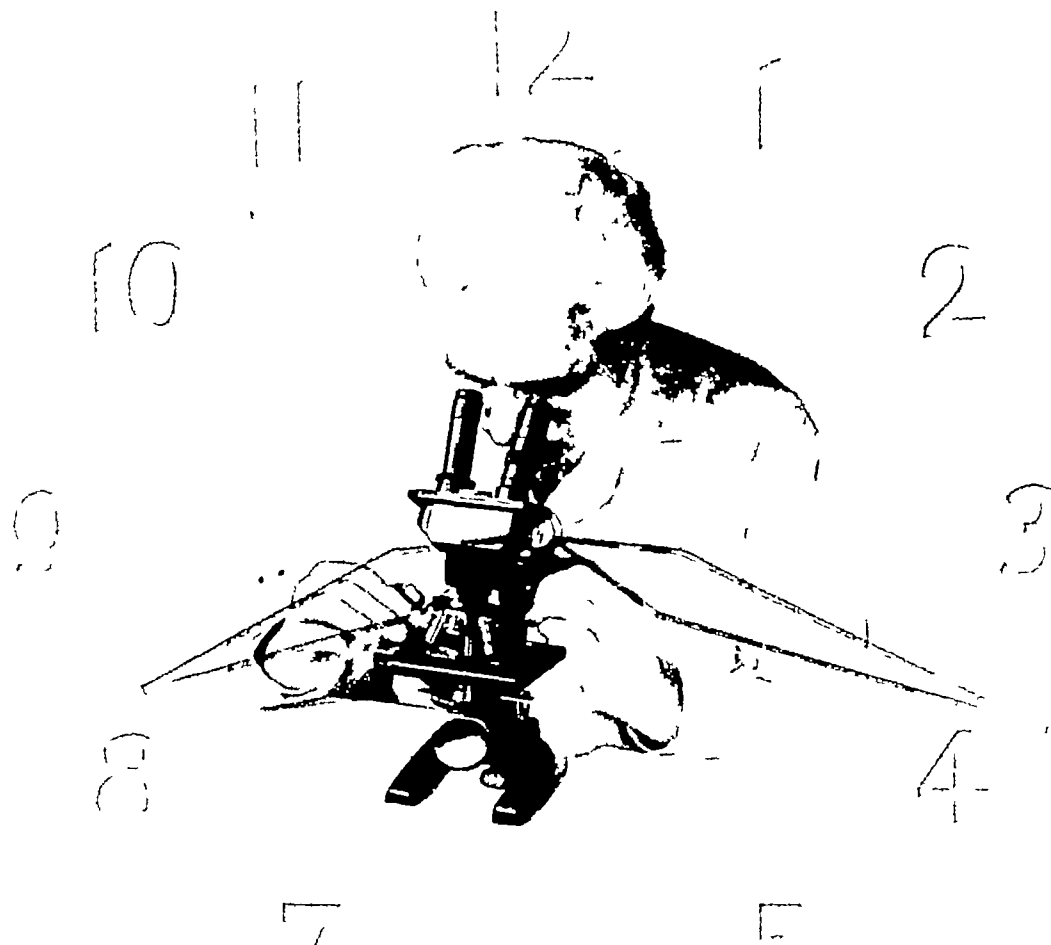
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The subscription price is \$4.50 (\$5.25 foreign) payable in advance. Single issues may be purchased, if ordered in advance, at the following prices: No. 1 (Part I, March, Abstracts), \$3.00; No. 1 (Part II, March, Program), 75¢; Nos. 2 and 3 (June and September), \$1.50 an issue; No. 4 (December), \$2.25 an issue. Subscriptions and orders should be sent to the Federation of American Societies for Experimental Biology, 2101 Constitution Ave., Washington 25, D. C.

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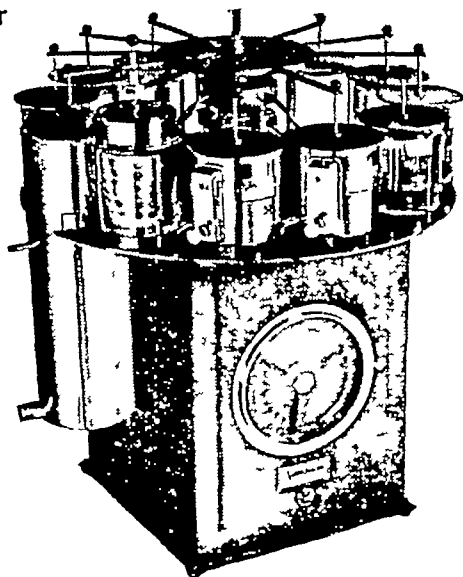
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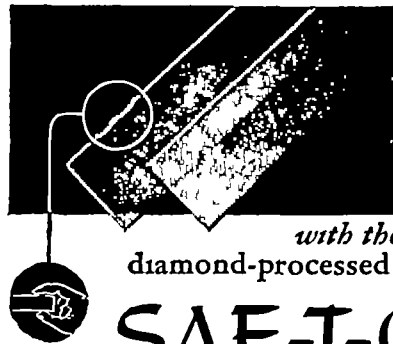
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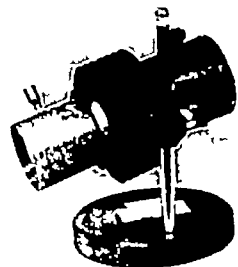
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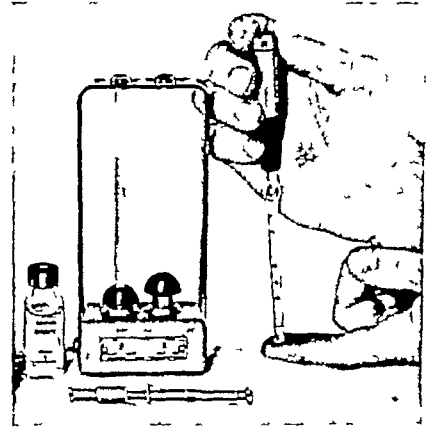
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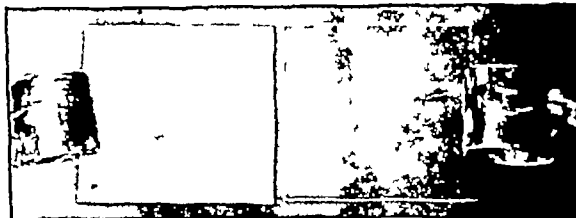
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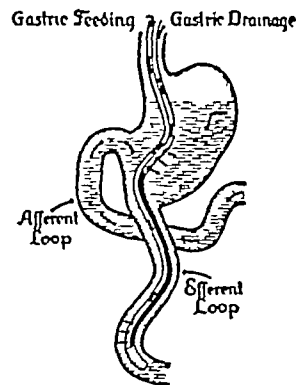
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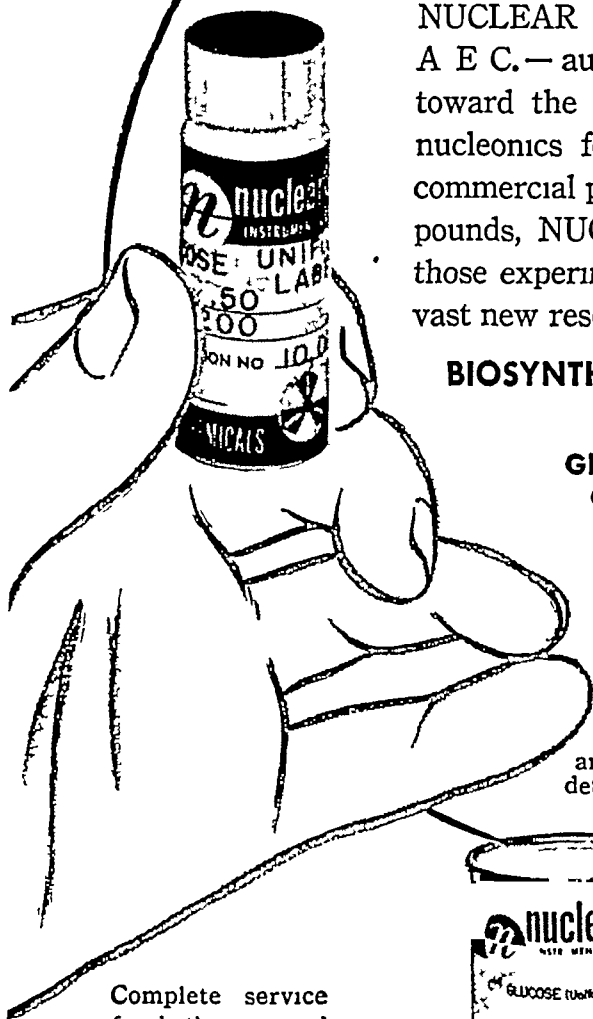
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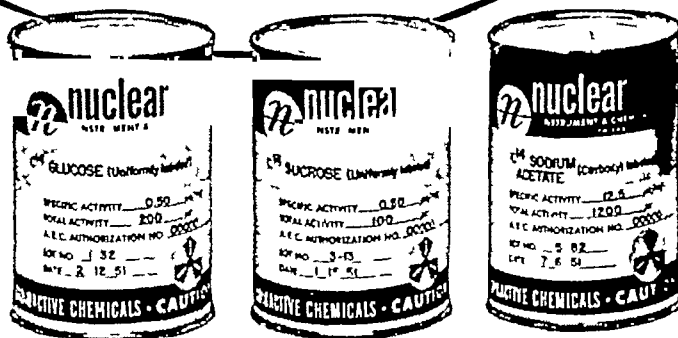
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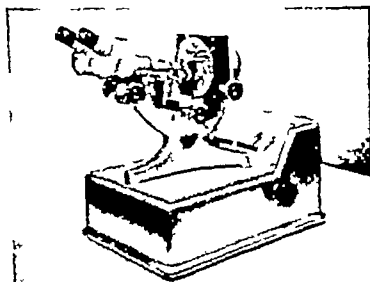
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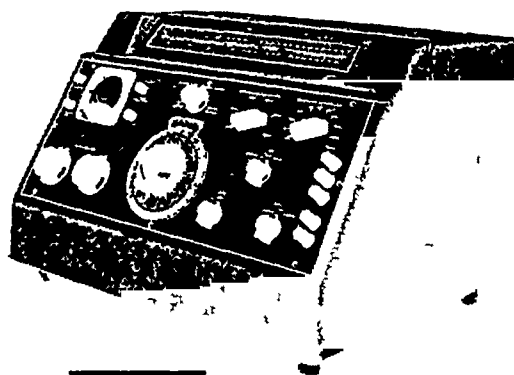
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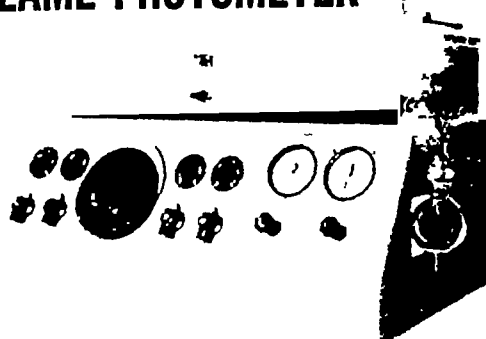
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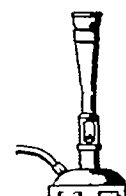
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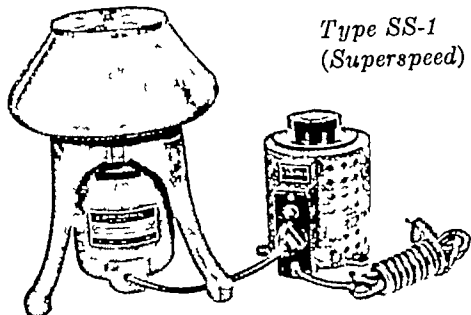
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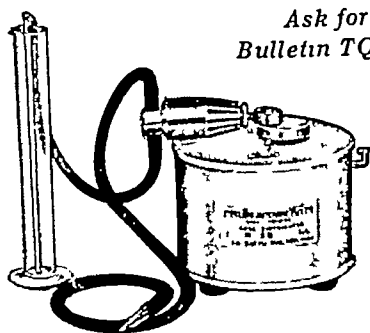
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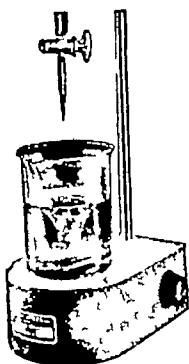
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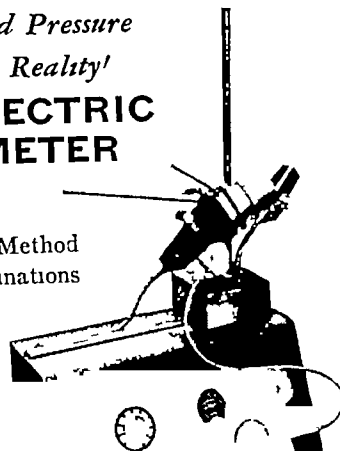
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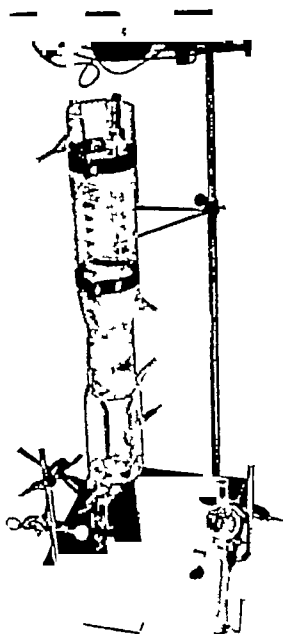
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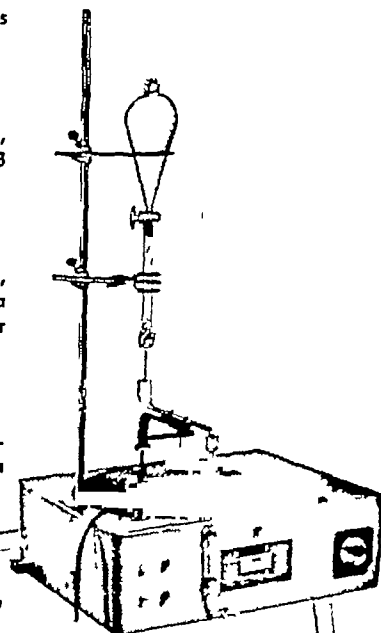
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Basically all efforts should be directed toward preventing seizures and the tendency to chronic invalidism.

The management regimen should include¹

- I Good physical and mental hygiene
 - a) Wholesome diet, Regular bowel habits, Avoid noxious materials, e.g. alcoholics, Moderate physical activity
 - b) Help overcome feeling that epilepsy is shameful, Encourage normal work and recreation, Regular sleep without overprotection

II Anticonvulsant medication selected on the basis of Exact Diagnosis

'Certain drugs are more effective in one type of seizure than they are in another, and it is necessary to use proper drugs. The dosage must be individualized for each case.'¹

Thus, the best guide in selecting medication for a patient is the type of seizures present. (Frequently several drugs in combination is most effective.)

The table shows which drugs are indicated for each type

of seizure. It will be noted that the newer hydantoin, Mesantoin, is effective for *grand mal*, Jacksonian and focal seizures.

MESANTOIN DOSAGE is adjusted to the lowest level adequate to attain freedom from seizures. Start with 1/2 or 1 tablet daily during the first week, increase the daily intake by 1/2 or 1 tablet during the next week. Continue this up to optimal dosage. When another drug has not given good results, Mesantoin is added by the same procedure, then the old drug is gradually reduced.

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- b No Mesantoin, if white cell count below 4000
- c caution if (1) rash or blood changes
(2) History of drug sensitivity
- d discontinue drug if (1) bleeding of gums or vagina
(2) Sore throat

TYPE OF EPILEPSY	PRIMARY CLINICAL MANIFESTATIONS	MEDICATION	AVERAGE ADULT DAILY DOSE*	LIMITING SIDE EFFECTS	SIGNS OF SENSITIVITY
GRAND MAL	Aura followed by loss of consciousness, tonic clonic convulsions and autonomic disturbance e.g., in bladder function.	-Phenobarbital	1 1/4 grs h.s. Usual max 3 grs per day	Sleepiness	
		-5,5 diphenyl hydantoin	1 1/4 grs t.i.d. p.c. Usual max 6 grs per day	Diplopia, staggering (may increase petit mal)	Overgrowth of gums, body hairs, itching, rash on extremities, gastric irritation
JACKSONIAN	Convulsions begin in one area and spread outwards (Jacksonian march). No loss of consciousness. ²	-3 methyl 5 5 phenylethyl hydantoin (mesantoin)	Adults 2 to 6 tabs Children 1 to 4 tabs	Sleepiness	For full details see Psychomotor cross column below
		-phenylacetylurea ³	2 Gms per day Max dose 6 Gms per day ³	Drowsiness	Central nervous system affected, nausea, vomiting and abdominal pain ⁵
PSYCHOMOTOR	Period of amnesia, actions apparently purposeful but mechanical, incoordination. Mild tonic spasms. In children 'behavior problems'. ³	-3 methyl 5 5 phenylethyl hydantoin ⁴ (mesantoin)	Adults 2-6 tabs Children 1-4 tabs	Pharyngitis, mucous membrane bleeding, lymphadenopathy, measles like rash with itching and fever in severe reaction, blood dyscrasia	
		-phenylacetylurea ³	See phenylacetylurea—above		
PETIT MAL TRIAD	Attacks in series (rapid succession), abrupt onset, few sec. duration, no aura nor impairment of consciousness. Rhythmic twitching (e.g. eyelids).	-3 5 5 trimethyl oxazolidine 2,4 dione	0.9 to 1.2 Gms	Inability to see in a bright light, Drowsiness	Hiccough, acneiform rash, leukopenia
		-N methyl derivative of phenobarbital	6 grs	Sleepiness	

*Although an average daily dose can be stated for each of these drugs, to obtain best results it is necessary that the physician determine the dose required by each individual patient for maximum control of his seizures.

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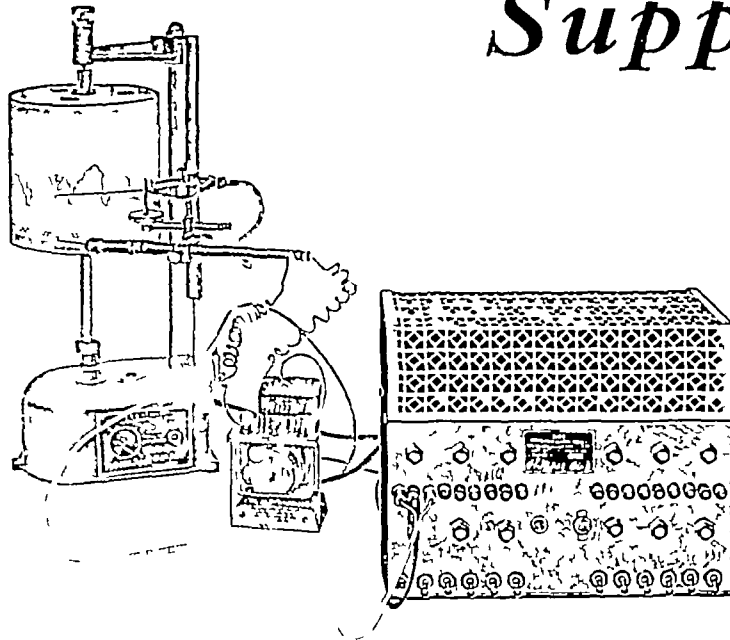
Bibliography: 1 Carter S. in Conn H. Current Therapy 1949 Phila Saunders Co., p. 495. 2 Kaufman I. Dis Nerv System 11: 99 1950. 3 Lennox W. Science & Seizures ed 2 N.Y.C., Harper & Bros 1946. 4 Harris, T & Otto J. Texas J Med 43: 328 1947. 5 Little S & McBryde R. Am J M Sc 219: 494 1950. (Except as otherwise indicated the data tabulated is from Gibbs F. Ann Int Med 27: 548 1947.)

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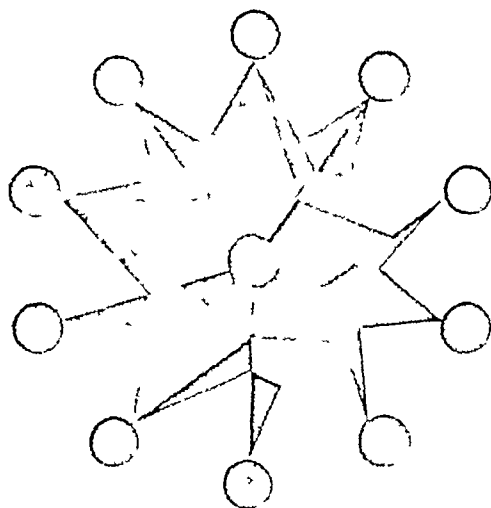
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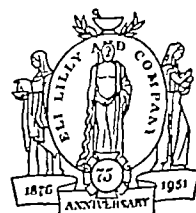
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MECHANISMS OF BIOLOGICAL EFFECTS OF PENETRATING RADIATIONS

CORNELIUS A. TOBIAS¹

From the Donner Laboratory, Division of Medical Physics, University of California, Berkeley

TO THE biophysicist, the study of the biological effects of penetrating radiation is a particularly interesting field because of the intimate connection of the radiation effect with the problem of growth. I wish to mention a few of the unique properties of radiations.

First, some radiations can penetrate the cell wall and interact with internal components of cells. The cell wall provides protection against many chemical agents.

Secondly, radiation seems to inhibit the mechanisms controlling growth before it seriously interferes with other cell functions. Radiation thus becomes a suitable tool for study of cell division.

Thirdly, the amount of energy which the cell must absorb if cell division is to be inhibited is extremely small compared to the energy turnover of the cell as a machine, or compared to the amount of energy needed from other sources to cause lethal effect. For example, many cells are inactivated by an amount of penetrating radiation which, as heat, would be only sufficient to raise cell temperature by a few thousandths of a degree. In contrast, thermal death due to raised temperature would take 10,000 times as much absorbed energy as radiation death. In fact, for some cells it is known, since the pioneer work of Dessauer (1, 2), that production of a single ion pair can cause lethal effect in the cell. In these cases, at least, lethal effect must be due to a chain of reactions.

Fourthly, as is generally known, a single inter-

action with ionizing radiation is able to make an inheritable change, or mutation, in the gene complement of cells. Radiation induced mutations are usually unfavorable, occasionally they can make cell metabolism more efficient. Thus radiation may have an important role in both destruction and evolution of life.

In order to comprehend at least a few of the complex phenomena involved in radiation effects on animals, it is useful to look at the explanation of the effects on unicellular organisms, and in order to understand these, one should study the physicochemical effects of radiation in inert chemical systems. The field is so large that all I can hope to give you is a list of some of the recent experimental approaches, then to describe, in a little more detail, some experimental findings of special interest to me. Excellent review articles by Bacq and by Curtis are available.

INTERACTION OF RADIATION WITH MATTER

Penetrating radiations either produce ionization directly in their passage through matter, as beta rays, alpha rays and protons, or they produce ionizing secondary particles as x-rays, gamma rays or neutrons. In the process of ionization, a negative electron is knocked out of an atom, leaving it positively charged. The negative electron attaches itself to a molecule, so that within 10^{-14} seconds a positive-negative ion pair is formed. The ions of the ion pair in liquid media often dissociate or interact with neutral molecules and form radicals or more stable chemical compounds. In addition, a number of atoms also

¹ The author's work was supported by the Atomic Energy Commission.

become 'excited' due to the passage of an ionizing ray, that is, one of the orbital electrons absorbs energy and is elevated to an excited state. The molecule in which the excited atom is located then might dissociate, emit a light quantum, interact with another molecule, or degrade the excitation energy by collisions with other molecules into vibrational and rotational energy. Dose may be defined, for the purposes of this discussion, as the energy absorbed in form of ionization and excitation per gram tissue. The radiochemical and radiobiological action of the ionizing radiation depends not only on dose, but on the geometrical distribution of ion pairs along each track. The number of ion pairs along a unit length of track is the 'specific ionization.' Among radiations available to experimentation, the specific ionization varies over a range of 100,000.

DIRECT ACTION

As to the mode of biological action of radiations, many early studies indicated that direct interaction of the ions with biological material occurs in some instances, causing splitting or fragmentation of large biological molecules. Classical example of such a process is the splitting of hemocyanin molecules as demonstrated by Svedberg and Brohult (3).

Experimentally, breakdown of macromolecules can be accomplished with different radiations: neutrons from the chain reacting pile, γ -rays, electrons, deuterons and alpha particles from the cyclotron, it can be performed on dry, biologically active molecules in the crystalline state, or in liquid suspensions. Some intriguing results to date include the following findings:

a) Breakdown of macromolecules by radiation sometimes occurs in fragments of well definable molecular weight (4).

b) In addition to breakdown, association of macromolecules can occur (5).

c) Quantitative study of the action of cyclotron-produced protons on crystalline enzymes leads to a novel method for determination of molecular weight (6).

d) When insulin was subjected to intense radiation with neutrons, a large part of the biological activity was retained in molecular fragments which had a variety of molecular weights (7).

The above instances indicate the potentialities of the 'molecular fragmentation' method in the breakdown study of biologically important molecules.

INDIRECT ACTION

In the second type of interaction of radiation with living matter, the primary ionization or excitation occurs at some distance from the molecules affected. As first clearly demonstrated by Dale (8), the indirect action occurs when the short-lived ions disappear, and give rise to radicals and intermediate chemical compounds in the liquid medium. The intermediates have much longer lifetimes than the ions themselves, and they can, in turn, chemically interact with protein molecules. In very dilute aqueous suspensions of enzyme molecules, the primary radiation effect is on water, and most of the effect is due to water decomposition products.

The decomposition of pure water, in its main features, is quite well understood, due to the work of Lind (9), Fricke (10), Weiss (11) and others (12, 13). We know that ionized water molecules almost instantly decompose into H and OH radicals, that these can readily interact, in the absence of oxygen, some hydrogen gas and hydrogen peroxide is formed. When oxygen is also present, dissolved in water, the radical HO_2 forms, which in turn enhances hydrogen peroxide formation. In the presence of organic matter, the formation of H_2O_2 is not observed, but the evolution of hydrogen is greater. When the water contains sulfhydryl enzymes with active SH groups, these are oxidized to form S-S bonds, presumably via interaction with the radicals.

Because the yield, lifetime, and interactions of intermediates depend on the chemical composition of the cell medium, one expects large variation of radiosensitivity due to environmental factors, such as temperature, pH, and composition of the medium. In contrast, the 'direct' effect is independent of these factors. Indeed, in living cells, striking environmental influences of radiosensitivity have been demonstrated.

Radiation sensitivity of an enzyme system depends on the composition of the aqueous medium. When substances are added to the medium that can compete for the radicals and other intermediates produced by radiation, due to the competition, the enzyme system can be partially protected from inactivation. An example, given by Barron *et al.* (14), is the protection from γ -rays of phosphoglyceraldehyde dehydrogenase with added glutathione. The same protective phenomenon was demonstrated in mammals by the brilliant work of Harvey Patt (15).

and his associates, who have recently shown that the amino acid cysteine, if administered to animals shortly before irradiation, increases their tolerance, so that twice the x-ray dosage is needed for lethal effect. Similar results have been achieved by Belgian workers with cyanide (16). At this meeting, workers from the Oakridge National Laboratory (17) presented data on an impressive array of organic substances which protect bacteria.

Indirect biological effects also depend on specific ionization (18). Spacing of ion pairs influences the yield of intermediates. In oxygen free water, for example, the dense ionization of alpha rays produces a high yield of H_2O_2 molecules. The yield of H_2O_2 remains small when sparsely ionizing x-rays are used. The enzyme phosphoglyceraldehyde dehydrogenase is partially protected from α -rays by catalase (19). Many biological systems are more affected by dense rather than by sparse ionization.

If some of the actual biological effects are caused by intermediate chemical compounds and not directly by ionization processes themselves, it should be feasible to produce similar effects with applied chemical compounds. Several types of radiation effects, notably chromosome breaks, mutations and carcinogenic effects (20, 21), have indeed been reproduced by a variety of chemicals. Typical agents that have similar effect to radiation are nitrogen mustards and peroxides.

Substances that contribute to formation of intermediates after exposure can increase the destructiveness of ionizing radiation. Thoday and Read (22) have shown that oxygen dissolved in the cell medium is known to increase radiation sensitivity. Lacassagne (23) demonstrated that baby rats are more resistant under anaerobic conditions than in the presence of oxygen, and Dowdy *et al* (24) showed that anoxic rats survive higher doses of radiation than normal ones.

Barron recognized the possibility that if radiation effects are due to chemical reactions, they should be, at least in part, chemically reversible. By the addition of glutathione he was able to reactivate some sulphydryl enzymes previously inactivated by radiation. A similar approach might possibly be of import in future therapy of radiation effects.

In the process of ionization or excitation, certain molecules receive a good deal more energy than is necessary for an ordinary chemical reaction. The new field of radiation chemistry con-

cerns itself with such unusual reactions. It is expected that ionization causes a number of irreversible reactions which would otherwise only occur at high temperatures and pressures.

Recently Garrison *et al* (25) have shown that carbon dioxide, dissolved in water and irradiated by alpha particles, produces some organic molecules, notably formic acid. Other investigators have for some time been studying a number of other interesting reactions. Mund (26) has shown polymerization of simple carbon compounds with occasional formation of cyclic molecules from straight chain substances. Dainton (27) has shown that certain low molecular weight monomers (e.g., acrylonitrile) in aqueous solution become polymerized into long chain polymers.

MECHANISM OF EFFECTS OF RADIATIONS AT THE CELLULAR LEVEL

In view of the great complexity of radiochemical reactions, many of which involve higher order reactions, it is surprising that the relationship of dose to certain types of biological effects is often relatively simple.

In many cases the dose-effect relationship has the properties of a monomolecular reaction. There are, however, cases which deviate from this simple relationship. The lethal effect in unicellular organisms may occur in the irradiated cells themselves, e.g., they fail to divide, and after the lapse of some time, they might undergo lysis. In another form of the lethal effect the formation of colonies is inhibited, but the irradiated cell lives on. Although it was known for some years that irradiation of the cell nucleus brings about lethal effect more effectively than irradiation of the cytoplasm (28), it seems likely that inhibition of cell division is due to genetic damage only in some species of cells and to extragenic destruction in others.

In association with Professor R. E. Zirkle of the University of Chicago, the Donner Laboratory group 2 years ago initiated a study of the mechanisms involved in the inhibition of yeast cells, *Saccharomyces cerevisiae*. French workers, Laterjet and Euphrussi (29), as well as Beam (30), have independently worked on the same problem and our respective results are not in disagreement. The approach was to test the relationship of dose to inhibition of cell division in haploid cells and compare the results of data obtained after irradiation of vegetative diploid colonies.

The morphological nutritional requirements

and growth rate of the haploid and diploid colonies were closely similar, and it was assumed that any differences in response to radiation effect would be due to the presence of paired genes and chromosomes in the diploid against the presence of one set in the haploid cells. It was further reasoned that if the radiation effect is most important in extragenic and extranuclear components of the cells, there should be no great difference in the radiation sensitivity of haploid and diploid cells. Detailed results of this investigation will be published elsewhere (31). We may, however, note here some of the conclusions. One great difference was found in the radiation sensitivity and in the dose affecting the relationship between haploid and diploid cells, the latter being about five times as resistant as the former. The relative shapes of the survival curves of cells in which cell division was not inhibited were compared, and the conclusions were mathematically in fair agreement with the following concepts:

a) Cell division is controlled in haploid cells from a number of mutually independent 'sites' (loci sensitive to radiation). Inactivation of any of these sites brings about inhibition of cell division. Inhibition can be the result of interactions initiated from a single ionization event. The probability of inhibition is proportional to dose, and the survival curve is a simple exponential function of dose.

b) In diploid cells, cell division is controlled from a number of mutually independent pairs of 'sites' which have similar functions to the sites in haploid cells. To inhibit cell division, it is necessary to inactivate both sites of an essential pair (allele). At least two ionization events are needed to accomplish this. The probability of inhibition of cell division is not proportional to the dose. The survival curve is of a sigmoid type.

c) Comparison of haploid and diploid survival curves can be made from the relative shape of the curves and the assumption that each 'site' is inactivated with the same probability. One concludes that in the haploid cells, there are about 16 sites essential to cell division, and in the diploid, 16 pairs of such sites.

Biochemical synthesis in cells is known to be under genetic control (32). It seems plausible to associate the sites sensitive to irradiation with independent biochemical processes controlled by genes.

The number of radiation sensitive sites is only an approximation which is expected to vary with environmental factors. In a medium very rich in various nutrients, there may be alternatives for each biochemical pathway so that the number of sensitive sites might be different. Yet it is of interest to note that this number is much smaller than the estimated total number of genes, and no proof is available as yet showing that single genes are affected. A mechanical disturbance, such as a chromosome break with loss of fragments during attempted cell division might be an acceptable concept of the mechanism involved.

From the above considerations, two important conclusions follow. In the first place, one would expect cells of higher ploidy of the same strain to show higher radiation resistance. The second consideration follows from the statistical nature of the distribution of radiation induced defects. If a colony of diploid cells is irradiated by a single dose, a number of the cells in which cell division is not inhibited will carry impaired genetic defects corresponding to inactivation of impaired radiation sensitive sites. Such defects should be hereditary and would make the cells less efficient in aspects of growth and metabolism, and more sensitive to radiation than normal diploid cells with no defects. Indeed, this was found to be the case experimentally. In certain experiments, as much as 50 per cent of the progeny of preirradiated diploid yeast cells showed an inheritable increase in radiosensitivity, accompanied in some cases by reduced vigor of growth and metabolism. Haploid cells surviving a preirradiation do not show these changes, which is a strong argument in favor of the recessive nature of such radiation induced effects.

The implication of these findings lies close at hand. By studying the biochemical nature of the abnormalities in the recessively defective diploid cells, one should get valuable data, not only concerning the components of radiation effects on cells, but also relating to essential steps in cell division. Since the general types of defects are limited to about 16 kinds (and combinations of these), it may be possible to proceed systematically. The further pursuit of this kind of study may also be of interest since it might uncover some clues concerning the nature of interaction between allelic gene pairs.

The experiments on yeast cells, carried out under a variety of environmental conditions, give clear indication that the mechanism in-

volved is indirect the ion pairs do not need to be produced at the 'site' of their action

The probability that an ion pair, through some intermediate process, can find an essential genetic component of the cell, is rather small, hence, many other ion pairs and interactions are produced elsewhere within the cell before the inhibition of cell division occurs. These, collectively, may be responsible in the yeast cells for diverse effects of radiation, delay in cell division, swelling, etc. In many species of cells they may even be responsible for the actual lethal effect.

When a large colony of diploid cells is irradiated, even with a dose much larger than the 50 per cent LD, one finds some cells which have received no genetic defect at all. This is due to the statistical spatial distribution of ionization. Such cells may play an important role in the 'recovery' phenomenon well known in radiobiology. When many generations of growth have passed between one irradiation and the next, most of the cells present in the preirradiated colony exhibit normal radiation resistance. This may be due to the fact that diploid cells left unaffected by radiation, and those which mutated back to normal, outgrow the ones with genetic defects.

It would be desirable to have detailed information on the mechanism of radiation effects on other microorganisms. Much work is being done in this field, unfortunately we do not as yet have sufficient information to indicate whether or not the process of inhibition of cell division is similar in each case.

RADIATION EFFECTS ON MAMMALS

There is a very long step between physiological simplicity of yeast cells and the complexity of mammals. Many experiments will have to be performed before the radiation effects are understood in the latter. However, there are certain similarities I wish to point out.

When a part of the body of a young animal is irradiated with a sublethal dose, one often finds a prolonged retardation of growth of that tissue lasting for several generations of cells. For example, an irradiated limb in a baby animal may remain stunted for a long period of time. Though there are many factors involved, e.g. radiation effects on vascular bed and connective tissue, some of the surviving irradiated cells have possibly unpaired genetic defects which slow the rate of growth and metabolic efficiency of each

cell. Histologically, regeneration in such tissues often follows a curious pattern. In the middle of a seemingly devastated area of impaired cells, a small island of normal young cells appears. These cells appear to originate from a single cell, possibly one that did not suffer any genetic damage from the irradiation. This phenomenon is observed in therapeutically irradiated tumor tissue as well. One might speculate on the frequency and importance of impaired genetic defects produced by radiation in the diploid germ cells of mammals. By reduction division these produce haploid gametes carrying lethal genetic defects, which in turn would give rise to recessive lethals in the offspring (32). The presence of such processes will have to be proven experimentally.

Radiation effects on the tissues of higher animals should not be directly compared to those of microorganisms. The functioning of each organ in the body depends on the functioning of other organs. When the total body is irradiated, one obtains not only direct radiation effects on the cells of each tissue, but indirect effects as well, due to changes in internal environment. The internal environment is chiefly modified by humoral agents. Investigations carried out with partial body irradiation of small animals have shown the validity of some fairly general rules.

a) There are important, remote effects on various organs when they are themselves shielded from radiation, but other parts of the body are irradiated. These effects probably originate from deficiencies in the blood composition or toxic substances carried by the blood. According to Hevesy (33), nucleic acid synthesis is diminished if a remote part of the body is irradiated. Kelly and Jones (34) amplified this finding by showing that selective irradiation of the liver with a radioactive colloid materially affects nucleic acid synthesis in tumors. Van Dyke and Huff (35) irradiated one member of a pair of parabiotic rats. Epilation of the hair extended not only to the irradiated animal, but also to its shielded twin. They have thus demonstrated that a toxic substance or a depletion of some essential blood constituent has transmitted the effect via the cross-circulation between the animals.

b) Animals survive a greater dose of partial body irradiation than of whole body irradiation. The data of Bond *et al* (36) show that partial irradiation of the abdomen increases the LD 50 in rats by a factor of 2. Similar recent informa-

tion was gained by Jacobson *et al* (37, 38) who have shown that shielding of the spleen alone increases the 50 per cent LD by a factor of 2 and that shielding of almost any part of the body (e g head) affords some protection against radiation effect. When one member of parabioc twins is irradiated, the lethal dose for that member is very significantly increased (35). All these results strengthen the assertion that in mammals, a significant fraction of acute radiation effect must be extragenic and mediated by the humoral agents.

Selective irradiation of a portion of the animal body is not easy to perform with x-rays. The Donner Laboratory group (39) is utilizing high energy deuteron ion beams which allow irradiation of a small deep-seated region of the body without materially affecting the overlying tissues. In one of the experiments, done in collaboration with the Institute of Experimental Biology (40), the anterior pituitary gland of rats was irradiated, and a study of the overall and differential effects on secretion of pituitary hormones is under way.

A few years ago attempts for therapy of the acute radiation syndrome seemed to be quite hopeless. With the realization that many of the

symptoms are due to humoral agents, we can now look forward with some confidence to the development of at least partial therapeutic measures. One should diligently continue the inquiry into the nature of blood constituents that are most affected by radiation, and further study the effects of partial body irradiation. There is already wide knowledge of the impairment of the blood clotting mechanism (41). In addition Rosenthal (42) recently reported opalescence in the blood serum of rabbits which showed high correlation with subsequent death of the animals due to radiation effects. The Donner Laboratory group (43) has identified the molecules causing opalescence as a group of abnormal lipoproteins. Very recently Jacobson *et al* (38) have demonstrated that lead-shielded spleens partially protect rats from lethal effects of radiation even if the circulation of the shielded spleen is clamped off during exposure to x-rays, and released soon after exposure. Further they showed that transplantation of normal baby rat spleen into the peritoneal cavity of whole body irradiated rats also gave significant protection. This 'therapeutic' influence seems to originate from humoral agents secreted from the spleen.

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AMERICAN PHYSIOLOGICAL SOCIETY

*Symposium on Demand and Supply of Energy for Function**

Chairman JOHN O HUTCHENS

THERMODYNAMIC PRINCIPLES AS APPLIED TO THE ANALYSIS OF BIOLOGICAL SYSTEMS¹

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AMONG the general purposes of this symposium is the discussion of the free energy (or work) requirements of certain biological processes and the examination of the relation between such requirements and the free energy made available by spontaneous, metabolic processes

It is the particular purpose of this paper, in addition to furnishing a brief thermodynamic background, to formulate the above stated problem, to make clear certain concepts encountered in such formulations, to discuss the form which the laws of thermodynamics must take when applied to the kind of system met with in biology and to acknowledge (with no pretense of here solving in detail) problems which arise from the inherent nature of biological systems and in the statement of the general problem posed in this symposium

It is clear that the above outlined objectives and the constraints of reasonable space are incompatible unless some other condition is imposed. It might then be anticipated that treatment here will be brief and include frequent reference to published and forthcoming work. Any attempt to avoid formal development is doomed from the start and would result in an unbearable clumsiness of expression. The attempt has been made, however, to include only such formalism as is demanded by reasonable standards of rigor and by the fact that physical intuition is no safe guide in all situations

1 SECOND LAW AND CRITERIA OF SPONTANEITY

It will be recalled that the First Law of Thermodynamics is a statement of the conservation

of energy in which recognition is taken of the equivalence of heat and work. The usual statement is

$$dE = dQ - dW \quad (1)$$

where dE is the increase in internal energy of the system, dQ the increment of heat absorbed by the system from the environment and dW is the increment of work done by the system on the environment. Sight should not be lost of the fact that dW is external work and is zero unless provisions are made for the system to perform work against external forces. The First Law places no limitations upon the transformation of energy from one form to another but demands only that energy be conserved.

On the other hand, the Second Law imposes a limitation upon the interconvertibility of energy. The statements of this limitation are many and familiar (1-3). They are all equivalent, as can be demonstrated (2, pp 127-9, 3, pp 31-35), to this statement: No process can occur the only result of which is the conversion into an equivalent amount of work of heat extracted from a source which is at uniform temperature throughout. This statement, based upon invariant human experience, completes the inductive stage of thermodynamics. The law is then stated in the form of an axiom and the methods of logic and mathematics are brought into play to develop the consequences, producing an assemblage of theorems or propositions. These comprise the well studied array of formulae, equations, etc., of thermodynamics which cover a vast range of physical situations and are of remarkable generality. This department, in some ways simplest of all, is the so-called pure branch of thermodynamics.

The usual point of departure in discussing the consequences of the Second Law is the statement

$$T dS \geq dQ \quad (2)$$

* April 30, 1951, Cleveland, Ohio

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where dS is the change in entropy of the system and T is the absolute temperature at which the increment of heat dQ is absorbed. The equality sign in equation 2 holds for a reversible process, the inequality for a spontaneous or irreversible process. If the system is isolated such that $dQ = 0$, equation 2 becomes

$$dS \geq 0 \quad (3)$$

which states that the entropy of an isolated system can never decrease. If the process is reversible, the equality in equation 3 holds and the system suffers no change in entropy. It must be remembered that equation 3 applies to an isolated or adiabatic system for it is possible, with the aid of an external system, to decrease the entropy of a given system. The conditions under which equation 3 applies may be achieved by isolating the system in a manner such that its energy is fixed and it can do no work upon the environment. For example, if the system can perform only pressure-volume work (PV work) then equations 1 and 2 show

$$dS_{E,V} \geq 0 \quad (4)$$

thus the entropy at constant energy and volume can never decrease. The system can in principle always be so isolated by 'including the environment'. Thus the entropy of the system plus environment never decreases, as frequently stated—the entropy of the universe increases as a result of an irreversible process. By 'universe' is meant the system and that part of the outside world with which it may interact. A process which occurs under such conditions of isolation is plainly spontaneous in that it occurs of itself, i.e. with no external aid.

Now the conditions under which the entropy change may be used as a criterion of spontaneity are difficult to achieve and almost never dealt with in laboratory practice. The usual experimental conditions are constant temperature and pressure or, occasionally, constant enthalpy and pressure (e.g. in constant pressure, adiabatic calorimetry, $dQ = 0$). The most useful function will then be the (Gibbs) free energy, F , defined by

$$F = H - TS \quad (5)$$

where

$$H = E + PV \quad (6)$$

is the enthalpy or "heat content" and P and V the pressure and volume. If we write the work as

the sum of PV work and work of other kinds, then for a reversible process

$$dW_r = dW'_r + P dV \quad (7)$$

where dW'_r will include reversible electrical, length-tension, etc., work. It follows from equations 5, 6, 1 and 2 that for a reversible process at constant temperature and pressure,

$$dF_{T,P} = -dW'_r \quad (8)$$

It is a direct consequence of equation 2 and the fact that dE is the same for a reversible and irreversible change that

$$dW_r \geq dW \quad (9)$$

thus

$$dF_{T,P} \leq -dW' \quad (10)$$

Equation 10 applies to any process at constant T and P . If $dW' = 0$, e.g. if the system can perform only PV work, then $dF \leq 0$. If the system is allowed to do work on the environment (i.e. the environment does *not* perform work on the system) then dW' is positive or zero, and in this case too, $dF \leq 0$. Thus the free energy of a system, at constant T and P , which is permitted to do work on its environment can never increase. When the free energy reaches a minimum no further change (without work by an external agency), can occur for this would entail an increase in free energy. The system is then in stable equilibrium. Further, according to equation 10, as long as the free energy can decrease, useful (non PV) work can be performed and when equilibrium is reached no further work can be performed. All of this is well known. The point which is here stressed is that the derivation of equation 10 and the conclusions drawn from it are based squarely upon equation 2. We cannot insist that the entropy increase except in an isolated system. But for every statement about the entropy of an isolated system there is a corresponding statement about the free energy of the same system at constant T and P . In fact it can be shown (4), cf. SECTION 3, that the simplest manner in which to compute $dS_{E,V}$, equation 4, is by computing $dF_{T,P}$. Moreover it can similarly be shown (5, cf. 6 pp. 62–3) that the straightforward and simplest calculation of the entropy change at constant H and P (under which condition T changes as a result of the process) hinges upon the calculation or knowledge of $dF_{T,P}$. The 'simplicity' in these cases is due to the agreeable properties of the chemical potential.

In view of these last remarks and the role of the free energy change in the assessment of dissipation of energy in irreversible processes (SECTION 3) we now turn to a discussion of

2 FREE ENERGY CHANGE OF CHEMICAL REACTION AND TRANSPORT

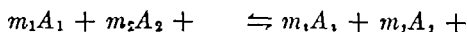
For the usual² chemical system the free energy is completely specified when T , P and the number of moles of each chemical species are prescribed. This evidently requires that T and P be uniform throughout the system. Even when this condition is satisfied additional information is needed if the system is spatially distributed (4, pp 96-8). The statements in what follows immediately can be modified for spatially distributed, open systems with no essential change in principle. Denote by n_k the number of moles and by μ_k the partial molal free energy (or chemical potential) of the k th substance. The free energy is given by

$$F = n_1\mu_1 + n_2\mu_2 + \dots = \sum_k \mu_k n_k \quad (11)$$

The chemical potentials μ_k , will be taken as

$$\mu_k = \mu_k^0 + RT \ln C_k \quad (12)$$

where R is the molar gas constant, C_k the molar concentration and μ_k^0 the so-called standard chemical potential of the k th substance. The μ_k^0 are functions of T , P and the nature of the solvent. Equation 12 embodies all of the assumptions³ regarding perfect, dilute solutes. Consider the chemical reaction



in which, stoichiometrically, m_1 moles of A_1 , m_2 moles of A_2 etc react to give m_4 moles of A_4 , etc. If we adopt the convention that the m 's are assigned negative values for reactants and positive values for products then

$$dn_k = m_k dN \quad (13)$$

where N is the "degree of advancement of the reaction" (9) and dn_k the number of moles of substance k produced when the reaction proceeds by the amount dN . The composition of the system is describable in terms of the initial number of moles, n_k^0 , and the single parameter, N . With

² We must exclude strained elastic solids, systems in which surface energy is an appreciable part of the total, etc.

³ In particular equation 12 implies (4, p 93, 7, 8, p 189) that there is no heat or volume of dilution

no loss in generality we assume that initially $N = 0$, then

$$n_k = n_k^0 + m_k N \quad (14)$$

If F_2 and F_1 are the values of free energy in the final and initial states in which the concentrations are C_k and C_k^0 respectively, then it follows straightforwardly from equations 11, 12 and 14 that $F_2 - F_1 = \Delta F$ is given by

$$\Delta F = N[\Delta F^0 + RT \sum_k m_k \ln \frac{C_k^0}{C_k}] + \sum_k (n_k^0 + m_k N) RT \ln \frac{C_k}{C_k^0} \quad (15)$$

or

$$\Delta F = N[\Delta F^0 + RT \sum_k m_k \ln C_k] + \sum_k n_k^0 RT \ln \frac{C_k}{C_k^0} \quad (16)$$

Where ΔF^0 is the standard free energy change. In equation 15 the quantity in brackets gives the free energy change, per equivalent,⁴ at the initial concentration, the remaining terms give the free energy change of adjusting the final number of moles, $n_k^0 + m_k N$, from initial to final concentrations. Similar remarks apply to equation 16. In spite of complications which may arise in applying equation 15, or a modification of it, to biological systems a fortunate, simplifying condition is frequently met in practice (10). Evident from equation 15 is the fact that if some reactants are completely consumed so that the final number of moles is zero and if the concentrations of some reactants do not change and if, further, the concentrations of none of the products change, then the terms in equation 15 associated with the changes in concentration vanish (e.g., substances such as O_2 and CO_2 are under usual conditions present at constant concentration, products such as starch, glycogen and fat are present at constant, saturated, concentration, and added substrates such as acetate, ethanol, etc are completely consumed). It can be shown (11) that rather restrictive numerical limitations can be placed upon the terms, in equations 15 and 16, associated with concentration changes and numerous special cases can be discussed.

If many reactions are proceeding, as is usually the case in practice, then the extension of the above discussion is as follows. Let N_j be the degree of advancement of the j th reaction and

⁴ Clearly, from equation 14, $N = (n_k - n_k^0)/m_k$ is the number of equivalents of any reactant converted or of any product formed.

m_{kj} the stoichiometric coefficient of substance k in the j th reaction Then

$$\left. \begin{aligned} dn_k &= \sum_j m_{kj} dN_j \\ n_k &= n_k^0 + \sum_j m_{kj} N_j \end{aligned} \right\} \quad (17)$$

For the total change in free energy one then obtains a sum of expressions, each of which is similar to equation 15 or 16 This causes no difficulty However, when, and this is the rule in biological systems, certain substances participate simultaneously in several reactions some special discussion (see SECTION 4) is needed for the calculation of 'efficiency'

Finally it follows (12) from equations 11, 12, 13 that the time rate of change of the free energy is given by

$$\frac{dF}{dt} = \sum_k \mu_k m_k \frac{dN}{dt} \quad (18)$$

where dN/dt is simply related to the usual chemical kinetic expression for the reaction rate If consecutive reactions, branched or coupled, are involved the summation is extended over all steps involved (12)

Consider a system consisting of two uniform phases Let n_1^0 and n_2^0 be the initial number of moles of a given solute and C_1^0 and C_2^0 the initial concentrations in the phases If n moles are transferred from 1 to 2, the free energy change is

$$\Delta F = nRT \ln \frac{C_2^0}{C_1^0} + (n_1^0 - n) RT \ln \frac{C_1}{C_1^0} + (n_2^0 + n) RT \ln \frac{C_2}{C_2^0} \quad (19)$$

where C_1 and C_2 are the final concentrations and it has been assumed that the standard chemical potential is the same in the two phases If several solutes are transferred the total ΔF is obtained by summing expressions such as equation 19 Strictly speaking, when ions are transferred, in addition to substituting activities for concentrations in equation 19 there should be added a term $nz\mathcal{F}\Delta\mathcal{E}$ where z is the valence, \mathcal{F} the Faraday and $\Delta\mathcal{E}$ the electrostatic potential difference between the phases In summing over all species involved, however, such electrical terms must cancel (8, 13)

If the system under investigation is not spatially uniform then the treatment in this section requires modification In the first place the free energy is no longer given by equation 11 for the values of the μ_k depend upon the point in the

system at which they are evaluated The total free energy is now given (4) by

$$F = \int_v \sum_k \mu_k C_k dV \quad (20)$$

when the μ_k and C_k are functions of the spatial coordinates and the integral is extended over the volume of the entire system If there are gradients in concentration there will in general be flow of matter, although this is not necessarily true of every solute In any event the μ_k and C_k are determined at every point by the rates of diffusion, of chemical reaction and of flow of matter across the surface of the system The value of F may not be changing with time, as is the case (4) in the steady state, but the value of equation 20 is still determined by the rates at which processes occur within the system Here we see an important example of the interplay of 'thermodynamics proper' and rates The time rate of change of F for a spatially distributed, open system is simple to obtain formally, often difficult to apply It can be shown (4) that

$$\begin{aligned} \frac{dF}{dt} &= \int_v \left\{ J_x \frac{\partial \mu}{\partial x} + J_y \frac{\partial \mu}{\partial y} + J_z \frac{\partial \mu}{\partial z} \right\} dV \\ &+ \int_v \mu q dV + \int_s J_s \mu ds \end{aligned} \quad (21)$$

where J_x is the flow, per unit area, per unit time, in the x -direction and similarly for J_y and J_z , q is the rate of production, per unit volume, J_s is the flow in the direction perpendicular to the surface, s , of the system Equation 21 has been written for one solute such expressions for all solutes are summed in the general case The first integral gives the contribution from the flow or transport of substances in the system due to the fact that gradients exist, the second integral is the contribution due to chemical reaction and when q is expressed according to chemical kinetics the integrand is a sum similar to equation 18, the last integral is the contribution due to the flow of matter, across the surface, into the system An important special case is the evaluation of the first integral in equation 21 for the case of a region (cell) in which concentrations are uniform, separated by a barrier (membrane) from another region (environment or extracellular space) which is likewise uniform There will be no gradients except the 'concentration drop' across the membrane We may assign the membrane a finite thickness, attribute to it the properties of a

bulk phase⁵, or take it in the limit as infinitesimal, or plead complete ignorance of the dimensions and properties of the barrier. The appropriate expression can be shown (4) to be

$$\left(\frac{dF}{dt}\right)_{diff} = A J R T \ln \frac{C_0}{C_i} \quad (22)$$

where A is the area of the membrane, J the flow (per unit area and time) out of the cell and C_0 and C_i are the concentrations outside and inside the cell respectively. The product AJ is the total flow (moles/time) out of the cell and (13) may be evaluated empirically without reference to the formal law of flow across the membrane. Equation 22 is for a single solute and embodies the assumptions discussed for equation 19. It should be mentioned that if concentrations are not uniform within the cell then additional terms, frequently of the same order of magnitude as equation 22 are contributed. The use of equation 22 may then be a fairly rough approximation but the error is not easily evaluated.⁶

3 FREE ENERGY, DISSIPATION OF ENERGY AND AVAILABLE WORK

According to the Second Law, the entropy, (unlike energy, volume, etc.), is not conserved when irreversible processes take place. From what has been said in SECTION 1, the entropy gain of a system is not equal and opposite to the entropy loss of the environment unless the process is reversible. If the process is irreversible, we are assured by the Second Law that the entropy of the system plus environment increases. Irreversible processes thus result in the creation of entropy. This is in essence the basis of the equality statement of the Second Law (9, 6, 17, 4). It may thus be stated that

$$TdS = dQ + dS^* \quad (23)$$

where dQ^* is the so-called uncompensated heat (9, 17). Equation 23 in conjunction with the inequality equation 2 shows that

$$dQ^* \geq 0 \quad (24)$$

⁵ This will lead to the usual permeability expressions (14-16)

⁶ To make the comparison between contributions from gradients inside and from the concentration difference across the membrane one must assign a permeability coefficient, a diffusion coefficient and dimensions of the cell. The results clearly depend upon these choices

Further,

$$dS = dS_r + dS^* \quad (25)$$

where dS_r is the entropy reversibly communicated to the system from the environment and dS^* is the entropy produced by irreversible processes which occur in the system, and is given by

$$T dS^* = dQ^* \geq 0 \quad (26)$$

The total entropy of a system does not necessarily increase but the irreversible production of entropy is positive or zero.

If one considers a system at constant energy and volume the entropy change for such a system must correspond to the irreversible production of entropy. For a process which can perform only PV work it can be shown (4) that

$$dS_{E,V} = -\frac{dF_{T,P}}{T} = dS^* \geq 0 \quad (27)$$

or for a finite change

$$\Delta S^* = -\frac{\Delta F_{T,P}}{T} \quad (28)$$

The physical significance of the irreversible production may be stated as follows. It can be shown that $T dS^*$, or $T \Delta S^*$ for a finite change, represents the dissipation or 'degradation' of energy which accompanies an irreversible process, i.e., this quantity represents the amount of energy rendered unavailable for useful work as a result of irreversibility. It is clear then that the irreversible production of entropy represents, in the above sense, the waste or inefficiency of the process in question, a matter with which accompanying papers (10, 13) are concerned. From equations 1, 23 and 26 it follows that

$$dE - T dS = -T dS^* - dW \quad (29)$$

Since, cf. discussion in (17) dE and dS depend only upon the initial and final states, the quantity $T dS^* + dW$ has a set value. If $T dS^*$ decreases dW increases correspondingly. Equations 1, 23 and 26 likewise imply that

$$dW = dW_r - T dS^* \quad (30)$$

which shows $T dS^*$ to be an amount of work which could have been performed had the process been reversible or the difference between the maximum possible work, dW_r , and the actual work, dW , in the irreversible process.

It can be shown (5) that for a process which can perform non PV work, that

$$dS_{E,V} = dS^* = -\frac{dF_{T,P} + dW'}{T} \geq 0 \quad (31)$$

from which

$$dW' = -dF_{T,P} - T dS^* \quad (32)$$

Equation 32 furnishes the equality statement corresponding to equation 10. It also states in view of equation 8, that the useful (non PV) work is the maximum or reversible work minus the energy dissipated.

It is difficult to exaggerate the simplicity of equations 27 and 31 and their derivations (4, 5) when comparison is made to the general formulation of the irreversible production of entropy (18) or the detailed derivation for special cases (6, 19) to which these results apply. We are in a position to write down at once the rate of dissipation of energy

$$T \frac{dS^*}{dt} = - \frac{dF}{dt} \quad (33)$$

for diffusion⁷ or chemical reaction by substituting equation 22 or 18 respectively into equation 33 and correspondingly for the cases of finite changes. It is easily shown (4) directly from the simple laws of diffusion that the rate of dissipation due to diffusion is the rate of work done against the forces of resistance to flow.

It is sometimes stated and often implied (e.g. 20-22) that the free energy dissipated in irreversible processes or the free energy not 'harnessed' or the energy wasted appears as heat. It is clear from the foregoing that this is not correct. The actual heat is related to the energy dissipated by equation 23. For a spontaneous chemical reaction the dissipation is numerically equal to the free energy change. In the special case of constant temperature and pressure when $\Delta S = 0$ then the dissipation is given by the heat evolved, viz., $-\Delta H$, which further implies that $\Delta F = \Delta H$ which is known not to be true in general. In the case of diffusion the dissipation is again numerically equal to the free energy change. If the solute is perfect² there is no heat change.

It is not legitimate to regard heat evolved as 'waste energy' nor does dissipated energy necessarily appear as heat.

4. CALCULATION OF EFFICIENCY

Consider an overall process which is spontaneous and which proceeds with a free energy change ΔF . Let it be capable formally of representation as the 'sum' of two component processes

⁷ In general the dissipation due to diffusion is given by the first integral in equation 21 preceded by a minus sign.

with associated free energy changes ΔF_1 and ΔF_2 . Then, since free energies are additive

$$\Delta F_1 + \Delta F_2 = \Delta F \quad (34)$$

Assume now for definiteness that ΔF_1 is positive and ΔF_2 negative. It will then be said that the second process 'drives' the first, and further, that the free energy 'available' from the second process is $-\Delta F_2$ and that 'used' in the first is ΔF_1 . The ratio

$$X = \frac{\Delta F_1}{-\Delta F_2} \quad (35)$$

is called the 'efficiency', i.e., the efficiency in the overall or 'coupled' process of the utilization of free energy made available by a spontaneous process in driving a non-spontaneous process. This quantity, which we will refer to hereafter as simply the efficiency, is also called the 'machine efficiency' (20, 10) and, more properly, the 'thermodynamic efficiency' (23).

The calculation and interpretation of efficiency as defined above requires at most what may be an inordinate amount of knowledge about the process and system and at least great care and thorough qualification in stating what is meant. Only in the simplest cases is it possible to unambiguously identify the quantities in equation 35 and assign them the meanings discussed above. In the remainder of this section the various factors and difficulties involved in the calculation of efficiency will be discussed. First, however, some remarks on the definition given in equation 35 will be made. The 'machine efficiency' was first discussed (20) in connection with the problem of whether or not organisms operate within the Second Law. It is easily shown from equations 34 and 35 that an efficiency of more than 100 per cent ($X > 1$) implies that ΔF is positive. It is well known that the effect of 'coupling' of processes is one of conservation of free energy. This is implicit in the definition of X , since⁸

$$X = 1 - \frac{\Delta F}{\Delta F_2} \quad (36)$$

Thus the smaller the loss in free energy, ΔF , the greater the efficiency. The term 'machine efficiency' is somewhat misleading since in many cases no external work is involved (e.g., expenditure of free energy in maintaining concentration gradients, chemical synthesis, etc.). The

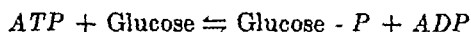
⁸ This equation shows that, since ΔF_2 is negative, if ΔF is positive X exceeds unity.

use of this term for X as defined above may be clarified as follows. If the non-spontaneous component process is to occur alone this is possible (equation 8) only if some external agency does work in amount at least ΔF_1 upon the system. When the spontaneous component process occurs it is possible for the system to perform work in amount $-\Delta F_2$ upon some external agency. The efficiency is the ratio of these theoretical works, although in the actual coupled process it may be that no external work is performed. Finally, it can be shown that, in terms of the concepts discussed in SECTION 3, X is a measure of energy dissipated. From equations 27 and 36 it follows that

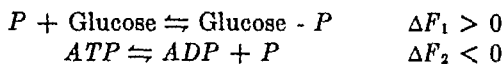
$$X = 1 - \frac{T\Delta S^*}{-\Delta F_2} \quad (37)$$

which states that the efficiency is unity minus the fraction of the (available) energy which is dissipated.

a) **Component processes** For the simple process



assume that conditions are such that



There is no question here that the component processes are those shown. In more complicated cases, however, (10) the overall process may be partitioned into component processes in several ways. Each partition leads to a different ratio of free energies and hence a different efficiency. The choice of component processes is usually clear from known pathways of intermediary metabolism. These problems are discussed elsewhere (10).

b) **Assignment of ΔF to a given reaction** A problem closely related to the one above is the following. The substance A is converted simultaneously to substances B and C



The calculation of the total free energy change is simple by the method already discussed (SECTION 2). We wish however to calculate the free energy change due *only* to the conversion of A to B . The concentration of A at any stage of the reaction is determined by how much A has been converted over *both* paths. The increment of free energy due to converting a small amount of A to B will then depend upon the amount of A

which has been converted over both paths. Clearly the quantity which we wish to compute depends upon the relative *rates* of the two reactions. If we define $f_1(t)$ and $f_2(t)$ as the number of moles of A which have at time t been converted to B and C respectively then it can be shown (11) that

$$\Delta F(A \rightarrow B) = \int_0^t \left\{ \Delta F^0 + RT \ln \frac{[B]}{[A]} \right\} \frac{\partial f_1}{\partial t} dt \quad (38)$$

is the desired quantity where $[B]$ and $[A]$ are the concentrations of B and A at any time. If n_A^0 and n_B^0 are the initial number of moles of A and B , equation 38 gives

$$\begin{aligned} \Delta F(A \rightarrow B) = & f_1 \left(\Delta F^0 + RT \ln \frac{[B]}{[A]} \right) \\ & + n_B^0 RT \ln \frac{[B]}{[B]_0} + n_A^0 RT \ln \frac{[A]}{[A]_0} \quad (39) \\ & + RT \int_0^t \frac{n_A^0 df_2 + f_2 df_1 - f_1 df_2}{n_A^0 - f_1 - f_2} \end{aligned}$$

The terms in equation 39, except the integral, are identical with the terms obtained when equation 16 is applied to the reaction in question. It can be shown that if the functions f_1 and f_2 are proportional, the last two terms in equation 39 give

$$\frac{f_1}{f_1 + f_2} n_A^0 RT \ln \frac{[A]}{[A]_0}$$

Thus the usual term in equation 16 contributed by the change in concentration in A is multiplied by the fractional amount of A converted to B . The problem can in principle be solved whenever the rates are specified whether they are empirically determined or otherwise known. In general such simple results as the above cannot be expected. This problem, illustrated by the very simple example above, is a prominent feature of the calculation of 'efficiency of synthesis' in which, for example, a substrate is simultaneously oxidized and assimilated.

c) **Available free energy** Perhaps the most vexing problem to be encountered in the calculation of efficiencies is that of deciding the amount of free energy 'available' for driving a given process. At least account must be taken of the resting, non nutrient or maintenance level of metabolism. If the available free energy is taken as that available from spontaneous processes above and beyond the maintenance level (10) it is then assumed that the cost of maintenance is not altered by the activity or synthesis in

question. Certainly circumstances can often be achieved wherein the 'correction' for resting or non-nutrient metabolism is negligible (10). But, in principle, one must entertain the idea that the cost of maintenance is not invariant under imposed conditions of activity. While, in the light of present knowledge, it cannot be asserted that activity and resting metabolism are even qualitatively the same it is probable that they are not utterly independent. As discussed elsewhere (12) it can be shown that various processes subserved by common energy-rich intermediates compete for such compounds. Thus the enhancement of a given process can suppress others with which it competes and conversely. If maintenance processes are not 100 per cent efficient, and this must be granted, then it is possible to divert energy from maintenance pathways without actually impairing maintenance.

The real problem here is that of identifying what may be called 'unavoidable waste'. We know that for spontaneous processes, processes proceeding at a finite rate, there must be dissipation. But what now is unavoidable waste?

Consider for example that among the processes competing for energy-rich intermediates there occurs wasteful splitting. It is known that such occurs and is even catalyzed by specific enzymes. Now the cell makes best use of available energy when the useful processes compete most successfully with wasteful splitting. This is governed by relative rates and those processes which are among the most successful competitors are most efficient. If wasteful splitting occurs this is just the type of information we wish to have reflected in an efficiency calculation. This is bad management on the part of the cell.

On the other hand consider the following situation. In computing the free energy of spontaneous processes if the calculation is made for the concentrations existing in the medium it is then assumed that a given amount of free energy is available. It is known that in general, but not necessarily (14), the concentration of a consumed substance is *lower* in the cell than in the medium. Calculation at the cellular concentration yields a different free energy. Moreover (equations 21 and 22) the difference corresponds to dissipation in the diffusion field. In general we would insist that this free energy is wasted insofar as syntheses are concerned. This is poor architecture on the part of the cell, open, non-uniform systems are naturally inefficient, this is unavoidable waste

consequent upon the structured nature of the system. But all of this is true if we are interested in a given synthesis and nothing else. It has been shown (4, 14) that as a result of the metabolism of substrates, non-metabolized solutes can be maintained at a higher concentration inside the cell than in the medium. More significantly, the cost of this maintenance is derived from free energy of the diffusion field of metabolized solutes. We cannot then regard the free energy of diffusion as wasted to the *total* economy of the cell. Conversely if the total free energy derived from metabolized solutes be regarded as available for maintaining concentration differences, the computed efficiency of this maintenance is quite low and under the circumstances discussed (4, 14) it is known that it is the free energy of diffusion alone which is responsible for this maintenance. The rates of free energy change due to diffusion and reaction are (equation 21) inseparably linked and assignment of free energy available for separate processes is often difficult.

It has also been shown (14) that soluble catalysts will distribute in a definite manner in the diffusion field of the cell. Often this distribution is such that the rates of catalyzed reactions are different (either greater or less) than would be the case if the same number of moles of reactant and catalyst were placed in a closed uniform system of equal volume. Such distributions are maintained by the diffusion free energy and, in addition to being regulatory devices, contribute to the cost of maintenance as certainly as do more obvious maintenances of structural integrity. An important feature is that such distributions and hence the cost of maintenance are shifted when new conditions are imposed upon the cell. It is a direct consequence of the general laws of diffusion and kinetics (14) that the rate at which a given process occurs is dependent upon the rates of other, chemically *independent*, reactions. The efficiency of a given synthesis will then be different when other syntheses are occurring.

Further examples cannot be discussed here. It should be clear from the foregoing that although efficiency calculations are based upon classical thermodynamics, often certain extra-thermodynamic information is required. In particular, rates, nature and concentration of intermediates and 'geometry' or structure play a major role in determining how well the cell can dispose of available energy.

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FACTORS IN NERVE FUNCTIONING

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RECENT developments emphasize a close relation between nerve activity and ion movement. Hodgkin and his associates have presented the evidence in favor of their view that spike production is related to sodium entry, this is believed to be followed closely by the known exit of potassium (1-4). Keynes (5, 6) has obtained direct analytical data for sodium influx as well as for the previously established potassium escape. As recognized by these investigators, the data are either highly indirect and dependent on a number of assumptions or seriously dissociated in time from the bioelectrical events assumed to be involved.

Earlier studies (7-12) have led to the conclusion that slow changes in the polarization of both frog and crab nerve, under a large variety of conditions, are caused primarily by alterations in the potassium concentration at the fiber surface which result from the release and active uptake of potassium. If this were substantiated one would have at hand the opportunity to study and to correlate electrical phenomena simultaneously with ionic transfer and thereby to evaluate concisely the factors governing the kinetics and energetics of the system.

The purposes of this report are a) to describe briefly recent results of the study of potassium and sodium transfer in relation to the earlier bioelectrical observations, b) to provide a more precise approach to the kinetics of ionic and electrical phenomena for comparison with available data, and c) to employ these principles for a preliminary survey of the energetics of the resting and active state. Full details will be given elsewhere.

RESULTS

Anoxia BIOELECTRICAL OBSERVATIONS Oxygen lack causes progressive depolarization which is slowed by glucose but accentuated by iodoacetate poisoning whether glucose is present or not (7, 11-15). Return to oxygen leads to repolarization, usually with overshooting (7, 11, 15, 16), in glucose the amplitude of repolarization is less (11, 12).

ANALYTICAL DATA Almost simultaneously evidence was reported for frog and crab nerve (17, 18) that anoxia causes potassium release, thereby refuting earlier claims to the contrary (19, 20), reabsorption was noted during the period of oxidative recovery. Glucose was found to reduce the ionic shifts in invertebrate nerve (18).

More details have now been obtained for frog nerve. Table 1 confirms the finding by Fenn and Gerschman that anoxia causes potassium release. In addition it demonstrates that glucose retards this loss and that iodoacetate poisoning inhibits the sugar effect. Because of its importance for the theoretical development later, mention must be made of our failure to confirm the conclusion by Fenn and Gerschman (17) that potassium release is not a continuous process throughout anoxia. Our experiments, now numbering in the hundreds, involve repeated analyses of the media from the same nerves as well as of the nerves themselves, none has failed to demonstrate a continuing loss of potassium.

Table 2 illustrates that removal of the external sheath improves potassium escape during anoxia only slightly but is essential for a demonstration of potassium uptake during a single hour of recovery. The unimportance of the sheath for the anoxic release indicates that another boundary, such as the individual fiber membranes, may be limiting potassium movement under these conditions. When potassium shifts are rapid, as may be the case during recovery or during stimulation, the epineurium would be expected to be the limiting factor.

Table 3 summarizes sodium and potassium data obtained by analyses of the nerves immediately following anoxia. In general, the escape of potassium is accompanied by an uptake of sodium, the latter being greater as described for stimulation (6). The protective action of glucose extends to sodium as well as to potassium. Within the error of our measurements of dry and wet weights, water movement usually was not significant.

Veratrine and Cocaine BIOELECTRICAL OBSERVATIONS These alkaloids have opposite effects

on the rate of anoxic depolarization, the anesthetic decreases, the 'unstabilizer' increases it (9, 11, 12, 21). Many other compounds (22), including procaine and antihistamines (12, 21), duplicate the action of cocaine and may be called 'stabilizers', at physiological concentrations, such as employed here, no respiratory effects are demonstrable (23). Veratrine, however, increases the respiratory rate (24). The antagonism be-

TABLE 1 POTASSIUM LOST IN N_2 TO RINGER¹ CONTAINING THE INDICATED CONSTITUENTS

<i>R. pipiens</i>				
HOURS IN N_2	SUC	GLUC	IAA + GLUC	DIFF ²
5	10.8 ± 0.5 ³	7.0 ± 0.5 ²		3.8 ± 0.2
2		0.7 ± 0.4 ⁴	2.7 ± 0.3 ⁴	1.97 ± 0.3

Data in $\mu\text{M/gm}$ wet weight

Suc = sucrose, Gluc = glucose, IAA = iodoacetate. Variability in this and all following tables expressed as the standard error based on at least 6 paired nerves.

¹ Composition as previously noted (7), potassium concentration 1.7 $\mu\text{M/ml}$. ² Standard errors computed from differences of paired nerves. ³ Sugar concentration 10 $\mu\text{M/ml}$. ⁴ Glucose concentration 20 $\mu\text{M/ml}$, IAA concentration 1 $\mu\text{M/ml}$.

TABLE 2 POTASSIUM LOST DURING ANOXIA AND THE FOLLOWING HOUR IN OXYGEN BY INTACT (I) AND DESHEATHED (D) NERVES
R. pipiens and *R. catesbeiana*

	2 HR N_2 LOSS		1 HR O_2 LOSS	
	I	D	I	D
D-I	1.3	2.2	0.24	-0.85 ¹
	0.9 ± 0.3	0.3	-1.1 ± 0.11 ¹	

Data in $\mu\text{M/gm}$ wet weight

¹ Potassium absorbed from medium

tween veratrine and cocaine is shown particularly clearly by the prevention of the depolarizing action of higher concentrations of the alkaloid mixture by low concentrations of the stabilizer (12).

ANALYTICAL DATA Figure 1A illustrates a) the increased escape of potassium in veratrine concentrations which cause depolarization and b) its complete prevention by cocaine in concentrations which prevent veratrine depolarization. Figure 1B demonstrates the ability of cocaine to

block veratrine action even after the alkaloid mixture has acted for some time, the cessation of potassium release in this case is followed by a significant absorption. Table 4 provides typical data showing the increased release of potassium during anoxia in veratrine concentrations too low to cause either depolarization or potassium release in oxygen. Table 5 shows the behavior of both sodium and potassium. As in anoxia, sodium entry usually exceeds potassium exit in veratrine, both are prevented by cocaine. The anesthetic also reduces sodium and potassium transfer during anoxia, but its effectiveness is substantially less than in veratrine. In all of these cases water shifts were not significantly greater than the experimental error of the weight determinations used for their measurement.

Stimulation BIOELECTRICAL OBSERVATIONS The after-potentials of crab nerve are particularly suited for correlation with other less accessible characteristics because of their typical slowness. Under special conditions the well-known depolarization during a tetanus (14) is followed by recovery leading to an overshooting of polarization level which apparently represents a positive after-potential (11). The relation of the depolarization to the negative after-potential is shown by its increase with veratrine, the amplitude of the repolarization is correspondingly greater (11, 25).

ANALYTICAL DATA These were obtained as in most of the previous experiments by analyses of small volumes of media recirculated past the nerves and collected at regular intervals, the perfusion units employed permitted concomitant observation of the spikes with stimulation. It has been found that in addition to the potassium escape long known to accompany activity, potassium reabsorption occurs during recovery, moreover, these shifts, like the corresponding electrical changes, are accentuated by veratrine (25).

In frog nerve potassium release also results from stimulation (26). The earlier work has been considered questionable because rather extreme conditions of stimulation were necessary. Table 6 illustrates the considerable increase in the potassium released per impulse when the external sheath of bullfrog nerve is removed. Milder stimulation, practicable in the absence of the sheath, gives about the same loss per impulse as a more rigorous tetanus. As in crab nerve, low veratrine concentrations increase potassium es-

cape during tetani Potassium uptake following stimulation has not been seen in this tissue, however this may have been obscured by the potassium loss which occurs from *R. catesbiana* nerve, whether sheathed or desheathed, even when at rest in oxygen

Studies restricted to desheathed fibers must be regarded as tentative pending the evaluation of the significance of the considerable swelling they

measurements on the assumption that accumulation of potassium in the extracellular spaces, such as would occur in the usual moist chamber situation if potassium did actually leak out, could be computed from the known depolarizing action of excess potassium when applied by way of the external medium Although the predictability of potassium behavior is now in favor of the original hypothesis, our more recent finding that sodium

TABLE 3 SODIUM AND POTASSIUM CONTENTS OF NERVES, PAIRED WITH RESPECT TO INDICATED CONDITIONS, AFTER 6-HOUR EXPOSURE TIME

R. pipiens

	Na		K	
	N ₂	O ₂	N ₂	O ₂
N ₂ -O ₂	82 0 ± 2 3	66 3 ± 1 7	27 1 ± 1 3	38 4 ± 1 2
	15 7 ± 1 5		-11 3 ± 1 3	
	N ₂ + 10mm Gluc ¹	N ₂ + 10mm Suc ¹	N ₂ + 10mm Gluc ¹	N ₂ + 10mm Suc ¹
Gluc Suc	58 7 ± 1 8	67 9 ± 2 0	28 7 ± 1 1	25 5 ± 0 8
	-9 2 ± 3 3		3 2 ± 0 7	

Standard errors of the differences are computed from the differences of paired nerves

Data in $\mu\text{M/gm}$ wet weight

¹ Sugar replacing an osmotic equivalent of NaCl in the medium

undergo in 3 to 4 hours their weight increase approaches a maximum of 30 per cent relative to the initial weight with sheath, and of 40 per cent relative to that without sheath Sodium is also gained in amounts equivalent to the entry of isotonic saline These effects may be extrafibrillar for a) the potassium content relative to the dry weight is little changed, b) the spike heights and the ability to withstand repetitive activity are not affected significantly, and c) the improved absorption of potassium following anoxia is hardly attributable to damage Fortunately, invertebrate and intact vertebrate nerve provide a check on the results with desheathed fibers

In view of the data which have been presented, the conclusion appears unavoidable that potassium shifts—and corresponding exchanges by sodium where these have been followed—are intimately correlated both in direction and time with the after-potentials and the changes in resting potential

THEORY AND RELATED DATA

Sodium vs Potassium BIOELECTRICAL OBSERVATIONS The observed movements of potassium were predicted from the earlier electrical

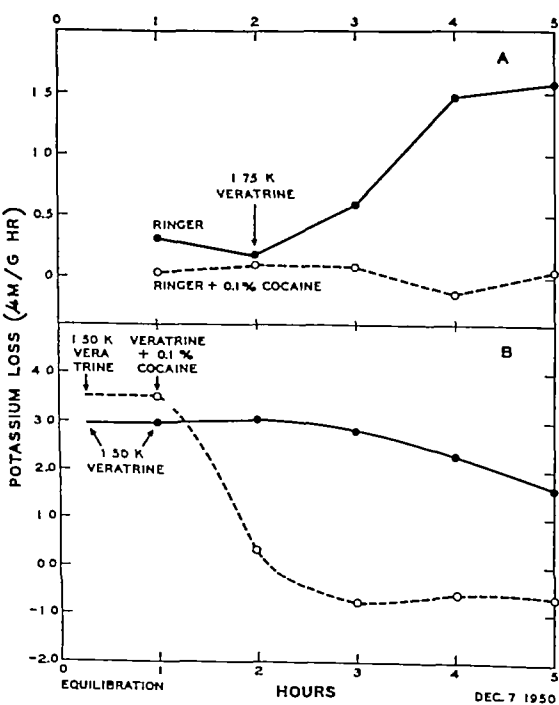


Fig 1 A EFFECT OF VERATRINE on rate of potassium release by frog nerve in the presence and absence of cocaine B Rate of potassium release in veratrine compared with that following application of cocaine

TABLE 4 COMPARISON OF POTASSIUM RELEASED HOURLY BY CONTROL (R) AND VERATRINIZED NERVES (V) SUBJECTED TO SUCCESSIVE EXPOSURES OF OXYGEN AND NITROGEN

GAS	R	v ¹	R	v ²
O ₂	0 0	0 0	0 0	0 0
O ₂	0 1	0 0	0 0	0 0
N ₂	1 0	4 3	1 1	3 1
N ₂	1 5	4 0	1 5	3 9
O ₂	-0 6	0 0	-1 0	1 7

Data in $\mu\text{M/gm}$ wet weight

¹ 0.5 mg % veratrine ² 0.17 mg % veratrine

potassium accumulation or from its prevention or decrease. In this connection Feng's observations on the failure of conduction during anoxia and iodoacetate treatment and the utilization of lactate during iodoacetate poisoning only when oxygen is present, parallel perfectly later findings on the state of polarization (15). This is in accord with the very early conclusion that the polarization level is an important element in conduction. The recent experiments of Lorente de N6 (16) lend further support to this view as well as to the postulate that conduction failure, where depolarization is known to occur, is an index of such

TABLE 5 SODIUM AND POTASSIUM CONTENTS OF PAIRED NERVES SUBJECTED TO DIFFERENT CONTROL (C) AND EXPERIMENTAL (X) CONDITIONS FOR 6 HOURS

R. pipiens

CONDITIONS		Na		K	
		x	c	x	c
X = N ₂ + 0.1% cocaine C = N ₂	X-C	69.9 ± 1.6	77.7 ± 2.3 -7.8 ± 2.1	35.0 ± 1.8	29.8 ± 1.5 5.2 ± 0.5
X = 1.50k ¹ veratrine C = Ringer	X-C	80.9 ± 2.1	60.8 ± 2.2 20.1 ± 2.4	16.8 ± 0.8	36.1 ± 1.8 -19.3 ± 1.2
X = 1.50k ¹ ver C = ver + 0.1% cocaine	X-C	84.4 ± 2.2	62.5 ± 1.9 21.9 ± 1.4	20.6 ± 1.3	37.3 ± 0.5 -16.7 ± 0.9

Standard errors of the differences computed from the differences of paired nerves

Data in $\mu\text{M/gm}$ wet weight

¹ k = 1000

moves in the opposite direction—particularly in the light of Ussing's suggestion (27) that sodium extrusion is the primary process responsible for frog skin potentials—raises the question as to which ion, if either, is immediately linked with metabolism and concerned with the electrical changes.

Several observations in the literature, and some which we have recently made, seem to provide definitive answers. With regard to anoxic failure, Feng and Gerard (28) reported that the mere washing of anoxic frog nerve with oxygen-free solution caused partial recovery. Feng (29) reported two observations to the effect that conduction failure occurred more quickly when nerves exposed to iodoacetate were mounted in a moist chamber than when they were kept in a large volume of the same iodoacetate Ringer. Similar observations for anoxia have recently been reported for frog nerve (30). These are obviously results such as would follow from

TABLE 6 POTASSIUM LOSS PER IMPULSE AS DETERMINED BY STIMULATION OF INTACT AND DESHEATHED BULLFROG SCIATIC NERVE, IN THE PRESENCE OR ABSENCE OF 0.05-0.1 MG % VERATRINE

CONDITIONS OF STIMULATION	100 SHOCKS/SEC FOR 60 MIN	20 SHOCKS/SEC. FOR 30 MIN	
		RINGER (R)	R + VERATRINE
Desheathed nerve	1.8×10^{-5}	2.0×10^{-5}	4.6×10^{-5}
Intact nerve	1.3×10^{-5}		

Data in $\mu\text{M/impulse}$, gm wet weight

depolarization. His demonstration that an anodal current restores conduction and repolarizes in the continued presence of experimental agents which have been demonstrated to cause potassium release, may now be taken as evidence for the integrity of the semipermeable properties of

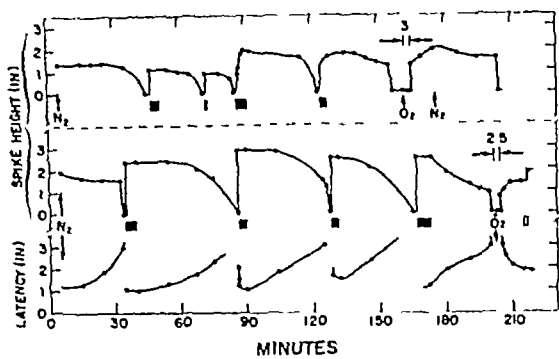


Fig 2 TEMPORAL CHARACTERISTICS of functional changes of a squid axon, only partly cleaned, a) in nitrogen gas, b) upon washing with nitrogenated sea water ■, c) upon return to oxygen gas, and d) upon washing with oxygenated sea water □ Spike height and latency (including conduction time) in inches of oscilloscope deflection

and conduction by washing and anodal currents appears irreconcilable with Ussing's proposal that failure of sodium extrusion leads to depolarization, conversely, the accumulation of excess extracellular potassium is in simple accord with known facts and provides a consistent explanation for the observations

ANALYTICAL DATA The possibility of distinguishing between a mechanism concerned with potassium uptake rather than with sodium extrusion is afforded by observations on the effects of a low sodium level in the medium on ion transfer With the sodium concentration reduced to that of potassium, the sensitivity attained for detection of sodium shifts is almost equal to that for potassium, moreover, analyses can be carried

TABLE 7 POTASSIUM LOSSES TO RINGER, AND SODIUM AND POTASSIUM LOSSES TO A LOW SODIUM RINGER, MEASURED AT SUCCESSIVE INTERVALS FIRST IN OXYGEN THEN IN NITROGEN

R. pipiens

CONDITION	RINGER K LOSS	LOW SODIUM RINGER ¹	
		K LOSS	Na LOSS
1 hr O ₂	0.24 ± 0.10	0.34 ± 0.15	3.88 ± 0.19
1 hr O ₂	0.08 ± 0.11	0.31 ± 0.13	2.49 ± 0.25
2 hr N ₂	2.02 ± 0.19	2.13 ± 0.20	1.91 ± 0.18 ²
2 hr N ₂	3.50 ± 0.15	2.58 ± 0.19	1.05 ± 0.24 ³

Data in $\mu\text{M/gm}$ wet weight
¹ NaCl replaced with sucrose or choline chloride ² The expected sodium loss calculated from oxygen data is 2.10 $\mu\text{M/gm}$ ³ The expected sodium loss calculated from oxygen data is 0.86 $\mu\text{M/gm}$

the fiber membranes and for the transport back into the fibers by the current of the potassium which had leaked into the surrounding space
The effects of washing have been examined in a system where barriers to electrolyte transfer are close to a minimum—the nerves and giant axons of the squid Figure 2 illustrates the dramatic effects noted for the action potential of a partially cleaned giant axon Washing with oxygen-free solution, after failure in nitrogen gas, restored the spike height and normal conduction velocity practically instantaneously and this could be repeated many times, return to oxygen from nitrogen gas, on the other hand, required 2½ to 3 minutes for the spike to first appear and longer still for it to approach normal amplitude It is significant that the changes in spike height and conduction velocity in nitrogen are such as to be expected from an excess of potassium (31, 32)
The restoration of both the resting potential

out for both sodium and potassium on the same medium samples
Table 7 demonstrates the behavior of nerves first in oxygen then in nitrogen after one-hour equilibration in Ringer or in a low sodium Ringer The potassium losses during anoxia are about the same in either Ringer—perhaps somewhat less in the low sodium Ringer if allowance is made for the initial tendency of potassium loss in this solution to be maintained at a somewhat higher level than in the control Sodium liberation, which is chiefly or exclusively from the extracellular spaces (33), occurs at a continually decreasing rate in accord with the exponential function observed in controls and with no indication of an additional decrease when nitrogen is introduced Experiments with longer equilibration periods in regular and low sodium Ringer were also tried but were unsatisfactory because potassium losses in oxygen were very high in the latter solution, respiratory inhibition probably

is involved (34) The experimental results therefore demonstrate that potassium release in nitrogen is not contingent upon sodium entry, from this it may be concluded that potassium exit is the primary consequence of metabolic inhibition and sodium influx is secondary In the absence of analytical data, the same relationships may be assumed to apply to potassium reabsorption during recovery

KINETICS THE RESTING STATE From the available facts we may conclude that a nerve in apparent equilibrium with its environment with respect to potassium and sodium achieves this by the absorption of potassium at a rate exactly equal to the tendency of this ion to escape Evidence has been presented (8, 10, 11) for regarding the enzymatic process of uptake to be directly related to the potassium concentration at the fiber surface, K_s , as a first approximation the relation may be considered linear The rate of escape would be governed by physical characteristics of the system such as permeability and concentration differences The rate of increase of the intracellular concentration, K_i , due to metabolism is therefore

$$\frac{dK_i}{dt} = kK_s, \quad (1)$$

and the rate of decrease because of physical factors is

$$\frac{dK_i}{dt} = -P_1(K_i - K_s), \quad (2)$$

in the steady state these are equal and opposite, hence, since $K_s \ll K_i$,

$$\frac{k}{P_1} = \frac{K_i}{K_s} \quad (3)$$

The rate constant, k , for metabolism should therefore be greater than the permeability constant P_1 (which also includes geometrical factors) by the ratio of intracellular to extracellular potassium concentrations On the basis of the potassium-potential relationship, an approximate indication of the relative magnitudes of these constants may be obtained from the maximal rates of post-anoxic repolarization and of anoxic depolarization These are of the order of 100 1 in frog nerve, which is roughly the potassium ratio in this tissue (see below)

Equation 3 is of importance in indicating that alterations in metabolism as well as of the permeability will change the equilibrium If valid it provides one means of evaluating permeability

changes which may be induced by experimental agents like cocaine that leave respiration unaltered This is presently under investigation

ANOXIA The washing and anodal repolarization experiments previously mentioned indicate that structural characteristics related to the functional activity of nerve remain intact for long periods during anoxia Glycolysis serves as a source of energy, but only to a limited extent, according to Feng (35) lactic acid production accounts for the residual energy release of frog nerve during anoxia The combination of anoxia and iodoacetate therefore may be regarded to achieve practically complete cessation of the metabolic machinery for potassium uptake Let us assume that structural integrity is still maintained¹ Figure 3 gives the simplified model of a nerve and the differential equations derived there-

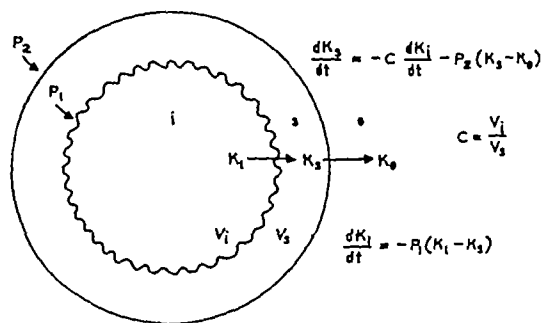


Fig 3 SIMPLIFIED MODEL of a nerve in cross-section and the equations assumed to underly potassium transfer in the absence of metabolism

from for potassium diffusion into a stirred medium, o The individual fibers are considered to constitute a single giant fiber of volume V_i bounded by a membrane whose physical characteristics are described by the constant P_1 ² Surrounding this is the lumped extracellular space, of volume V_s , bounded in turn by a surface which in frog nerve is represented chiefly by the epineurium and by the constant P_2 ² That the epineurium interferes with ionic movement was shown many years ago by the direct analytical data of Fenn *et al* (33) chloride and most of the sodium (which few will deny exist largely in the extracellular space) diffuse from frog nerve into an electrolyte free medium with an exponential time course When the chloride data

¹ Functional recovery was noted after 12 hours under these conditions and after 24 hours in Ringer

² $P_1 = h_1A_1/V_i$, $P_2 = h_2A_2/V_s$, where the h 's are the customary permeability coefficients

are plotted semilogarithmically a straight line with a time constant of 200 minutes is obtained, thus a single diffusion process is indicated and P_2 under their conditions is found to be $0.5 \times 10^{-2} \text{ min}^{-1}$. Our own preliminary determinations of P_2 from sodium measurements are closer to 10^{-2} min^{-1} , the larger value may be due to more effective stirring. The slowness of potassium escape during anaerobiosis, even when the nerves are desheathed, indicates that P_1 is substantially smaller and that the rate of anoxic loss provides a rough estimate of this constant. It may be taken as $\frac{1}{2} P_2$ for frog nerve. Additional data required are the intracellular concentrations of the cations and the volumes of the intra- and extracellular spaces. The shrinkage of frog nerve in Ringer made hypertonic with sodium chloride

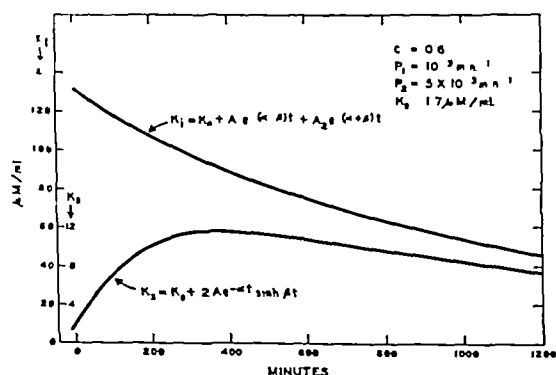


Fig 4 TIME COURSE of the intracellular (K_i) and extracellular (K_o) potassium concentrations derived from the equations in fig 3 and with the constants for frog nerve

(36) agrees with available chloride data (33) in assigning (in round numbers) the volumes given in table 8. From these and whole nerve analyses the axoplasmic concentrations shown have been computed. Estimates for corresponding data in crab nerve are also given.

Figure 4 illustrates the general forms of the solutions of the equations in figure 3.³ The specific curves shown were derived by assigning the experimentally available values for frog nerve to the constants shown. The surface potassium concentration K_o goes through a maximum while the intracellular concentration K_i declines according to a somewhat less than exponential time function.

Figure 5 illustrates the time course of polarization change when the membrane potential is

³ The author is especially indebted to Dr. Jacob Lieberman for a description of a method of solution.

assumed to obey the indicated relation for a concentration cell with shunt. On a logarithmic time scale an initial 'slow phase' is followed by a nearly linear decline, which in turn is succeeded by a 'slow phase' at very late times, this is exactly as found by Lorente de N6 (16) who conducted his experiments with the nerves in contact with solution. The linear time scale provides a more correct representation of the actual rates involved and is in keeping with time curves indicated by earlier experiments (15).

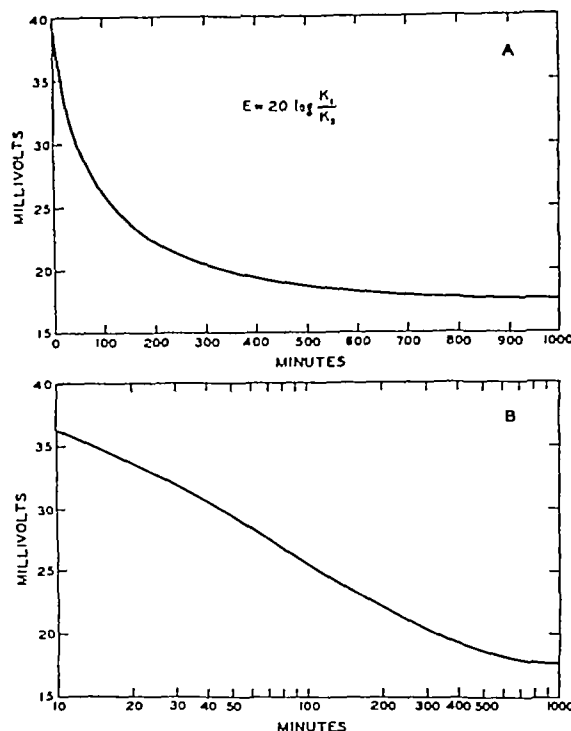


Fig 5 TIME COURSE of membrane potential change as obtained by the introduction of the theoretical values for K_i and K_o into the equation shown

The analysis reveals that it is erroneous to consider the very late slow depolarization as an approach to ionic equilibrium. Figure 6 summarizes data collected over a long period to test the validity of this analysis and compares them with a theoretical curve derived from the constants shown. The theory describes potassium release for the entire 24-hour period when iodoacetate is present, in Ringer there is an initial deviation, apparently because of glycolysis, but thereafter the theoretical time course is followed. It seems necessary to conclude that the physical restraints to potassium diffusion are essentially unchanged over these very long periods as originally assumed. Moreover, another procedure is at hand for evaluating changes in permeability

from alterations in the rate of potassium escape, it may also serve to distinguish between metabolic and permeability effects by experimental agents

POST-ANOXIC RECOVERY A description of events in this case is more complex. The time constant for metabolism, i.e. $1/k$, is small, which implies rapid potassium uptake, hence boundaries which were negligible during anoxia must be considered for an analysis of recovery. For example, the depolarization effects of applied potassium are still not instantaneous in desheathed nerve (35) but occur at a rate suggesting a time

figure 7 then suffice to describe the relationships. These lead to a third order differential equation, the solution of which for potassium at the fiber surface, K_a , is given in figure 8. The variations in the concentration possess the rapidity, the overshooting, and subsequent decline to be expected from the corresponding electrical behavior (7, 15). The chief value of these calculations at present lies in the demonstration that essential

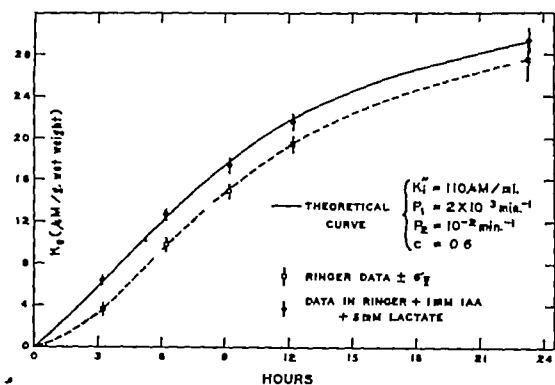


Fig 6 COMPARISON of the theoretical relation derived from the equations in figs 3 and 4, with experimentally obtained data for the net potassium release by frog nerve in nitrogen when iodoacetate (IAA) is present or absent

constant of 10 minutes, or $P = 0.1 \text{ min}^{-1}$. The relative volumes of the compartments into which such boundaries divide the extracellular space also must be known, the difference in the osmotic response of frog nerve to Ringer made hypertonic with sucrose and sodium chloride (36) may be assumed to indicate two such compartments and to provide their volumes, as given in figure 7.⁴ The lowered intracellular potassium concentration probably contributes to an elevated respiratory level following anoxia⁵ and consequently must be taken into account in a description of events. For mathematical simplicity, the nerve may be assumed to have been mounted in gas during anoxia and upon return to oxygen, the equations in

⁴ Gradients set up around individual fibers as a consequence of the diffusion restrictions by connective tissue may be a closer representation of the situation, the present assumptions may be regarded as a convenient rough equivalent

⁵ Conditions leading to a depletion of intracellular potassium (e.g. anoxia, calcium precipitants, or veratrine) augment respiration (37, 38, 24)

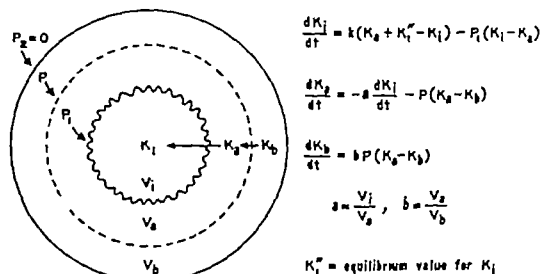


Fig 7 SIMPLIFIED MODEL of a gas-mounted nerve in cross-section and the equations assumed to underly potassium transfer during post-anoxic recovery

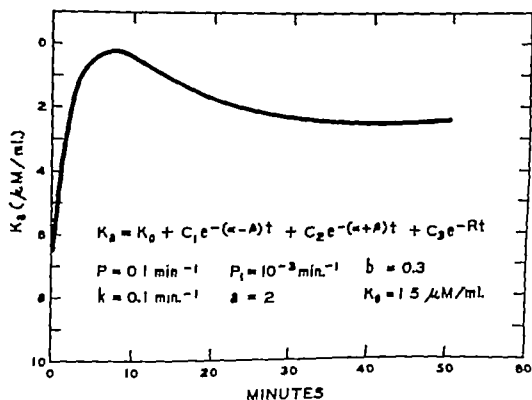


Fig 8 TIME COURSE of the potassium concentration at the fiber surface as derived from the relations in fig 7 and with the constants for frog nerve

features of post-anoxic repolarization are describable from known or measurable properties of nerve. An interesting feature of the derivation is that it is oscillatory. A concrete basis is therefore provided for examining the possibility that more rapid oscillatory bioelectrical phenomena, such as are observable in single fibers, are also due to the interplay of physical and metabolic factors, in this case the sheaths surrounding the individual axons may have an important part because of the rapidity of ionic shifts.

Energetics The encouraging results obtained in the above attempts to describe nerve behavior in terms of the ionic shifts seem to justify a

preliminary examination of some aspects of the energetics from the same standpoint. The computations to be described serve to indicate limitations of available data and to check the validity of the original assumptions.

In ionic exchange, where electrical terms cancel, the free energy change or the work done in the transfer of n mols of one of the ion species between two phases at concentrations C_i and C_o is given by

$$\Delta F = nRT \ln \frac{C_i}{C_o} \tag{4}$$

$$\Delta F = \frac{d(\Delta F)}{dt} = RT \ln \frac{C_i}{C_o} \frac{dn}{dt} \tag{5}$$

No assumptions other than those inherent in equation 4 are involved. Less general considerations, discussed previously in this symposium, lead to the same relation. The formula therefore employs the steady state concentrations (table 8) and the rate of ion transfer provided by media analyses corrected for potassium retained in the extracellular space.

In table 9 the figures obtained⁶ are compared with available heat data or with the caloric

TABLE 8 COMPONENTS OF CRAB AND FROG NERVE ESTIMATED FOR 1 GM WET WEIGHT TISSUE AT REST

SPECIES	DRY WT	V _e ¹	V _i ²	K _o ³	K _i ⁴	Na _o ³	Na _i ⁴
	mg/gm	ml/gm	ml/gm	μM/ml	μM/ml	μM/ml	μM/ml
<i>L. emarginata</i>	100	0.25	0.65	10.4	310	520	80
<i>R. pipiens</i>	200	0.50	0.30	1.7	131	110	32

¹ Volume of extracellular space ² Volume of intracellular space ³ Concentrations in the medium ⁴ Concentrations in the axoplasm

TABLE 9 RATES OF POTASSIUM AND SODIUM SHIFTS IN NITROGEN (CORRECTED FOR RETENTION IN EXTRACELLULAR SPACE), COMPUTED MINIMUM RATES OF WORK, AND RELATED EXPERIMENTAL DATA ON RESTING HEAT PRODUCTION

SPECIES	K	Na	ΔF _K	ΔF _{Na}	ΔF _{Tot}	HEAT RELEASE	EFFICIENCY
	μM/gm.hr	μM/gm.hr	cal/gm.hr	cal/gm.hr	cal/gm.hr	cal/gm.hr	%
<i>R. pipiens</i> ¹	3.4	4.7	8.8 × 10 ⁻³	3.4 × 10 ⁻³	1.2 × 10 ⁻²	8.28 × 10 ⁻² ²	4-13
<i>L. emarginata</i> ³	26	26 ⁴	53 × 10 ⁻³	29 × 10 ⁻³	8 × 10 ⁻²	69 × 10 ⁻²	10

Heat data from Feng (39)
¹ IAA present ² Based on the range of respiratory data (39) ³ No IAA present ⁴ Assumed

when a) the concentrations are the same as the activities and remain unaltered and b) the standard chemical potentials in the two phases are the same. Should one or both concentrations change appreciably—as they must under prolonged experimental conditions, particularly when the extracellular fluid volume is limited—then account must be taken of the dependence of C and hence of the chemical potentials on n . The total free energy change will be the sum of the free energy changes for each ion.

THE RESTING STATE According to the postulates underlying the kinetics, the cation redistribution when the metabolic machinery is turned off is a measure of the rate of ion transfer by metabolism during the steady state. The rate of work (or the 'free energy flux') is obtained by differentiating equation 4 with respect to time

equivalents based on respiratory measurements (39). In the case of frog nerve, where the lower members of the glycolytic cycle are a major source of energy, oxidation is found to yield a free energy change exactly equal to the thermal values (ca. 0.11 cal/μM O₂ consumed, as calculated from the data in reference 40). In this particular instance the heat figures suffice for the calculation of efficiency. Because of the sparsity of information on the specific reactions involved in the other cases to be considered, heat output

⁶ Where sodium data are lacking, sodium movement has been assumed equal and opposite to that of potassium. When both ions are followed, somewhat more sodium usually is found to be involved, hence another ion probably is undergoing transfer. It may be anticipated, therefore, that some error is present even where sodium data are available.

will be assumed to equal the total free energy change for the efficiency computations

Within the limitations of the data, the efficiency is about the same for frog and crab nerve, viz., 10 per cent, when the energy turnover in the steady state is assumed to be concerned exclusively with ion transfer. Allowance for the contributions of processes concerned with other than ion distribution to energy liberation may make these efficiency figures higher.

STIMULATION A satisfactory evaluation of the energy relationships of activity involves correction not only for potassium retention in the extracellular space but for potassium reabsorption during the stimulation period as well. The use of inhibitors selective for 'activity metabolism' (41) may serve to minimize the second complicating factor. The available data must presently be

change during recovery, we obtain an efficiency of about 45 per cent for crab nerve and of 5 per cent for frog nerve. The high value for invertebrate tissue indicates that the restoration of the normal ion balance is a major concern of recovery, the involvement of some recovery reactions in other processes would lead to a prohibitively high efficiency. It is hardly likely that the use of heat rather than free energy data in the calculations is in itself responsible for this high value. The low figure for frog nerve cannot be taken seriously until an estimate is available of the potassium failing to appear in the medium during stimulation because of active reabsorption, a high absorption rate would give a spuriously low efficiency.

These considerations do not imply a direct dependence of impulse transmission on recovery

TABLE 10 POTASSIUM AND ASSUMED SODIUM SHIFTS FOR A SINGLE IMPULSE (CORRECTED FOR RETENTION IN THE EXTRACELLULAR SPACE), CORRESPONDING FREE ENERGY CHANGES, AND AVAILABLE DATA FOR HEAT PRODUCTION

SPECIES	K	Na	ΔF_K	ΔF_{Na}	ΔF_{Tot}	INITIAL HEAT	RECOVERY HEAT
	$\mu\text{M/gm impulse}$		$\mu\text{cal/gm impulse}$			$\mu\text{cal/gm impulse}$	
<i>L. emarginata</i>	5.3×10^{-3}	5.3×10^{-3}	10.7	5.9	16.6	0.73	36
<i>R. pipiens</i>	2.9×10^{-6}	2.9×10^{-6}	7.5×10^{-2}	2.2×10^{-2}	9.7×10^{-2}	$6.7-26^2 \times 10^{-2}$	2

¹ At 20°C

² At 0°C

regarded on the low side. In the case of crab nerve this error is probably small because of the slow recovery process.

Table 10 gives available thermal (39) and calculated figures for the single impulse. In frog nerve the initial heat is of the same magnitude as the free energy decrease, but this may be regarded a coincidence since in an ideal solution no heat would be evolved as a result of mixing. Some heat transfer may be anticipated from the non-ideality of the system but, unless other than simple dilutions are involved within the fiber, this is probably small. It is noteworthy that the decline in initial heat with continued repetitive stimulation (39) is in the direction to be anticipated from the changes in concentration and in the associated activity coefficients.⁷

Again assuming that thermal data (i.e. the recovery heats) measure the total free energy

⁷ Dr. Hearon very kindly provided the theoretical basis for these remarks on initial heat

processes. This would be contrary to the conclusion reached earlier from the washing and recharging experiments. Rather, recovery may be regarded as concerned a) with minimizing the extracellular accumulation of potassium and b) with preserving an adequate reserve of intracellular potassium for impulse production. On this basis the interference with recovery respiration achieved recently with azide (41) need not be expected to impair conduction as long as the potassium levels within and without the fibers are not altered excessively, but, as in the case of inhibitors less specific for 'activity metabolism,' azide may be expected to hasten the onset of failure during continual activity.

NOTE ADDED IN PROOF

A series of publications by Feng and his associates in the 1950 issue of the *Chinese Journal of Physiology*, received subsequent to the submission of this manuscript, should be noted, of particular interest is the paper (*Chinese J. Physiol.* 17:247, 1950) corroborating the correlation be-

tween potassium release and depolarization indicated by our early indirect experiments and discussed with Dr. Feng prior to publication (7, 8). Limitations of space have compelled a scrupulous neglect of recent pertinent data on muscle, however, attention must be called to the work of Fleckenstein and his associates, recently summarized in detail (*Arch Exper Path Pharmacol* 212: 54, 1950). Their results are of particular importance in indicating significant relationships between electrochemical changes and muscle short-

ening under conditions similar to those which have been described for nerve.

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MACHINE EFFICIENCY OF ASSIMILATIVE PROCESSES

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THE EFFICIENCIES with which living organisms convert food materials to protoplasmic constituents or energy stores during growth, repair, or maintenance are of economic importance. They also carry important theoretical implications for students of intermediary metabolism. Investigations dealing with this problem have been conducted using organisms ranging from mammals to microorganisms (Phototrophic nutrition poses special problems and will be neglected here). The purpose of this paper is to analyze some of the results obtained in an attempt to estimate the efficiency with which such conversions can or do occur. The varied and frequently arbitrary conventions used by different workers will be examined, and the doubts and difficulties which beset students of the problem will be emphasized. Conventions which make possible the application of thermodynamic as well as thermochemical analysis to studies of heterotrophic nutrition are introduced. It is hoped that these will encourage further investigation and thermodynamic analysis particularly in mammalian nutrition.

The discussion which follows is divided into five parts: 1) Definition of the machine efficiency of assimilation; 2) problems connected with ascertaining this efficiency; 3) efficiencies reported or calculated here for microorganisms; 4) factors affecting assimilation efficiency; and 5) calculation of a machine efficiency of assimilation in the rat.

The literature cited is intended to be illustrative rather than comprehensive and it is left to the reader to consult original papers for details of methods and results. All free energy calculations are based on values to be found in previous publications (1, 2).

1. DEFINITION OF MACHINE EFFICIENCY OF ASSIMILATION

Two widely differing measures of assimilation efficiency are in common use. Thus, in mam-

malian nutrition studies, efficiency is ordinarily calculated (3) as $100 \times \text{calories stored} - \text{assimilable calories ingested}$. In studies on microorganisms (1, 4, 5) efficiency has been calculated as $100 \times \frac{\text{free energy cost of assimilative reactions}}{\text{free energy available from catabolic reactions}}$. An efficiency calculated in either way is commonly called the 'efficiency of growth'. Growth in the complete sense involves cell division which requires energy (movements of cytolinesis and karyokinesis, creation of new surface, etc.) and organization with an unknown entropic contribution. Most of the studies referred to below, however, involve simply the accretion of protoplasm or of stored materials such as glycogen or fat. I shall therefore speak of efficiencies of assimilation rather than of growth.

The two different customs referred to above represent basically different approaches to the problem of assimilation. On the one hand, the calories in corn conserved by a cow are of economic interest. On the other, the ratio of free energy required to that made available for assimilation may either support or embarrass particular ideas about intermediary metabolism and the mechanisms whereby energy is trapped and utilized. I shall refer to efficiencies calculated in the first way as 'caloric conservation efficiencies' or to the 'caloric conservation coefficient'. For the quantity $100 \times \frac{\text{free energy cost of synthesis}}{\text{free energy available from catabolism}}$ I shall use the term 'machine efficiency of assimilation' (6).

By free energy available from catabolism I shall mean that over and above a basal level² observed in nonassimilating control organisms. In microorganisms this 'basal' level is ordinarily a fasting level (1, 5). In mammals, as we shall see, the maintenance level of metabolism serves

² Subtraction of the endogenous metabolism of microorganisms may be questionable in other connections (7). For the present purpose, failure to do so would imply that the cost of other processes during assimilation is zero. In many cases the correction is inconsequential, particularly if the endogenous metabolism is a small fraction of the exogenous.

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best as the base (S) Subtraction of a maintenance metabolism in microorganisms would be preferable, but this is difficult to establish The fact that metabolism during assimilation in microorganisms is many times (5- to 40-fold) greater than the endogenous rate minimizes the error involved

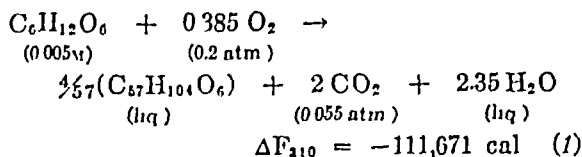
2 POSSIBILITY OF ESTABLISHING MACHINE EFFICIENCIES OF ASSIMILATION

At first glance, calculation of the efficiency with which free energy available from catabolism is captured for purposes of assimilation appears simple Assimilation in principle consists of the chemical conversion of one or more compounds to some other compound(s) Given the free energies of formation (ΔF_{form}) of reactants and products, their activities, and the number of mols converted, the free energy required is calculable The same being true for catabolic reactions, the energy available and thence the machine efficiency of assimilation emerge A closer inspection of the details involved, however, forces a more pessimistic outlook

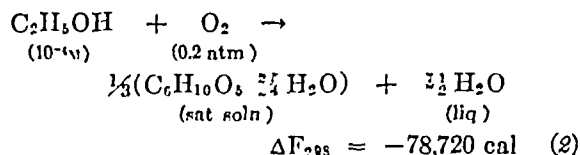
The most glaring present-day deficiency in the knowledge needed for this purpose is our complete ignorance of ΔF_{form} values for proteins Indeed the values for many amino acids are unknown, and are not calculable by usual procedures (9) Entropy values associated with structural improbabilities in proteins further complicate the matter It is no wonder therefore that those who have attempted to estimate assimilation efficiencies have either eliminated protein synthesis by omitting a nitrogen source (2, 5) or have made arbitrary assumptions regarding the energy cost of protein synthesis (1, 4)

The above difficulties regarding the cost of protein synthesis could probably be removed by appropriate experimental work Heat-capacity measurements at low temperatures on a few amino acids and a few crystalline proteins would permit calculations regarding entropies from the third law of thermodynamics There would still remain certain difficulties not so easily overcome These concern carbohydrate and fat formation as well as protein formation

What shall we say is the work done when an animal converts a food, say glucose, to fat? The overall process is exergonic Thus for glucose to triolein assuming passage through a C_2 stage with the necessary generation of 2 CO_2



If we accept this at its face value, no work is done and the efficiency of assimilation is zero or indeterminate This situation is not confined to mammalian nutrition or to foods like glucose Thus Blum *et al* (2) were faced with the problem of assimilation of ethanol with starch being synthesized Again the overall reaction under their conditions was exergonic



The escape from the above dilemma proposed (2) is something more than a convention Invoking the known intermediary metabolic steps and the fact experimentally demonstrable in their experiments that acetic acid appears at least transiently in the conversion of ethanol to carbohydrate the authors reasoned as follows Energy is released in the oxidation of ethanol to acetate, some further energy is released by the complete combustion of a fraction of the acetate, to convert the remainder of the acetate to amylose is an endergonic process Taking the work done as that of converting the acetate to amylose, and the energy available as the sum of the two oxidative processes, these authors found an efficiency essentially the same as that found for acetate oxidation and assimilation in *Chilomonas*

The above line of reasoning can be applied to the glucose-to-fat conversion cited above Thus glucose in all probability is first converted to two C_2 fragments + 2 CO_2 + water To synthesize fat from these C_2 fragments is an endergonic process A more detailed analysis of this situation is given in section 5 The important point to be recognized is this Whenever in an overall exergonic process, a point is reached at which the remaining steps are endergonic, the problem for the biologist is to ascertain how efficiently the free energy yielded in the exergonic steps is captured to drive the endergonic ones This is no violation of the principle that the overall free energy change is independent of the path (10) This must and will still be true However, one does not have to be frightened into ignoring everything except the overall ΔF Common sense,

if nothing else, tells us that if endergonic steps occur, they must be driven. Other steps in the same overall process are candidates for this function on an equal basis with steps in parallel reactions.

The above convention introduces certain difficulties not encountered in simpler situations. Thus in the ethanol-starch conversion, the concentration of the intermediary acetate affects the actual ΔF values of both the exergonic and endergonic reactions. In the glucose-to-fat conversion the identity and concentration of the C_2 fragment are of critical importance. These however are transient difficulties to be overcome by future studies of intermediary metabolism and quantitative analyses of the cells or fluids concerned.

Finally we may consider the methods available for establishing the percentage of available food-stuff degraded and the percentage assimilated. These methods differ in detail but are in essence the same whether the assimilating organism be a bacterium or a mammal. Three mutually supporting methods are available.

1) Direct chemical analysis This is most easily practiced with mass cultures of microorganisms from which aliquots can be withdrawn from time to time. It is the least equivocal of the methods and knowledge of the details regarding conversions occurring is limited only by existing analytical methods and the experimenter's patience.

2) Calorimetry By measuring the extra heat produced by an organism presented with a limited and known amount of substrate, the degradation-assimilation ratio can be calculated under certain circumstances (2, 5). Ordinarily chemical analyses or respiration measurements must be conducted to confirm the assumptions regarding the reactions responsible for generation of the heat.

3) Respirometry The extra O_2 consumed and/or CO_2 produced can be used in place of heat production as above. Again auxiliary information from direct analyses or heat measurements may be required.

The practical difficulties met with in calorimetry and respirometry for the above purposes are well illustrated in figure 1 taken from an experiment carried out in my laboratory (11). This figure might just as well represent heat or CO_2 production as O_2 consumption.

It is a well-established fact that in such ex-

periments the extra O_2 , CO_2 or heat is less than that theoretically derivable from the added substrate (2, 5, 7). It is commonly assumed that when a sharp break occurs in the upper curve the added substrate has been exhausted through combined degradation and assimilation. With glucose as substrate this has been demonstrated analytically (5). With other substrates it seems the only explanation compatible with other known facts (7, 12). It would be comforting, however, to have direct analyses for other substrates at the time of this sharp break in the curve.

The difficulties inherent in this method of establishing oxidation-assimilation ratios stem from the following:

1) The endogenous rate of O_2 consumption falls off with time. In yeast this decline is first order (5). In *Chlamydomonas* there is usually an abrupt change in rate after 2 to 3 hours (lower curve, fig. 1).

2) The rate of O_2 consumption following the exhaustion of added metabolite exceeds even the original endogenous rate for some time. This excess in rate after the break is roughly proportional to the amount of substrate assimilated (7, 11).

The net effect of the above is to indicate a progressively greater percentage of substrate catabolized the longer one waits to assess the gas or heat increment. In my opinion the best choice one can make is ΔY_1 (fig. 1). To take the increment at any later time probably includes metabolism of material recently assimilated. This problem has long plagued experimenters in this field, but it has become particularly important in some of our recent studies.

So long as a large fraction of added substrate is oxidized and the calculated efficiency is low it carries no embarrassing theoretical implications. However, in experiments on *Chlamydomonas* metabolizing acetate we have recently recorded oxidation-assimilation ratios near 1.4. With starch as the end product this implies an efficiency near 50 per cent under our conditions. Furthermore, the energy captured is approaching the maximum which can be accounted for by high energy phosphate bond ($\sim P$) formation. Should the apparent efficiencies increase by much, the number and/or energy value of $\sim P$ bonds formed would have to be increased, other mechanisms of energy capture would have to be found,

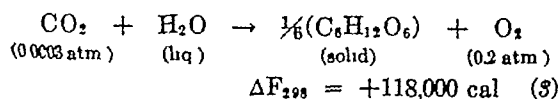
on our assumptions regarding the cost of synthesis would have to be revised

To summarize, in preparation for the next section. So long as the end product of synthesis is carbohydrate or fat, quite accurate calculations of the efficiency of assimilation frequently can be made. When protein is being synthesized no exact calculations are possible. Often the overall reaction of assimilation is exergonic. Only by splitting it into its exergonic and endergonic components can any calculations of efficiency be made. With these reservations I present the data on microorganisms below.

3. BEST PRESENT ESTIMATES OF ASSIMILATION EFFICIENCIES

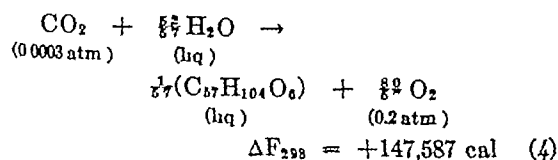
The most precise calculations of the efficiency with which assimilation occurs have been made using data obtained with microorganisms (1, 2, 4, 5). In a pioneering effort Baas-Becking and Parks (4) in 1927 analyzed the data of many workers who had studied the growth of autotrophic bacteria. The data available to them gave moles of CO_2 fixed per mol of inorganic energy yielding material oxidized. Defining efficiency of growth as I have defined machine efficiency of assimilation they obtained values near 6 per cent for most forms, hydrogen bacteria being exceptional with an efficiency of 26 per cent.³ Until the recent studies of Blum *et al.* (2) on *Chilomonas* the figures for hydrogen bacteria were the highest ever reported.

In making their calculations Baas-Becking and Parks (4) made the simplifying assumption that all of the CO_2 fixed was converted to solid anhydrous glucose. The equation used by them was



With CO_2 as a carbon source this simplifying assumption leads to a probably negligible error in view of other uncertainties. Thus, if the amylose hexose unit of glycogen or starch (1) is taken as the product in the above equation $\Delta F_{298} = +118,862 \text{ cal}$.

For that fraction of the CO_2 converted to fat, using triolein as typical



³ See also Burk (6)

In the absence of free energy values for protein formation we can do no more than state the value of $\Delta H = +126,000 \text{ cal}$ per mol of CO_2 converted to egg albumin based on a heat of combustion of 5.7 cal/gm. The efficiencies calculated (4) for autotrophic bacteria would therefore be increased to no more than 7 to 8 per cent for most forms if closer account were taken of the end products.

When we turn to heterotrophic nutrition, the assumption that solid anhydrous glucose is the end product of synthesis can no longer be employed without introducing an intolerable error.

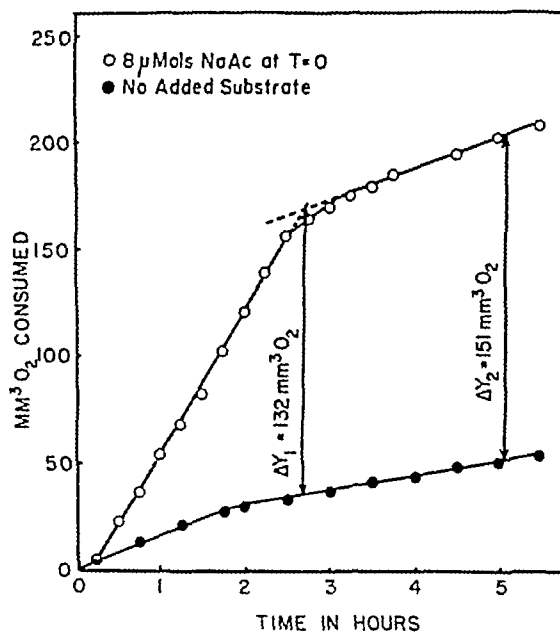
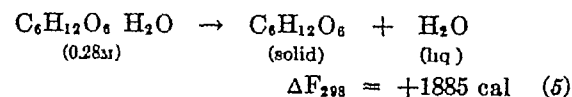
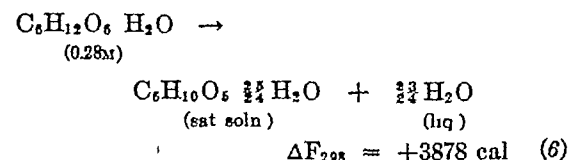


Fig. 1. TIME COURSE of O_2 consumption following addition of 8 μmols Na acetate to suspension of *Chilomonas* at pH 6.0. Temperature 25°C, 4.0 ml cell suspension 3.5×10^6 cells/ml.

Thus, if 5 per cent glucose (0.28 M) itself is the substrate



On the other hand, taking the actual final product as amylose hexose units in saturated solution yields



Large errors (a factor of 2-3) in estimating the

work done can easily be introduced in this way. In all of the studies summarized in table 1 adequate account was taken of the final product of synthesis. In making calculations from Barker's (7) data I have accepted his conclusion that only carbohydrate was formed and used the amylose hexose unit as the final product taking as its free energy of formation $-217,851$ cal (1). For lack of space I have dealt only with Barker's data involving acetate and butyrate. Data presented by Wilson and Peterson (13) and Fulmer (14) could also serve as the basis for interesting calculations but assumptions used would have to be explicitly stated.

It will be seen from table 1 that reported values for the efficiencies of assimilation of organic substrate by heterotrophic forms fall in the same range as those reported for autotrophic bacteria (4). Whether these are the highest possible efficiencies will be discussed below.

4 FACTORS AFFECTING ASSIMILATION EFFICIENCY

The natural tendency of investigators studying the efficiency with which substrates are assimilated has been to employ temperatures, pH values, salt concentrations, etc., optimal in the sense that they produce the most rapid growth of the organism. The carbon and energy source has frequently been below optimal in the above sense. There is no *a priori* reason to assume that conditions which lead to most rapid cell division or increase in mass of protoplasm also lead to the most efficient assimilation. Indeed there is evidence to the contrary.

Brücke (15) studying anaerobic assimilation of glucose by yeast found that monoiodoacetate (IAA) at a concentration which inhibited fermentation by 86 per cent reduced conversion of glucose to glycogen by only 57 per cent. The experiments were of such duration as to preclude any explanation based on temporary nonsteady state phenomena. The improved assimilation-fermentation ratio raised the efficiency of the process from ca 2 per cent to ca 6 per cent. A similar effect of fluoride on assimilation of glucose by yeast has been reported (16). These findings may be interpreted as follows. The fermentation of glucose is inhibited by IAA and fluoride while the assimilative reactions from glucose to glycogen are unaffected. Any existing excess of energy production over that actually required for synthesis would thus be reduced.

The converse of the above situation is seen in the effect of such inhibitors as IAA and fluoride in reducing the percentage assimilation of substrates such as acetate and butyrate (12). Here the assimilative pathway is blocked while energy release is essentially unaffected. Cyanide and azide reduce assimilation of substrates but their effect is not easily explained. Diminophenol in appropriate concentrations leads to complete oxidation of acetate in yeast (5) and *Pseudomonas* (12), i.e. zero assimilation efficiency. Presumably this results from interference with oxidative generation of $\sim P$ bonds (17).

The report of Blum *et al* (2) that the oxidation-assimilation ratio for acetate is greater in the presence than in the absence of a nitrogen source cannot at present be interpreted. Protein synthesis may require more energy than carbohydrate or fat synthesis. On the other hand it may be less efficiently accomplished. In any event the finding serves as a warning against accepting assimilation efficiencies obtained in the absence of a nitrogen source as a measure of the 'efficiency of growth' (5).

Two factors which might logically be expected to influence assimilation efficiency have been inadequately studied in microorganisms. These are temperature and the nutritional state of the cell. In my own laboratory we have been unable to detect any change in the efficiency of synthesis of starch from acetate by *Chilomonas* over the temperature range from 15° to 30°C. Regarding nutritional state, starving cells for 24 hours has little if any effect on percentage of acetate assimilated. Raising the starch content of the cells by prolonged exposure to acetate in the absence of a nitrogen source (to prevent growth) simply reduces the rate at which acetate is oxidized and assimilated rather than changing efficiency (11).

5 MACHINE EFFICIENCY OF CARBOHYDRATE ASSIMILATION IN THE RAT

As stated earlier it is common practice in studies on mammalian nutrition to calculate the caloric conservation efficiency. If calculations analogous to those given above for microorganisms have appeared, they have escaped my notice. I have attempted some crude and tentative calculations below with three objects in view. First, to stimulate others to make more precise calculations, second, to stimulate the reporting of data which will make such calculations possible, and finally to show that, to a first approxi-

mation, mammals do not differ radically from microorganisms in the efficiency with which they capture free energy for assimilative purposes

Out of the vast body of knowledge regarding mammalian nutrition relatively few studies are suitable for the present purpose. Measurements of the specific dynamic action of foods measured above the basal (fasting) level must be rejected. Between the basal and maintenance levels of metabolism food apparently serves largely to spare the body stores which otherwise would have to be metabolized. Only above the maintenance

On the basis of the above figures the mean caloric conservation efficiency is 77.5 per cent since only 22.5 per cent of the calories theoretically derivable from the extra fed starch appeared. In order to calculate a machine efficiency of assimilation we must make certain assumptions regarding the end product of the assimilation, the pathway of metabolism, and the conditions under which the conversions occurred.

That the end product stored was chiefly fat there can be little doubt. The rise in R:Q values produced by the extra starch indicates this, and

TABLE 1 MACHINE EFFICIENCIES OF ASSIMILATION OF HETEROTROPHIC MICROORGANISMS

ORGANISM	SUBSTRATE	PRODUCTS	METHODS	EFFICIENCY, %	REFERENCE
Yeast	Acetate	Glycogen	Heat prod	12.2	3
	Glucose (aerobic)	Glycogen	Heat prod	2.88	3
	Glucose (anaerobic)	Glycogen	Heat prod	4.62	3
	Glucose (anaerobic)	Glycogen	CO ₂ prod	ca 4.6	20
<i>Chilomonas</i>	Acetate	Starch	Heat prod	26.7	8
	Acetate	Starch (and fat?)	Heat prod	21.9	8
	Acetate	Starch (fat and protein)	Heat prod	13.1 ¹	8
	Acetate	Starch, fat and protein	Direct analysis	17.0 ¹	4
	Acetate	Starch	O ₂ cons and CO ₂ Prod	29-46	10
	Ethanol	Starch	Heat prod	20.2	8
<i>Prototheca</i>		Starch (and fat?)	Heat prod	17.1	8
	Acetate	Amylose	O ₂ and CO ₂	16.4	6
	Butyrate	Amylose	O ₂ and CO ₂	13.8	6

¹ Energy cost of protein taken as that of carbohydrate of equal carbon content

level does net synthesis occur. Forbes *et al.* (8, 18) have shown that above the maintenance level, i.e. when net synthesis is occurring, the specific dynamic actions of foods increase sharply. Above the maintenance level the extra heat produced per unit of ingested food is constant over a considerable range (8).

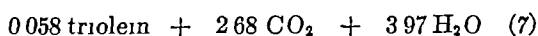
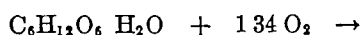
I have chosen as the basis of the computations which follow the data of Kriss *et al.* (19). In these studies rats were first established on a maintenance ration of calves' meal. The average daily heat production was 15,888 cal. When an additional 2.2 gm of starch having a caloric equivalent of 8228 cal were fed the mean daily heat production rose to 17,736 cal. The difference of the means was 1848 cal with a standard error of 242 cal.

storage of any appreciable portion of the starch as glycogen would produce an improbable rise in the glycogen content of the tissues. I shall assume therefore that fat was the product of synthesis and use triolein to represent this.

The remaining assumptions which I shall make can be quarrelled with in almost every detail. The absurdities are purposeful, however, in that the pathway and conditions chosen yield what is probably the maximum machine efficiency. Thus I shall assume that the C₂ particle derived from glucose which serves as the starting point for fat synthesis is the acetate ion at a concentration of 10⁻⁶ molar. Trapping the C₂ particle at an energy level above acetate would decrease the work of synthesis more on a percentage basis than it would decrease the energy made avail-

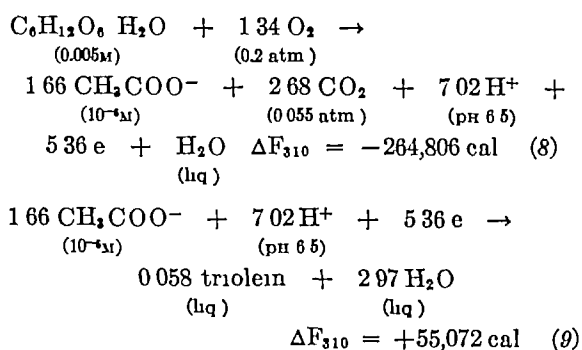
able for driving the reaction. Other assumptions can be similarly shown to work in this way.

I shall ignore the heat and free energy changes attendant on hydrolysis of the starch, and the hydration, solution and dilution of the derived glucose. Starting then with glucose at a concentration of 90 mg per cent (0.005 M) I shall write the following overall equation:



I have calculated $\Delta H_{310} = -176,926$ cal for this reaction. The heat value given by Kriss *et al* for their starch (3.72 cal/gm) corresponds to a little more than monohydrated amylose units (MW = ca 182). On this basis they fed 0.012 mols/day. The above equation therefore predicts an extra 2100 cal/day. This figure is well within the permissible range of 1364 to 2332 cal (mean ± 2 S.E.).

To estimate the energy available and required for the conversion I shall break the overall reaction into two components:



On this basis the machine efficiency is 20.8 per cent. As mentioned above alterations in the adopted scheme to make it more nearly compatible with the realities of intermediary metabolism will tend to lower the efficiency. I can only plead that I would have made such changes if I could. Until the identity of the C_2 fragment, its concentration, and energy of formation are known, a better calculation will be difficult. One point is perhaps worth comment. In classical schemes of representing carbohydrate to fat conversions generation of gaseous O_2 is employed in partial reactions. The scheme I have employed of reserving the H^+ -electron pairs for subsequent reductions in fat formation with generation of water is more realistic. It was to achieve balance in this regard without appealing to outside systems that I accepted a slightly high figure for heat production. Trapping the C_2 fragment at a more reduced stage than acetate would allow greater latitude in proportioning oxidation and fat synthesis.

It would thus appear that, employing similar conventions, the machine efficiency of assimilation in a mammal may be quite similar to those observed in microorganisms. Other and more adequate calculations of this type should of course be made. In particular, variations in food-stuff, feeding schedule, and end product synthesized may alter the efficiency. The examples cited in section 4 bear witness to the fact that the efficiency which does occur may have little relation to the maximum possible efficiency.

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AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS

Symposium on Cytochemistry

Chairman E L TATUM

LOCALIZATION OF SUBSTANCES IN CELLS

ARTHUR W POLLISTER, MARION HIMES AND LEONARD ORNSTEIN

From the Department of Zoology, Columbia University, New York City

IT is very gratifying to a cytologist to have an opportunity to address The American Society of Biological Chemists in a Symposium on Histochemistry. Until the later decades of the nineteenth century, cytology and biochemistry were not at all distinct, both were prominent parts of the broad field of physiology. The microscopic structure of tissues always appeared as part of the textbooks, and many of the great physiologists speculated on the possible localization of functions within the structural components of cells. Claude Bernard, for example, suggested that the cell nucleus, the only structure then recognized to be common to all cells, was concerned with synthetic functions—a view that was later brilliantly confirmed by the demonstration that when a single-celled organism is cut in two pieces, only the nucleated half can carry on the syntheses essential for growth. It was under the stimulus of such ideas that in 1869 Miescher devised a method of isolating masses of nuclei from the remainder of the cell, the cytoplasm, and subjected them to chemical analysis. Almost coincident with Miescher's discovery of the very unusual chemical composition of the nucleus, Abbé's greatly improved compound microscope became available—and in the hands of Flemming and others this instrument led to observations of the remarkable nuclear phenomena during cell division and fertilization, which marked the beginning of modern cytology. These early workers by no means regarded themselves as in distinct fields, each was well aware of parallel progress by the other group. Miescher, for example, performed experiments to show that the specific staining of nuclear substance was due to combination of his nuclein with the basic dye methyl green, while it was a cytologist, Altmann, who

gave the name nucleic acid to the acidic component of Miescher's nuclein. Beginning early in the twentieth century, however, there developed among both cytologists and biochemists a tendency to work under domination of particular standardized techniques rather than under stimulus of the broad problem of correlation of biological function with intracellular organization. Such specialization eventually led to the almost complete isolation of these two fields, which prevailed until fairly recently. Within the last 10 years happily there has been a reorientation, a return to recognition of the priority of the problem of the significance of intracellular organization. Today there are many cytologists who are anxious to work with biochemists, even to attempt to master some of their techniques, and there appear also to be biochemists who are willing to attempt to localize activities within morphological components like the nucleus, mitochondria, and microsomes.

It is the great charm, the fascination, of study of the microscopic structure of organisms that the shapes of tissue cells and intracellular morphological changes are often so beautifully expressive of the function of the cell. This is well shown by the exocrine glandular cell, such as that of the pancreas, which I shall later use to illustrate the methods of localization of nucleoprotein in cells. This cell is in the form of a pyramid, usually six-sided, with sides in contact with 6 other cells. The base of the pyramid is adjacent to the blood vessels, the apex is in communication with the duct system of the gland which opens into the small intestine. The fine details of cells are usually studied in thin sections, and the probable structures visible in such a thin section are shown schematically in figure 1. The

most conspicuous object is of course the nucleus, within which are scattered masses of chromatin, and a conspicuous nucleolus. The duct end of the cell is largely occupied by a mass of spherical secretory granules, concealed within which is a pair of centrioles, which are organs of cell division. Scattered throughout the cytoplasm are mitochondria, and in the basal region, perhaps also

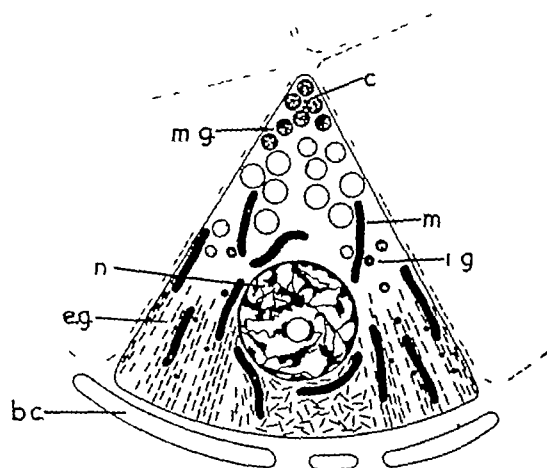


Fig 1 DIAGRAM of structural features in section of an exocrine glandular cell of the pancreas, after Pollister, 1951 (1). Parts of the boundaries of adjacent cells in the secretory unit, or acinus, are outlined and the blood capillaries near the basal end of the cell are indicated. Abbreviations: *c*, centriole, *eg*, basal ergastoplasmic zone, containing submicroscopic microsomes (The microsomes have not been observed in this type of cell. It is assumed from results on liver cells by Hogeboom, Schneider, and Palade, 1948 (2), that: *a*) the cytoplasmic region of high nucleic acid content must be the site of a major accumulation of microsomes, *b*) the microsomes are asymmetrical, probably thread-like, and *c*) these asymmetrical bodies tend to become oriented parallel with the long axis of the cell, as suggested in Pollister, 1940 (3)), *mg*, mature granule, *ig*, immature granule, *m*, mitochondrion, *n*, nucleus, and *bc*, blood capillary.

elsewhere, there is reason to believe there are rather densely packed sub-microscopic thread-like microsomes. The actual structure is shown in the phase contrast photomicrograph of an unstained one-micron section of a rat's pancreas (fig 2 A). This cell has one principal function, to elaborate the granules which are thrown out into the duct system when the gland secretes the pancreatic juice. This phenomenon clearly suggested that the granules are the precursors of the constituents of the pancreatic juice, for which reason they have long been called *zymogen granules*.

As you doubtless all know, this relation to the pancreatic enzymes is supported by the observations of Van Weel and Engel (4), that the peptidase activity of fresh slices of pancreas is directly related to the granule content of the cells. The central problem of intracellular pancreatic function, then, is how in a few hours the cell elaborates a large mass of zymogen granules, which has been estimated to be as much as one-fifth of its own dry weight. Morphologically this process has been well described in beautiful studies, first by Hirsch (5) and later by Duthie (6). In the quite intact gland, drawn out onto a warmed slide through a ventral incision in an anesthetized mouse, they followed the development of individual granules. The granule appears first as a small body in the basal zone, it slowly enlarges and becomes progressively more dense as it moves toward the final position for discharge. There are also marked concomitant changes in the nucleolar part of the nucleus, and in the appearance and staining reactions of the basal part of the cell.

The pancreas is a typical component of a specialized tissue, and like other cells has two categories of visible structures: *a*) the granules which are primarily an expression of the unique cell function, and *b*) the nucleus, mitochondria, centrioles, and probably microsomes which are much like those of all other cells, and which we accordingly are accustomed to consider as the essential cell organelles which are concerned with cell maintenance and other functions which underlie the special tissue activity. These structural features of cells are fairly well worked out, although the picture will surely soon be greatly enriched by developments in electron microscopy. The major unsolved problem is to make this picture meaningful in terms of cellular biology, to determine what specific functions may be attributed to the visible structures in cells. There was a period—not so long ago—when it seemed as if physiologists were satisfied to attribute a majority of fundamental intracellular activities to the properties of a formless sort of soup, which had as its chief asset the heritage of a fine old name—*protoplasm*. I suppose this was patterned after the distribution of hemoglobin in the erythrocyte, which is a most atypical cell indeed. At any rate, there has been in recent years a marked change of viewpoint. As some of the other speakers in this symposium will amplify, it is becoming evident that many important physiological mechanisms are as sharply restricted to

one particular cell component as the mechanisms for gene distribution and photosynthesis have long been recognized to be

The localization of substances within cells, to which I am to give particular attention, often clearly indicates the function of a particular part of the cell. There are two main ways by which such a qualitative chemical analysis may be carried out: one may deal with isolated masses of a particular structure, as Miescher did with nuclei, or one may by direct microscopic observation visualize the substance within a single intact cell.

Improved methods of isolation have added much information about the chemical composition of nuclei, mitochondria, and microsomes of animal cells and the plastids of plant cells. The sample may be large enough so that a variety of analytical procedures can be carried out to give a comprehensive average composition of structures from a population of billions of cells. If one performs the arduous task of analysis of all fractions in the isolation procedure, as Doctor Hogeboom and his collaborators (2) first did, it is possible to make the very important demonstration that a chemical compound, or a specific activity, is restricted to a particular category of cell component. Thus, it has been repeatedly demonstrated that the desoxyribose nucleic acid of a mass of tissue is all recoverable in the isolated nuclei. Furthermore, if one counts an aliquot from the sample, he can estimate the number of components in the sample, and by division of the figures from the analysis of the sample arrive at an average amount per component. Boivin, Vendrely and Vendrely (7) first did this with isolated nuclei from a variety of beef organs and from spermatozoa. They noted the average amount per tissue nucleus was the same for all organs, and was approximately twice that of the spermatozoon (table 1). Since this was identical with the relationship between the double set of chromosomes of the tissues and the single set of the spermatozoon, they concluded that the amount of nucleic acid was constant for each chromosome set. Davidson and Leslie (10) have suggested that with the DNA determination as a base, one can estimate the number of cells in a sample, and by reference to this express other analytical results as amount per cell. This will, of course, indicate the real situation within each cell only if there is reason to suppose that all cells are alike. The method must therefore often

tend to obscure the very aspect of chemical composition which may be most important, namely the variation of cellular composition with cyclical or progressive cell phenomena.

Most workers seem to be well aware of the fact that there are further difficulties in extrapolating to the cell, which arise from the procedures used in isolating components. The purity of the isolation is always problematical, and even though the fraction may obviously contain only a negligible contamination with other structural elements, there is always the possibility that material may be either washed out of the components or adsorbed from other sources.

TABLE 1. DNA PER NUCLEUS IN BEEF TISSUES

ORGAN ¹	AMOUNT ¹ ($\times 10^{-9}$ mg.)	NO. OF NUCLEAR TYPES	PROBABLE CHROMO- SOME NO.
Thymus	6.4	1	Diploid
Liver	6.4	2	Diploid
Pancreas	6.9	6	Diploid
Kidney	5.9	10	Diploid
Sperm suspension	3.3	1	Haploid

¹ After data of Vendrely and Vendrely, 1948 (8). The major number of nuclear types are estimated from common textbook descriptions of the histology of the organs. Table reproduced from Polster, Swift, and Alfert, 1951 (9).

Assuming that these difficulties are obviated, the best that the method of isolation alone can yield is data as to the overall average composition and the restriction of a substance to some members of one category of cellular component, it obviously falls far short of giving specific information at the level of a single cell. Thus, from the data of table 1 alone, one could not say whether there might not be in any sample a considerable, though constant, fraction of the nuclei which had no desoxyribose nucleic acid at all. As a matter of fact, such a question has not, so far as I know, ever been raised, because, as everyone knows, there is excellent cytological evidence from application of the highly specific Feulgen reaction for desoxypentose that every single nucleus contains an abundance of desoxyribose nucleic acid. While, as Dr. Hogeboom will tell you, chemical analyses indicate that certain important intracellular respiratory mechanisms appear to be confined to the mitochondrial fraction, it by no means follows that these occur in every individual mitochondrion. The most conclusive evi-

dence that this is so appears to come from the cytological observation that when cells are stained vitally with a dye Janus Green B, which is readily reducible to the colorless leucobase, every mitochondrion remains brilliantly colored in sharp contrast to the colorless remainder of the cell. I respectfully request that you keep in mind for a few minutes these excellent illustrations of the interdependence of the isolation and cytological techniques, while I describe our efforts at adaptation of some chemical methods to the study of individual cells. Compared with the certainty and variety of the analytical techniques which can be used upon a sample of several milligrams of isolated components, our resources will seem extremely limited. Few though they are, however, these are the only gateway which certainly leads directly inside the individual cell.

The possibility of applying analytical methods to achieve the visualization of substances *in situ* within the cell has intrigued many cytologists, histologists and pathologists, and there have been numerous sporadic outbreaks of enthusiasm for one or another supposedly satisfactory method, the devotees of which have proceeded to describe the localization of the particular substance in a wide range of cell types. Many of these are evaluated critically in Lison's *Histochemie Animale* (11). The ideal localization is of course that which can be carried out upon the intact living cell, but in practice this is necessarily limited to substances with strong natural absorption, which occur in relatively high concentration. When this is visible color, like that of chlorophyll or hemoglobin, visualization is obviously easy. It has long been known that with ultraviolet light, absorption within the living cell is very pronounced in the chromatin of the nucleus (fig. 2D). In 1936 Caspersson (15), in his masterly doctoral dissertation, proved that the chief basis of this was the natural high specific absorption of the purine and pyrimidine components of the nucleic acid of chromatin. Originally, this seemed to offer an opportunity for localization of nucleic acid within living cells, but it is now becoming generally recognized that the interpretation of the ultraviolet absorption spectra of living cells is by no means unequivocal. A great variety of intracellular substances (for example nearly all aromatic compounds) absorb the same part of the ultraviolet spectrum as does nucleic acid, and there seems no way of distinguishing among these in the living cell. Other

difficulties arise from non-specific light loss and from the injurious action of the radiation (see Ris and Mirsky, 16). Consequently it is clear, as Caspersson himself has stated in his latest publication (17), that living cells are as a rule unsatisfactory objects for ultraviolet absorption analysis. It is far better to analyze fixed tissues, in which the specificity of the ultraviolet absorption can be checked by subjecting the cells to a variety of solvents and enzymes, as well as to confirmatory specific color tests and stains.

We must then, work mainly with what is called fixed material, and our efforts must be of course confined to those analytical procedures which will not alter the cellular structure beyond recognition. This immediately excludes a great many methods which are too drastic to apply to sections. A further limitation arises from the fact that during the process of fixation there is abundant opportunity for loss of and extensive redistribution of inorganic and smaller organic molecules. This has been repeatedly demonstrated to take place, and the necessity for caution in interpreting the results of microchemical tests for these substances has been frequently stressed¹. Thus, localization is practically limited to the compounds of large molecular size, which are precipitated or coagulated by fixation. By some special methods certain lipoidal substances can be rendered insoluble, but in most cells the common acid fixation may be assumed to leave essentially unchanged in amount and distribution only the proteins and nucleic acids. These are, in fact, the basis of the common histological picture of the cell, in which the nucleus is stained with basic hematoxylin, the cytoplasm with acid eosin. From the standpoint of nucleoprotein analysis, such fixation is of great advantage, for it is a sort of preliminary fractionation. The specific methods of demonstrating the distribution of protein and nucleic acids in the glandular cell, which was described previously, will illustrate the modern application of qualitative chemical cytology.

Analyses of animal tissue have shown that the bulk of the cellular material, roughly 60 to 80 per cent of the dry weight, is protein. This pro-

¹ Localization of certain mineral elements does seem possible if frozen-dried material is ashed (see Scott, 18) and many other elements can be localized by x-ray absorption analysis of frozen-dried tissue. Also the method of radioautography can probably be extended to the levels of intracellular localization.

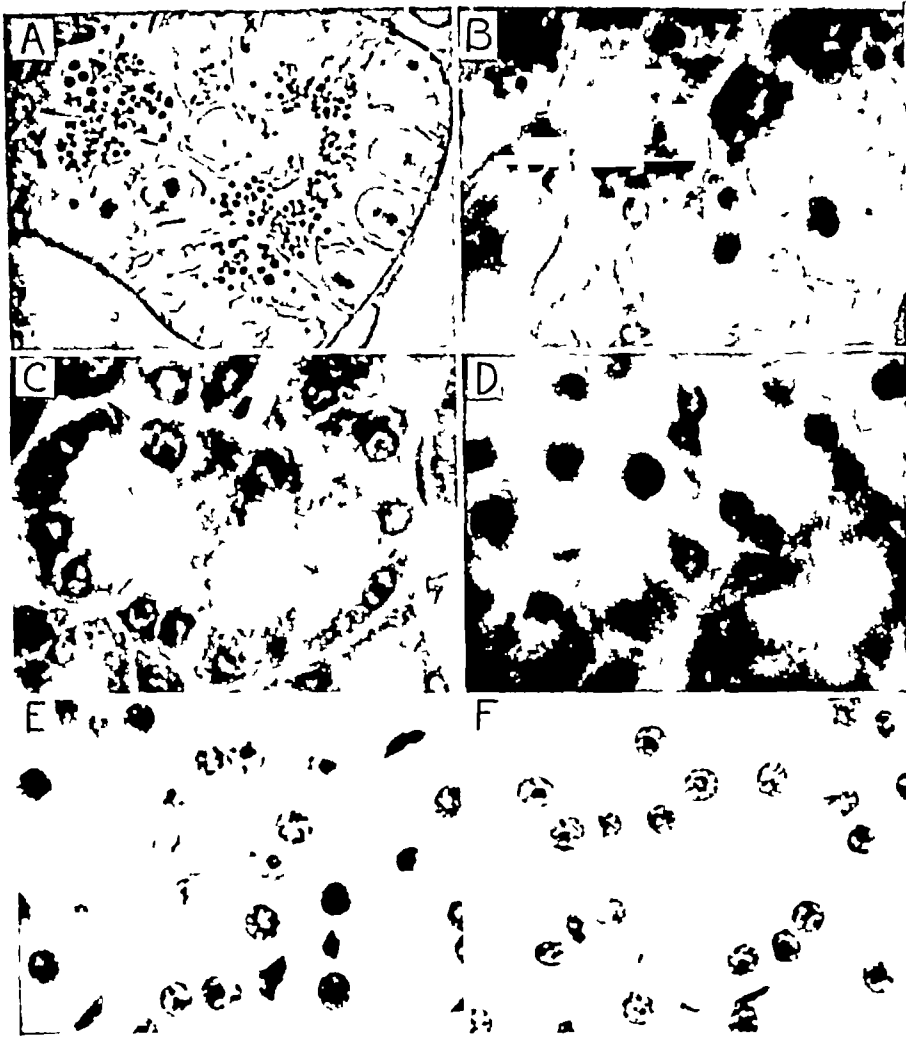


Fig 2 SERIES of photomicrographs of pancreatic glandular cells, to show structure and the results of various techniques of localization of nucleic acids and proteins

A From a tadpole of the green frog, *Rana clamitans*, tissue well fixed to show all structural details Technique Double fixation in osmic acid followed by formalin, embedded in n-butyl methacrylate and sectioned at 1μ , using a Spencer microtome with slow feed attachment and a glass knife, mounted in Cargille's refractive index oil (n_D 1.460), photographed with Zeiss phase contrast equipment, using a Pointolite lamp, Wratten 58 filter, and Kodak M Plates, $\times 800$ B From the salamander, *Necturus*, after the Millon test for protein Technique Fixed in Champy's osmic-chromic-bichromate mixture, embedded by the methyl benzoate-celloidin-paraffin method, and sectioned at 6μ , Millon reaction for total protein as described in Pollister (12), photographed on Wratten M plates, using an AH4 type mercury vapor lamp, from which the 365-6 radiation was isolated by Corning glass filter No. 5840 in 2.4 mm thickness, $\times 850$ C From an adult male white rat, after staining with the basic dye Azure A with a method which is specific for chemical staining of nucleic acids Technique Fixed in acetic alcohol (1:3), embedded in paraffin, and sectioned at 5μ , stained as described in (12), photographed on Kodak M plates, using a tungsten ribbon filament lamp and a Bausch and Lomb daylight glass filter Magnification $\times 800$ D From the same block of tissue as C, photographed by ultraviolet light to show the high absorption in regions of nucleic acid concentration Technique Sectioned at 3μ and mounted on a fused quartz slide, paraffin removed with chloroform, and section mounted in glycerin saturated with chloral hydrate, as recommended by Kohler (13), photographed on Kodak Process plates, at $254m\mu$, with the apparatus described by Pollister and Moses (14), using a Cooke, Troughton, and Sims 81 \times monochromatic objective, $\times 800$ E Adjacent section to C, which has been digested with ribonuclease before staining with Azure A Technique Digestion for 2 hrs at $37^\circ C$ with Worthington's crystalline ribonuclease, concentration 0.2 mg/ml pH 6.0, boiled before use with saturated $(NH_4)_2SO_4$ to destroy protease activity, staining, photography, and magnification as in C F An adjacent section to C, after the Feulgen reaction for desoxyribose Technique Feulgen reaction as described in (12), photographed as in C, using a Wratten 58 filter instead of the daylight glass

tein is the basis of the staining of cytoplasm with acid dyes, like eosin. A more satisfactory chemical method of showing intracellular distribution of protein is by use of a protein test, like the Millon reaction, which is specific for tyrosine and tryptophane. As figure 2B shows, there is protein throughout all parts of the cell, with especially conspicuous concentrations in the zymogen granules and the nucleolus. Such a photomicrograph as this has no chemical meaning unless it can be shown that the method is specific for protein. It is a major distinction between the attitude of many cytologists of the past and of those of today that this point is always critically examined. That the Millon color on slides is formed with the reactive protein groups is demonstrated by the fact that protein masses which are known to be deficient in tyrosine and tryptophane, such as collagen and protamine, are Millon-negative on slides in which adjacent proteins are strongly positive.

The old method which demonstrates the intracellular distribution of nucleic acid is by staining with an acid solution of a basic dye, such as Azure A, shown in figure 2C. Histologists call this basophilia. There is strong stain in the chromatin and nucleolus of the nucleus, and in the broad basal part of the cytoplasm. The last reaction has been known for a long time, and a special name, *ergastoplasm*, was given to it before the chemical basis of the staining reaction was understood. Strictly speaking there are two sorts of strongly acid basophilic substances in cells, the phosphoric acid esters which include the two nucleic acids and the sulphuric acid esters which include such substances as mucopolysaccharides. The latter are relatively rare and easily distinguished from nucleic acid when they are present. For precise characterization of the basophilic material in any part of the cell further cytological tests are always necessary. For example, after a companion slide to the last has been digested by ribonuclease the basal zone and nucleolus are no longer stainable with Azure A, while the chromatin still stains as heavily as in an undigested slide (fig 2E). If the ribonuclease digestion is followed by desoxyribonuclease, then the slide will not stain at all with Azure A.²

² It should be pointed out that it is dye-binding capacity which the Azure A staining indicates. This property depends to a certain extent, perhaps variable from tissue to tissue, upon the association of the nucleic acid with protein, hence a weak or negative basophilia does not necessarily always indicate a low concentration of nucleic acid.

That the nucleic acid of chromatin is largely if not entirely of the desoxypentose type is confirmed by the highly specific Feulgen reaction for this sugar. With this method, as with the azure stain after ribonuclease, there is only a nuclear pattern to indicate the cellular structure of the pancreas (fig 2F). As would be expected, after predigestion of a slide with desoxyribonuclease the Feulgen reaction is negative.

In addition to the basophilia due to phosphoric acid groups of nucleic acid and the Feulgen reaction of the desoxy-sugar the strong natural absorption of the purine and pyrimidine bases has proved to be, in Caspersson's hands, a useful method of localization of polynucleotides. Figure 2D, which is a companion slide to that from which 2C was photographed, clearly shows, as did the basophilia, the nucleic acid localization in the chromatin, nucleoli and basal zone of ergastoplasm. The method of Caspersson (17) has been to measure complete ultraviolet absorption curves of points in different parts of the cell, and from the curve shape to draw conclusions about the approximate nucleoprotein composition. For example, the basal zone shows a nucleic acid curve with distortion of the long wavelength slope due to the protein, while a region of the zymogenic granules shows only protein ultraviolet absorption. As with basophilia, it has been shown (e.g. by Davidson, 17a) that nuclease digestion reduces specifically the ultraviolet absorption of areas like either the basal zone and nucleolus or of the chromatin, while absorption in all these regions is reduced by pretreatment of the section with both enzymes, or with hot trichloroacetic acid. Unlike basophilia, the ultraviolet light loss is by no means all due to specific nucleic acid absorption, a certain amount at any wavelength is due to protein absorption, and often as much as half of the light loss is non-specific because of scattering and internal reflections in the dense protein mass (1, 17).

The variety of techniques which may be used to localize nucleic acids within the cell may be briefly summarized. Both pentose and desoxypentose nucleic acid are basophilic, absorb ultraviolet light strongly, and are soluble in hot trichloroacetic acid, the specificity of the nucleases and the Feulgen reaction serve to demonstrate whether the nucleic acid in a given region is predominantly of the pentose or the desoxypentose type.

Application of these nucleoprotein methods has completely clarified the questions of distribution

of nucleic acids in cells. Independently Caspersen and his fellow-workers, using ultraviolet absorption and the Feulgen reaction, and Brachet and his associates (19), using bisophila, ribonuclease, and the Feulgen reaction, have made it clear that a) chromatin is the site of major intracellular concentration of deoxyribose nucleic acid, b) ribose nucleic acids are especially characteristic of the cytoplasm and the nucleolus, and c) that there are striking changes in amount of ribose nucleic acid in cells in which protein synthesis is especially rapid, as in growth or synthesis of a glandular product. The cell shown above had completed synthesis of a batch of granules, if this were about to begin it would have been noted that the basophilic ergastoplasm would have been much increased, extending distally into the region level with the nucleus.

The qualitative cytological methods which have just been discussed lead to localization of a substance within a cell by its absorption, which is detectable visually. Cytologists usually speak of the intensity of a stain or color reaction as weak, strong, very dark, etc.—and, of course, these terms imply semi-quantitative evaluation of the concentration of the component which is responsible for the color. When two objects are side by side in an evenly illuminated microscopic field, or in the two fields of a comparison eyepiece, visual matching appears to be as accurate as objective photometric measurements. With objects of the same size, proper visual comparison is then a rough indication of relative amounts, if two objects are equally dark they may be assumed to have approximately the same amount, and if they appear different the darker one may be taken to contain more reacting substance. The same conclusions regarding relative concentrations may be drawn of two bodies of equal vertical thickness (equal absorbing path). More often, the quantitative question which faces a cytologist cannot be answered even roughly by visual comparison. For example, one often wishes to know relative amounts in two objects of very different size. It is uncertain to what extent by visual study a microscopist can even determine whether two such unequal objects have the same intensity (a rare condition probably), for the relative sizes of the contrasting surroundings introduce considerable difficulty. If this match could be accurately made, then from the dimensions of the object one could compute a fairly good estimate of how much more substance the larger object contained.

The above examples illustrate the range of visual microscopic comparison. If, to cite a very common experience, one cytological object is larger and more lightly colored than another, the cytologist is almost completely helpless to answer the obvious question of whether the decrease of color is entirely due to dilution in the larger mass. The relation of volume to light absorption is easily computed from an actual figure, a measurement of extinction, but such a quantitative datum is absolutely necessary. No amount of experience can train a cytologist's eye to operate as an objective microscopic photometric device. These measurements must be made with objective photometers.

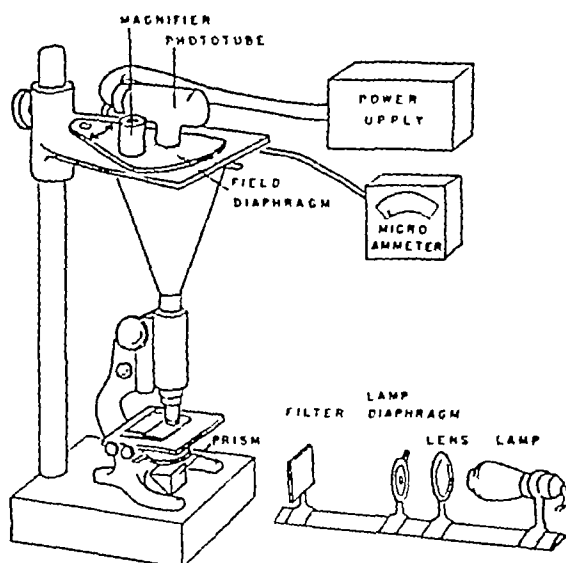


Fig 3 DIAGRAM of a simplified apparatus for photometric measurements of cytological preparations. After Swift (20)

At the Columbia Laboratory we have for some time been exploring the possibilities of escape from these limitations of visual estimation by adapting quantitative photometric analysis to cytology. We hoped to be able to substitute objective relative values for the differences in absorption which the eye or a photographic plate detects as different intensities. The photometry can be made by carefully controlled photography and densitometry, but it is easier and more accurate to measure the absorption directly by a phototube mounted in the optical axis directly above the microscope (fig 3). Measurements are made at a single wavelength, near the absorption maximum.

If methods of demonstration of nucleoprotein are to be used in this quantitative fashion ob-

viously they must, in addition to being specific, be reproducible to a known extent. For example, the staining technique must be standardized so that it comes to a sort of end point, always reaching approximately the same intensity with a given sort of cytological material. When I started to measure cell structures it at once became apparent that the individual absorption measurements on any object within a single section were variable over a considerable range—a situation which has likewise been found by all other workers who have given attention to the question. The data for any one object group themselves into

that from cell counts (table 1) Boivin and the Vendrelys (7) concluded that the nuclei of tissue cells of any one species of animal all contained approximately the same average amount of DNA. This was fully confirmed by Swift's (20) photometric measurements of individual nuclei of 11 different tissues of the rat (table 3). Essentially similar results have been reported by Pasteels and Lison (23) and by Leuchtenberger, Vendrely and Vendrely (24). It does indeed appear that specializations of cells do not involve major changes in amount of desoxyribose nucleic acid.³ Swift's photometric measurements also confirmed

TABLE 2 DNA, HUMAN ERYTHROBLAST NUCLEI, FEULGEN

CASE NO	DIAGNOSIS	BEFORE B ₁₂		AFTER B ₁	
		No nuc	E ₄₄₀	No nuc	E ₄₄₀
1	Normal	30	231 ± 003		
2	Nutritional macrocytic anemia	20	250 ± 009	30	247 ± 007
3	Megaloblastic anemia of infancy	30	244 ± 007	5	244
4	Pernicious anemia	10	232 ± 007	20	242 ± 005
5	Pernicious anemia	35	249 ± 005	27	238 ± 005
6	Pernicious anemia	30	240 ± 004	35	240 ± 005
7	Pernicious anemia	35	231 ± 006	20	235 ± 007
8	Pernicious anemia	50	248 ± 005	20	242 ± 006
9	Pernicious anemia	40	256 ± 006	30	248 ± 005
10	Pernicious anemia, juvenile	30	231 ± 005	40	237 ± 006
Mean			241	Mean	241

Mean extinction values and standard errors of the mean Feulgen stained erythroblast nuclei, smears of human marrow, all nuclei 9.5 μ in diameter. The extinction, E_{440} , is a measure of the amount of nucleic acid in the nuclei. From data of Reissner and Korson, 1951 (22).

fairly normal looking unimodal distribution curves (21). Reproducibility of one of these cytochemical methods must therefore always be estimated by statistical analysis. The shapes of the distribution curves are the same from slide to slide, but reproducibility is more easily assessed by comparing mean values for each population measured. The methods now in use in our laboratory show means which are reproducible within about 10 per cent of the total extinction, as shown for example in Reissner and Korson's data (22) on the Feulgen reaction in human erythroblasts (table 2). Using this simple statistical method of studying slides, a change of composition is detected as a significant difference in mean value.

The results with this quantitative cytology have been most striking when applied to the cell nucleus, especially in measuring its content of desoxypentose nucleic acid. It will be recalled

the view of the constant relationship of DNA to number of sets of chromosomes and strengthened the validity of it by showing that polyploid nuclei with 4 and 8 sets of chromosomes had a corresponding greater amount of desoxyribose nucleic acid. Additional cytological researches have traced the synthesis of DNA in animal cells through the cycles of mitosis, meiosis, and fertilization (see Swift, 20, Alfert, 25, Pollister, Swift and Alfert, 19).

These results based upon measurement of the Feulgen reaction not only provide strong con-

³ But it must also be stressed that the cytological values are means of a population in which the highest computed value may be as much as 50% larger than the lowest. Part of this is undoubtedly due to technical difficulties, such as the inhomogeneities of the absorbing mass, but it is quite possible that there is also a considerable actual variation in DNA content from nucleus to nucleus.

firmatory evidence of one major aspect of the significance of desoxyribose nucleic acid in cellular biology but also validate the whole quantitative photometric approach very satisfactorily. If a biochemist is to use a colorimetric method of analysis, an essential calibration procedure is to set up a series of known concentrations, or standards, and to check how accurately his colorimeter readings reflect the known relative concentrations. With cytological photometry this cannot be done. Unquestionably within the cell the nucleoprotein mass is very different, in physical

TABLE 3 DNA CONTENT OF CLASS I NUCLEI OF RAT TISSUES

CELL TYPE	NO OF NUCLEI MEASURED	DNA FEULGEN ARBITRARY UNITS $\left(\frac{E\pi}{F}\right)$
Liver	21	3 34 \pm 0 05
Pancreas	20	3 10 \pm 0 06
Thymus	33	3 28 \pm 0 06
Lymphocytes	19	3 20 \pm 0 08
Sertoli cells	18	3 00 \pm 0 12
Kidney epithelium	30	3 14 \pm 0 04
Epithelium, small intestine	20	2 97 \pm 0 04
Spleen	33	3 12 \pm 0 04
Motor neurones	20	3 14 \pm 0 07
Testicular interstitial cells	20	3 05 \pm 0 08

After Swift, 1950 (20) In the formula for computing arbitrary relative values, column 3, E is extinction at 550 $m\mu$, r is the radius of the cylinder measured through the center of the nucleus, and F is the fraction of the total nuclear volume which the central cylinder represents

state and in concentration, from that when it is studied *in vitro* after extraction from the cell. This difference must have a profound effect upon not only natural absorption but also upon the course of tests and stains for the protein and nucleic acid. For further discussion of this point consult Pollster, (1). For a cytological calibration which will be comparable with that of an analytical biochemist we must look for a natural condition in which there is a wide range of concentrations. From the averages obtained by cell counts and gross analyses (table 1) it is clear that nuclei of non-dividing tissue cells may present just this desired situation. For, if a mixture of several types of nuclei of widely varying volumes (as in the kidney) show nearly the same average

DNA value as does a population of nuclei which is nearly homogeneous as to size (as in the thymus) then it must be true that in the larger nuclei the DNA has been diluted in proportion to the increase in cell volume. It is as if a biochemist had prepared his samples, not by dilution of one concentration, but by weighing out 10 one-milligram samples and dissolving them in a range of volumes from 1 to a 100 milliliters. The largest diploid nuclei which Swift analyzed cytologically were nearly 20 times the volume of the smallest. Since the computed DNA was approximately alike in the two nuclear types it is evident that the method can satisfactorily estimate relative DNA over a concentration range from 1 to 20⁴.

TABLE 4 NUCLEASE DIGESTION OF DNA IN NUCLEI

CYTOCHEMICAL METHOD	PART OF NUCLEOTIDE MOLECULE MEASURED	PERCENTAGE REDUCTION OF EXTRACTION
Natural UV absorption	Pyrimidine or purine	39 9
Feulgen reaction	Desoxypentose	39 6
Azure A basophilic	Phosphoric acid	37 7

Measurement of the effect of ribonuclease digestion (2 0 mg/ml), at high temperature (56°C), on 5 μ sections of the nuclei of liver cells of the salamander, *Amblystoma*, showing the same reduction of extinction, measured by 3 different cytological photometric methods. (Note such digestion of DNA by ribonuclease is very exceptional, as a rule ribonuclease preparations are highly specific for ribonucleic acid.)

A careful analytical chemist often checks his results with one method by comparison with others. This is readily done with the variety of techniques for estimation of DNA by cytological photometric analysis. Table 4 shows that when there was about 40 per cent reduction of the extinction of the Feulgen reaction, in this experiment with ribonuclease at high temperature, this

⁴ The average amount per diploid rat nucleus has been given from cell counts as 5.5×10^{-3} mg (Leuchtenberger, Vendrely, and Vendrely, 24). From this it may be computed that the average concentration within the normoblast nucleus (volume 24 μ^3 , after Swift) was of the order of 2.3×10^{-10} mg μ^3 (23%), and that within the nucleus of the motor neurone (volume, 460 μ^3) the DNA concentration was 1.2×10^{-11} mg μ^3 (1.2%). By contrast, about the highest concentration of DNA which is readily measurable by ultraviolet absorption in a cuvette one centimeter thick is less than 0.01%.

loss of DNA was also independently determinable by measuring either the capacity of the phosphoric acid to bind Azure A, or the natural ultraviolet absorption

The results which I have just been discussing were all expressed in arbitrary units, either extinctions or values computed from the extinctions, taking into account the geometry of the object measured. If the relation of the intensity of the reaction measured to the absolute amount of substance were known, from the extinctions the absolute amounts could have been readily computed. Very early in this work Ris and I (26) published what appeared to be this sort of validation of ultraviolet absorption measurements of individual nuclei—but this now appears to have been no more than the result of a fortuitous combination of errors. From the results of Swift and others it is apparent that in rat tissues, the Feulgen reaction bears a constant relationship to the DNA content, but it is not known to what extent this relationship holds from species to species. It is obvious, of course, that of itself the absolute amount in a given cell or cell component is of limited importance, most of the problems can be adequately solved if relative values can be determined.

The work on the desoxyribose nucleic acid content of animal nuclei has combined biochemical and cytological approaches to give a picture of this component of the nucleus as fairly constant, markedly altered only when the chromosome number is changed or the material for new nuclei is synthesized. A similar interdependence of the two approaches is leading toward clarification of the problem of the total nucleoprotein composition of nuclei (27). The older analytical data indicated that even in liver nuclei the protein content was not more than two or three times that of nucleic acid. The available cytological data, by contrast, showed indications that the protein content was much higher, up to 5 to 20 times the nucleic acid. Leuchtenberger and I

assumed that this meant that the washing incidental to the isolation of nuclei removed a considerable amount of the protein, and we showed by photometric measurements of nuclei in fresh slices of liver that such loss did indeed occur. From other analytical data, most of them more recent, and using different isolation procedures there may be computed considerably higher protein values which are in some cases (28) even higher than those we gave for liver. We now know that our ratios were probably high by a factor of two, but even with this correction the protein content is much higher than that from the older analyses. From the gross analytical data alone it could not be decided whether the low values were a result of loss of protein, or whether the high values indicated that protein had been adsorbed onto the nuclei from the surrounding cytoplasm. The comparison of the cytological and biochemical data, however, makes it highly likely that the nucleus does, as a rule, contain high protein concentration, quite comparable with that of the cytoplasm.

In closing, I should like to summarize briefly. The attempt to localize substances in cells is one approach to the broad problem of determining to what extent cellular structure reflects diversity in distribution of the physiological functions of the cell. Localization of substances can best be achieved by a combination of two approaches, analysis of isolated masses of cell components and direct localization by microscopic study of cells. In most cases the cytological study must be made on fixed cells, on which a variety of specific tests, stains, digestions, and chemical extractions can be carried out. Cytological localization methods are best developed for proteins and nucleic acids, and these have been adapted to yield quantitative estimations of relative amounts in cells. The quantitative techniques have been used most successfully in study of the composition of the cell nucleus.

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SEPARATION AND PROPERTIES OF CELL COMPONENTS¹

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CERTAINLY the most versatile approach to an understanding of the relation between cell structure and cell chemistry is the procedure of cell fractionation, whereby the cell is mechanically broken and its structural elements are segregated according to size and density by fractionation in the centrifuge. The main advantage of this procedure lies in the fact that cell fractions can be obtained in sufficient amounts to permit the direct application of quantitative biochemical methods of analysis as fast as these methods are developed. To the best of my knowledge, no other general cytochemical technique has this capability.

It is, therefore, simple enough to prepare a broken cell suspension, subject the suspension to increasing centrifugal force, isolate particles of various sizes, and carry out chemical analyses on the fractions obtained. Past experience has amply demonstrated, however, that it is not simple to arrive at firm conclusions relating the chemical data to the structural make-up of the cell, and this is the point where the cell fractionation procedure must be examined critically. Now what is liable to happen when the cell membrane is broken? First, the intracellular structures are dispersed into an aqueous medium bearing only a faint resemblance to that within the living cell. When so dispersed, the structural elements behave appropriately by displaying a pronounced tendency to undergo morphological alteration. (1) Aside from the fact that this phenomenon of morphological alteration can make it difficult if not impossible to identify cytologically the dispersed structures and thus to determine the composition of isolated cell fractions, a number

of other undesirable phenomena might be expected to follow, such as a redistribution of biochemical properties as a result of leakage of substances through damaged membranes, or adsorption of soluble compounds on particulate material.

These considerations have led to the establishment of several criteria defining an adequate isolation procedure, the first and most obvious of which is that a sound cytological background must be provided. Thus the morphological integrity of the cell structures must be preserved to such an extent that the composition of the isolated fractions can be directly determined by cytological study. It seems reasonable to believe that this principle also has some bearing on the possible occurrence of redistribution of biochemical properties after cell disruption. Thus the membrane of a cytologically intact structure, for example nucleus or mitochondrion, would probably be less likely to leak than the membrane of an obviously altered structure.

A second item of importance involves the question of yield. To isolate only a small proportion of the nuclei or mitochondria of a given tissue and attempt to interpret results obtained with that preparation in terms of the whole tissue is an extrapolation of questionable validity, particularly when one considers the possibility of heterogeneity of cells and perhaps even of nuclei or of mitochondria. It is therefore necessary to provide a means by which almost all of the cells of a tissue can be broken and to devise a fractionation procedure permitting reasonably clean separation of the various cell components and their isolation in good yield.

Two important additional points arise in connection with the biochemical analysis of the fractions and the interpretation of the biochemical data. First, it is essential to analyze both the original whole tissue and all the fractions obtained from it. By this means it is possible to draw up a balance sheet, which serves as a test of the validity of the analytical method, enabling one to detect the presence of inhibitors or activators or the possible role of more than one cell

¹ For the sake of brevity, the following abbreviations will be employed: DPN = diphosphopyridine nucleotide, TPN = triphosphopyridine nucleotide, ATP = adenosine triphosphate, ATPase = adenosine triphosphatase, AMP = adenosine-5-phosphate, AMPase = AMP phosphatase, DNA = deoxyribonucleic acid, PNA = pentonucleic acid.

² Federal Security Agency, U. S. Public Health Service.

component in a given biochemical reaction. The second requirement arises from a consideration of the possibility of adsorption of soluble compounds on particulate material and of an overlapping of fractions that may not be readily detected by cytological study. Thus in an investigation of the distribution of an enzyme among cell fractions, the presence in a single fraction of a relatively small proportion of the total enzyme should be interpreted with caution. The finding that the enzyme is concentrated in the fraction to a greater extent than in the original whole tissue is much more likely to be significant from the cytochemical standpoint.

Up to the present time, mammalian liver has been by far the most popular tissue for cell fractionation studies. Liver presents certain definite advantages over most other tissues. It is largely composed of what appears to be a single cell type and the individual liver cells are large and easily disrupted. The abundant cytoplasm contains many mitochondria (probably somewhere in the neighborhood of 700³ per cell), and apparently several types of submicroscopic structures which have recently been revealed by electron microscopy. Also present in the cytoplasm and visible in the light microscope are lipid droplets and a number of small spherical secretory granules which stain with neutral red and are usually located near the periphery of the cell. The cell contains a single nucleus containing 1 to 3 nucleoli.

By utilizing the mitochondrion as a test object, it has been possible to obtain a considerable amount of information concerning the proper conditions for fractionating the liver cell. The mitochondrion, which has almost as widespread a distribution among cells, plant and animal, as the nucleus, has certain characteristic cytological properties, including a usually elongated shape, the probable presence of a surface membrane, specific staining vitally with Janus Green B and after special types of fixation with certain other dyes, and a very pronounced sensitivity to mishandling. In the latter respect, both the cytological and biochemical properties of mitochondria are markedly affected by the composition of the suspending medium. The following facts became apparent in a study of the effect of various media on mitochondria (1). The use of

water or other hypotonic solutions causes prohibitive morphological alteration, and the use of solutions of electrolytes at the isotonic level produces aggregation of the particles to such an extent that they cannot be adequately separated from nuclei. An answer to these problems of morphological alteration and of aggregation was found, however, in the use of solutions of non-electrolytes. Two media evolved from the study: isotonic (0.25M) sucrose in which the mitochondria change from an elongated into a spherical shape but otherwise retain their normal cytological properties, and hypertonic (0.88M) sucrose in which the particles retain both their normal shape and staining properties. Subsequently, a comparative study of these two media has led to the conclusion that neither possesses any distinct advantage as far as the distribution of biochemical properties among the cell fractions is concerned. There are, however, difficulties associated with the use of hypertonic sucrose, including the necessary addition of large amounts of sucrose to reaction mixtures and, because of the high density and viscosity of the solution, a requirement for high centrifugal forces in the fractionation procedure. For these reasons we are at present using the more convenient of the two media, namely the isotonic sucrose. Perhaps I should point out, however, that we do not by any means feel that the choice of a proper medium for cell fractionation is a closed problem. Sucrose solutions are certainly far from physiological in the sense of duplicating the medium of the cytoplasm, and we are well aware of the possibility that artifacts may eventually be encountered as a result of the use of such unphysiologic solutions. The addition of various salts at low concentrations to the sucrose solutions in an attempt to improve the physiological characteristics of the media has, in our experience, invariably resulted in some degree of aggregation of particles and thus in difficulties in obtaining adequate fractionation.

The fractionation procedure (2) is, of course, carried out entirely in the cold, the temperature of the preparations being maintained at 5° or less. In our experience, the Potter-Elvehjem homogenizer (3) provides the most satisfactory method for disrupting cells, since its use results in the breakage of 80 per cent or more of the liver cells without damage to an appreciable proportion of the nuclei. The Waring blender is entirely unsatisfactory for the preparation of

³ This value was obtained by making simultaneous counts of nuclei and mitochondria in homogenates of mouse and rat liver.

homogenates suitable for cell fractionation studies. The subsection of liver, for example, to the action of the Waring blender for periods sufficient to disrupt most of the cells results in the breakage of many nuclei and even of some mitochondria.

By the use of increasing centrifugal forces, four fractions are obtained from homogenates of mammalian liver, first, a nuclear fraction containing about 15 per cent of the original total nitrogen and including all of the nuclei of the homogenate together with some residual intact cells and a variable but usually small number of free mitochondria, second, a mitochondrial fraction in which virtually all of the visible particles satisfy the cytological criteria for the identification of mitochondria and in which, as indicated by electron microscopy, there are apparently few submicroscopic particles (1). The mitochondrial fraction contains approximately 25 per cent of the original total nitrogen. The third fraction, the submicroscopic particles or microsomes, contains 20 to 25 per cent of the original nitrogen. The types of structures present in this fraction have not as yet been clearly defined, although recent studies carried out with A. J. Dalton with the electron microscope have indicated the presence of several types of particles of diameters ranging between 30 and approximately 150 millimicrons. The fourth fraction, a final supernatant, contains 35 to 40 per cent of the original nitrogen and is made up of the soluble material of the cell, possibly some very small particles, lipid droplets, and some of the secretory granules. It is our experience that most of the secretory granules do not exist as formed elements after disruption of the liver cell (1).

It is apparent, therefore, that this fractionation procedure is suitable for the isolation of the structural elements of the cytoplasm but does not yield a homogeneous preparation of nuclei. In fact, it seems unlikely that a satisfactory method for the isolation of cytologically intact nuclei in good yield has yet been developed. Although the use of the citric acid method, for example, permits reasonably clean separation of nuclei from cytoplasmic constituents, the nuclei so obtained are obviously morphologically altered (4). A similar picture is obtained by the use of the Behrens' procedure (5, 6). Recently, several modifications of the sucrose (7, 8) technique have yielded clean preparations of nuclei that appear cytologically intact, but all these

methods thus far permit the isolation of only a small proportion of the total number of nuclei of the tissue.

By far the most striking result of studies of the biochemical properties of cell fractions has been the partial elucidation, by work carried out in a number of laboratories, of the important role played by mitochondria in the metabolism of the liver cell. In this respect, one of the earliest discovered enzymatic properties of mitochondria was their content of cytochrome oxidase and succinic dehydrogenase (9, 10). Several experiments have suggested that these two enzyme systems are, in fact, exclusively a function of mitochondria, the relatively small amount of activity shown by other fractions probably being the result of contamination by mitochondria (1). Other enzyme systems and related substances that are concentrated in this fraction of liver are ovalacetic acid oxidase (11), octanoic acid oxidase (12, 13), glutamic dehydrogenase,⁴ DPN cytochrome reductase, TPN cytochrome reductase (14-16), adenosinetriphosphatase (9, 17), catalase (18), uricase (19), cytochrome *c* (2, 20), riboflavin (21), and vitamin B₆ (22). The system catalyzing the synthesis of *p*-aminohippuric acid from *p*-aminobenzoic acid and glycine was largely recovered in the mitochondrial fraction (23). Recently, Kielley and Kielley (24) in an interesting study of oxidative phosphorylation have shown that in the presence of an oxidizable substrate, such as α -ketoglutarate, liver mitochondria are capable of synthesizing ATP from AMP and inorganic phosphate at a very rapid rate, the net uptake of inorganic phosphorus at 28° being approximately 2 μ M/min/mg of mitochondrial nitrogen. In this study it was also shown that the 'myokinase' activity of liver is largely, if not exclusively, associated with mitochondria and plays a prominent role in the phosphorylation mechanism (24). The adenosinetriphosphatase (ATPase) of mitochondria was found to be non-operative under the conditions of ATP synthesis and became operative only when the particles were aged or subjected to procedures causing structural alteration and at the same time a loss of their ability to carry out the phosphorylation reaction. The latter finding brings up the interesting possibility that ATPase may under appropriate conditions actually function as a synthesizing system.

⁴ Hogeboom, G. H. and W. C. Schneider. Unpublished experiments.

Of the enzyme systems that are concentrated in mitochondria, several are apparently not exclusively associated with this cellular element. The cytochrome reductases, for example, are also concentrated in microsomes. ATPase is also concentrated in the nuclear fraction, catalase and cytochrome *c* in the final supernatant, and the activity of mitochondria in the oxidation of oxalacetate, and to a lesser extent of octanoate, is enhanced by the addition of other fractions which by themselves are inactive. The situation with respect to α -ketoglutarate oxidation is not entirely clear, since a satisfactory method for the assay of this system in homogenates is not available. Isolated mitochondria, however, are capable of oxidizing α -ketoglutarate in the presence of AMP and magnesium ions at a reasonably rapid rate, the Q_{O_2} in terms of milligrams of total nitrogen at 28° being approximately 400 μ l of O_2 /hour (24). It thus seems possible that mitochondria are largely responsible for this reaction.

Another interesting study having a bearing, from the negative standpoint, on the properties of mitochondria, was carried out by LePage and Schneider (25). These investigators found that over 50 per cent of the anaerobic glycolytic activity of rabbit liver was recovered in the final supernatant. No other fraction showed appreciable activity except the nuclei, which, of course, were contaminated by intact cells. When the fractions were recombined, the activity of the supernatant was enhanced greatly by the addition of microsomes but only slightly by the addition of mitochondria. It would thus appear that mitochondria are not intimately concerned with the anaerobic phases of glycolysis.

Another finding of interest from the negative point of view is that mitochondria contain only a relatively small amount of pentose nucleic acid (1, 9, 17). Approximately 25 per cent of the total mass of mitochondria appears to be composed of lipide, of which about two-thirds is phospholipide (26-28).

It seems apparent, therefore, that mitochondria are chiefly concerned with the oxidative metabolism of the cell, including some of the reactions of the Krebs cycle. Perhaps their most interesting biochemical property, arising from the studies on phosphorylation, among others, is their ability to carry out reactions capable of supplying energy for synthesis.

As far as the enzymatic properties of the nucleus are concerned, the results of investiga-

tions of the nuclear fraction have been largely negative. Although in all cases some enzyme activity is always shown by the nuclear fraction, only in the case of ATPase (9, 17) and AMPase (29) is the concentration of enzyme sufficiently high to rule out contaminating cytoplasmic elements as the cell structures responsible for the activity. In general, the results of enzyme determinations carried out on nuclei isolated by other techniques are open to the same difficulty in interpretation (4). It is, on the other hand, entirely possible that the nucleus actually does contain a large number of enzymes in relatively low concentration, but proof that this is the case is certainly not a simple problem.

The submicroscopic particles or microsomes are characterized by strikingly high concentrations of pentose nucleic acid (PNA) and of lipide, which is mostly in the form of phospholipide. The concentration of PNA in microsomes is some 5 to 6 times that found in the mitochondrial fraction (1, 11, 12, 17, 28), and approximately 40 per cent of the dry weight of microsomes is lipide in nature (26-28, 30).

Until recently, the metabolic function of microsomes has been largely a matter of conjecture, but during the last few years several enzymes have been shown to occur in the fraction in a concentration exceeding that in whole tissue. These enzymes include both DPN and TPN cytochrome reductase (14-16), the former being present in considerably higher concentration than the latter. Omachi, Barnum, and Glick (31) have reported that the esterase activity of whole liver is largely recovered in the microsome fraction. Microsomes also play a role in certain other enzyme reactions, including anaerobic glycolysis (25), the oxidation of oxalacetate (11), and the reductive cleavage of *p*-dimethylaminoazobenzene (32).

It is obvious, however, that our present knowledge of the role of microsomes in cellular metabolism is quite limited and does not permit the type of generalizations that can be made in the case of mitochondria.

The fourth fraction obtained from mammalian liver, the supernatant, is, of course, a highly complex mixture containing all of the enzymes involved in anaerobic glycolysis (25), as well as isocitric dehydrogenase (16), acid and alkaline phosphatase (29), catalase (18), cytochrome *c* (20), and undoubtedly many other substances of biochemical importance. A detailed study of the types of proteins present in the soluble frac-

tion of the cell is a problem of major proportions in itself. Recently, some steps in this direction have been made by Sorof and Cohen through the use of electrophoretic and ultracentrifugal analysis (33, 34).

Perhaps it would be of some interest if I were to devote the last few minutes to a discussion of recent experiments designed to investigate further the properties of liver mitochondria. For a time it was somewhat disturbing to find that practically all of the enzyme systems associated with mitochondria were 'insoluble', i.e. easily sedimented from extracts of practically any tissue, not obtainable in a monodisperse state in true solution, and thus refractive to extensive purification. Notable examples were cytochrome oxidase, succinic dehydrogenase, ATPase, and DPN-cytochrome reductase. In view of evidence favoring the existence of a mitochondrial membrane (35, 36) and in spite of the cytological evidence for the integrity of isolated mitochondria, it seemed conceivable that the membranes of the particles were actually being damaged sufficiently during the fractionation procedure to allow the escape of soluble substances, leaving only an insoluble residue. Several lines of evidence have clearly indicated, however, that this is not the case. More recent studies have shown, for example, that several soluble proteins that can readily be purified, such as cytochrome *c*, TPN-cytochrome reductase, myokinase, and glutamic dehydrogenase, are concentrated to a considerable extent in the mitochondrial fraction. Finally, when the mitochondrial membranes are mechanically broken (37, 38) either by the use of intense sonic vibrations or by forcing the particles through a small orifice under high pressure, approximately 60 per cent of the total nitrogen is released into solution. Further studies of the soluble fraction of mitochondria have shown that it consists largely of proteins that can be separated and characterized in the analytical centrifuge. Different refractive index patterns are obtained by the two methods of disintegration of mitochondria, the only component that appears to sediment as a monodisperse system being released by sonic vibrations (38). This component which constitutes a large proportion of the total protein of mitochondria, and is characterized by

a sedimentation constant of 6.2 Svedberg units, is apparently either denatured or dissociated when the mitochondrial disintegration is carried out by forcing the particles through a small orifice.

Of some interest is the fact that the soluble fraction of mitochondria isolated from a liver tumor shows a qualitatively different protein pattern in the analytical centrifuge, in that the main component obtained from normal liver mitochondria appears to be absent (38), whereas other components are seen that correspond in sedimentation constant to those of the normal liver mitochondria. Furthermore, the two methods of disintegration produce identical patterns in the case of the tumor mitochondria but strikingly different patterns in the case of normal liver mitochondria. Attempts are now under way to isolate and characterize the component of normal liver mitochondria that cannot be detected in the tumor preparation.

The effect of the disintegration of mitochondria on the enzyme systems of the fraction is also of some interest. Myokinase and glutamic dehydrogenase are released into solution. Cytochrome oxidase, DPN-cytochrome reductase, ATPase, and, somewhat surprisingly, cytochrome *c* remain attached to particles that are sedimented with considerable difficulty, not being completely sedimented by 30 to 40 minutes centrifugation in an angle centrifuge at 150,000 *g*. Moreover, the specific cytochrome oxidase and DPN cytochrome reductase activities of the slowly sedimenting particles are much higher than the corresponding activities of the original preparation of mitochondria. It is obvious that without the availability of a high speed centrifuge permitting sedimentation with a minimum of convection, it could be erroneously assumed that these 'insoluble' enzymes had been brought into solution.

As I have attempted to illustrate, the procedure of cell fractionation, despite certain deficiencies, is capable of yielding a considerable amount of information both in the field of cytochemistry and in basic studies of the mechanism of biochemical reactions. It is our hope that future efforts in this field will emphasize the development of new methods capable of checking independently and extending the present data.

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INTRACELLULAR ENZYME DISTRIBUTION, INTERPRETATIONS AND SIGNIFICANCE¹

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It is now 9 years since the symposium entitled "Frontiers in Cytochemistry" was held in honor of Prof. R. R. Bensley at the University of Chicago and what little we know about enzyme distribution in cells has developed largely since that time, inasmuch as almost no data on intracellular distribution of enzymes were presented at that meeting. Indeed, the first observations on succinoylase distribution as reported there differ considerably from present views. Nevertheless a beginning was made and the time was ripe for further developments.

A recent review by Schneider and Hogeboom (1) has surveyed the literature on enzyme distribution up to this time, and therefore in covering the subject that was assigned to me for the present occasion, I shall feel free to spend more time on what we take to be the significance of studies in this field rather than to cover such details of enzyme localization as are available. Since this field is just beginning to establish itself, this may be a good time to take stock of our present position and to ask ourselves what we may hope to accomplish by these studies.

There are two approaches that are beginning to be widely applied in the field of intracellular enzyme localization. One is the so-called histochemical method, based on the classical methods of histology, and the other is the method that is based on the early experiments by Bensley and by Claude, namely, the method of differential centrifugation of homogenized tissue samples. We shall not attempt to interpret the findings obtained by the histochemical method except in one instance, in which the application by Novikoff (2, 3) of the method of differential centrifuga-

tion to the localization of alkaline phosphatase led directly to the rejection of the widely held view that the enzyme was localized in the nucleus, as indicated by the usual histochemical method. The same view has now been accepted by Gomori (4) and is apparently not a subject of controversy.

The early philosophy of the differential centrifugation school was summed up very neatly by Professor Bensley in the statements that "It would seem to be an axiom of analytic chemistry to separate separable things before proceeding to their analysis," and "The analysis of particulate components of cytoplasm is only as good as the species purity of the preparation" (5). Although it is apparent that Bensley regarded the immediate task as analytical, he was also well aware of the broader implications of the work. He said, "Obviously the possibility of separating mitochondria and particulates, and of isolating the structural proteins for chemical study, opens a rich field for further research. The localization of enzyme and carrier systems, vitamins and hormones, and the viruses, functional changes in composition, the tracing of radioactive isotopes into the interior of the cell, and the further fractionation of the submicroscopic particles by more refined methods all offer inviting opportunities to the inquiring mind" (5). It is the purpose of the present discussion to inquire whether the hopes and predictions of Bensley were justified, in the light of the subsequent 9 years of experimentation in this field. What are some of the objectives that we may hope to attain by further work in this field?

OBJECTIVES

When looked at as an analytical problem, that is, the determination of enzyme distribution in the obtainable cell fractions, it must be realized that anything less than the quantitative approach has very little significance. Thus the problem of enzyme distribution in cell fractions involves the same hazards and the same precautions that are

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met in applying the homogenate techniques to the determination of enzymes in tissues as pointed out in 1944 (6). This means that the reaction mixture must be fortified with such cofactors and adjuvants as may be required to make the given enzyme the rate determining factor. But it must be clear that the enzyme distribution studies are not an end in themselves. It is clear by this time that the localization of enzymes in cell fractions is no longer the main line of development in this field but that the methods of differential centrifugation can now be used as a powerful tool for the study of *intracellular physiology*, which is the underlying basis of physiology at all higher levels.

What can we hope to gain by knowing more about where the individual enzymes in a cell are located? Stated in the broadest terms, these studies have as their objective the understanding of events occurring at higher levels of organization.

In order to understand these events it is necessary to effect a fusion between the morphological or structural aspects of biology and the chemical or metabolic aspects, and to correlate both along the time scale. The understanding of the morphological and metabolic changes at various levels of organization is in our opinion *primarily a matter of understanding the control or regulatory mechanisms that are called upon*. In this connection, we think of enzyme action in terms of two main categories: 1) the *controlled enzyme activity* that is manifest *in situ* at the highest level of enzyme organization, namely, the intact animal, in which the manifold factors of the internal and external environment are brought to bear, and 2) the *potential enzyme activity* that the various enzyme systems can be shown to possess at lower levels of organization, such as the homogenate or the cell fraction. In this discussion we hope to show how the methods of differential centrifugation of homogenized tissues can contribute effectively to studies at both levels, and to mention various subsidiary problems that arise.

CONTROLLED ENZYME ACTIVITY IN SITU

An example of how these methods are proving useful for the study of controlled enzyme activity *in situ* lies in the field of nucleic acid metabolism. For some time evidence has been accumulating in the laboratory of Caspersson (7) and elsewhere that important interactions between the nucleus and cytoplasm are involved in nucleic acid synthesis. The studies based on microscopic

examination of individual cells have certain limitations for the study of metabolic processes, and various investigators, including Jeener and Szafarz (8), Barnum and Huseby (9) and Marshak (10) have attempted to supplement the findings on single cells with metabolic studies involving isotopic phosphorus combined with techniques of differential centrifugation. Their studies were complicated by the fact that phosphorus can follow many metabolic pathways, but nevertheless valid conclusions were reached as to the high metabolic activity of the nuclear PNA (pentose-nucleic acid). I will illustrate this type of experiment with data that are being obtained in

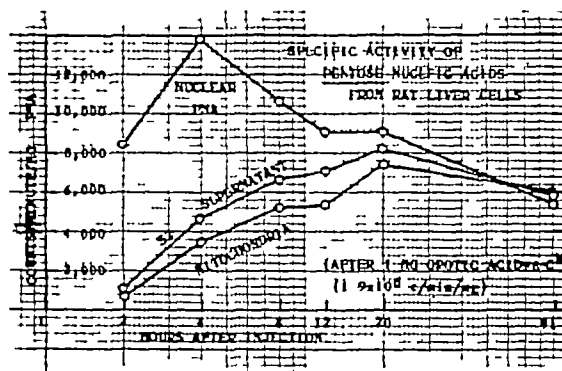


Fig 1 SPECIFIC ACTIVITY of PNA in rat liver cell fractions

our laboratory by Mr Huilbert. We injected rats with 1 mg of C^{14} labeled orotic acid (11), which has been shown to be converted into the pyrimidines of nucleic acids in high yield (12-15), while the purines do not incorporate this substance. Rats were killed at time intervals of 2, 4, 8, 12, 20 and 91 hours and the amounts of radioactivity in the internal organs, respiratory CO_2 and excretory products were determined. The livers contained most of the C^{14} retained in the body. They were homogenized and separated into nuclear, mitochondrial, and supernatant (S_2) fractions by differential centrifugation using the sucrose medium as employed by Price, Miller and Miller (16). The PNA and DNA of the nuclear fraction were separated. In the initial period, most of the C^{14} was in the nuclear fraction and was in the pentosenucleic acid (PNA) of this fraction. No C^{14} was detected in the desoxypentose nucleic acid (DNA) at this time. The results (fig 1) are shown in terms of the specific activities of the PNA of the nuclear, mitochondrial and supernatant (S_2) fractions. From this first approximation of the intracellular distribu-

tion it is clear that the label was first incorporated into the PNA of the nuclear fraction, then into the PNA of the cytoplasmic fractions. After 91 hours, the DNA had attained only a small fraction of the radioactivity in the liver. Thus the suggestions of earlier workers regarding the importance of the nuclear PNA (7-10), considered to reside largely in the nucleolus, is reinforced by these studies, more particularly because instead of using P^{32} the present study was carried out with orotic acid. This substance is incorporated with a comparatively high yield, and has alternative metabolic pathways that appear to be more restricted than those of phosphorus. From these data it is clear that the orotic acid is not being taken up at the same rate by all parts of the cell—it is being taken up by the nucleus, and indeed by a specific portion of the nucleus other than the DNA portion. By following the rates of incorporation of the label from orotic acid into the various cytoplasmic particles along the time scale, and under various conditions which augment the incorporation into the DNA of the chromosomes, it should be possible to test some of the current hypotheses regarding the origin and interrelationships among the various formed bodies found within the cell (cf. 8).

We feel that these data illustrate how the method of differential centrifugation combined with appropriate isotopic precursors can be a powerful tool for the study of the controlled activity of localized enzymes *in situ*, operating at the highest level of enzyme organization. This was the first main category of enzyme activity which we mentioned as an important field for the application of these methods to the problem of the control mechanisms.

POTENTIAL ENZYME ACTIVITY

The second main category in which the methods of differential centrifugation seem at present to be indispensable is the study of potential (and experimentally altered) enzyme activity at the lower levels of organization in terms of intracellular control mechanisms. In studying the activity of the cell components we can observe relationships that enable us to begin interpreting the data obtained by means of slices, whole homogenates and the washed residues, etc. It is now clear that many of the enzymes present in the whole organism or even in the tissue slice do not operate at capacity under usual conditions of study. When these enzymes are studied

by the homogenate technique or in isolated cell fractions they will be either *less active* or *more active* than they were *in situ*—less active if the necessary reaction components are in lower concentration than *in situ*, and more active if the reaction components are in optimum concentration. When the enzymes are studied at the level of the homogenate or the cell fractions, it becomes possible to study their requirements for maximum performance while maintaining them in their intracellular form. By learning just what factors are necessary for maximum performance, we are in a position to learn not only the *amount* of the enzyme present in a given cell fraction, but we also learn what factors the other parts of the cell would have to contribute in order to control the activity of the fraction under study. Although there are many ways in which the activity of an enzyme can be regulated (6,17,18), we believe that one of the more important is by the regulation of the supply of substrates and co-substrates (coenzymes), and this is obviously a mechanism in which the spatial distribution of enzymes could be decisive. We refer particularly to the delivery of co-substrates such as inorganic phosphate and phosphate acceptors, which appear to be necessary components and hence common denominators in a large number of reactions, with the result that any reaction that tends to use up the supply of either of these reaction components can shut off or regulate the rate of all the other reactions that are dependent upon the phosphate balance (6, 19, 20). The delivery of co-substrates can also be decisive in determining the choice between alternative metabolic pathways, as we have recently shown in the case of pyruvate metabolism (21).

Let us examine the data obtainable by various techniques. The first point that needs to be emphasized is that the comparison of slices with homogenates under conditions that are appropriate only for slices is a meaningless gesture. When a reaction can be studied in a homogenate, and the optimum conditions are worked out, the reaction tends to be faster in the homogenate than in the slice. In figure 2 the activity of the homogenate is given as 100 per cent, and the activity of the slice is given as a percentage of the homogenate data. Thus the slices compare with the homogenates as follows: urea formation in rat liver, 43 per cent (22), *p*-amino-hippuric acid synthesis in rat liver, 32 per cent (23), liver oxidation of pyruvate, 38 per cent (24-25), kidney

oxidation of pyruvate, 10 per cent (21, 25), liver synthesis of acetone, 79 per cent (21), and in the case of the only tumor so far studied in these terms, pyruvate oxidation was about equal by both techniques. The possible significance of the latter observation has been discussed elsewhere (26).

It is evident that in many instances the enzymes of the slice are not working at capacity, in comparison with homogenates from the same tissues. Once it is clear that this can be true, it is not too surprising to find reports in which the suppression of an enzyme *in vivo* is so pronounced as to make it appear that an organism lacks the enzyme, although extracts of the organism con-

chrome *c* reductases (29, 30) and isocitric dehydrogenase (30) in the various fractions and find large amounts of the reductases in the microsomes while a high proportion of the dehydrogenase is in the soluble fraction. They were of the opinion that the rate of oxygen uptake by the mitochondria was mainly a measure of cytochrome reductase activity. However, on the basis of the following studies, we believe that in the mitochondria alone the rate-limiting reaction is phosphate breakdown.

We carried out phosphate balance studies on kidney mitochondria, whole homogenates and mitochondria plus nuclei (31). The mitochondria did not break down the added ATP until the

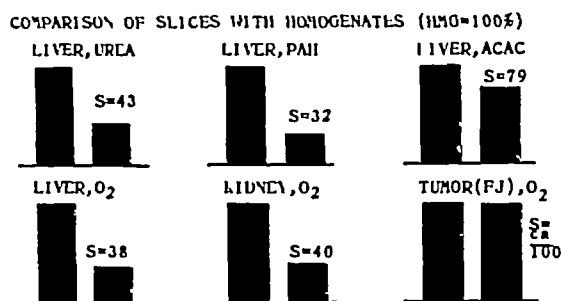


Fig 2 COMPARISON of slice activity with homogenate activity. Data from references 21-24

tain the enzyme in demonstrable form, as in the case of the pantothenicless mutants 5531 and 34556 of *Neurospora* (27). The more usual case will of course involve only modifications of enzyme activity.

The case of pyruvate oxidation in kidney may be discussed in somewhat greater detail, since studies with cell fractions are available for comparison (fig 3). The oxidation of pyruvate by rat kidney slices yields a Q_{O_2} of about 17, while that of the homogenate under optimum conditions is about 3-fold greater, or 52 (24, 25). When we examined the components of the kidney homogenates (28) we found that the nuclei and the S_2 supernatant were almost inactive, while the mitochondria possessed only 33 per cent of the activity of the homogenate. When the mitochondria were supplemented by either the nuclei or the supernatant fraction the activity was doubled.

In interpreting the stimulating effects of the other fractions in the presence of the mitochondria, it is necessary to consider what individual enzymes these fractions contain. Hogeboom and Schneider have studied the distribution of cyto-

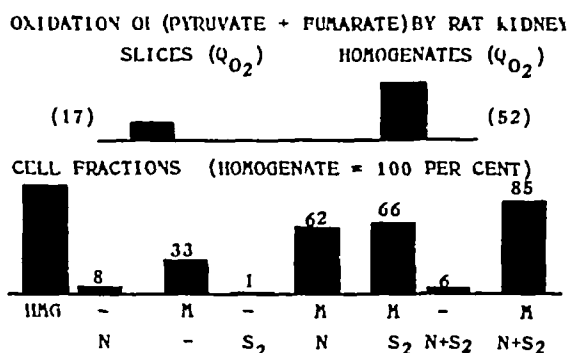


Fig 3 COMPARISON of pyruvate oxidation by kidney slices, homogenates and cell fractions

substrate was depleted, while the whole homogenate dephosphorylated the ATP much sooner. When nuclei were added to mitochondria, the oxidative rate was doubled and the phosphate breakdown was also accelerated. Since the nuclei do not appear to contain the oxidative enzymes (29, 30) to account for the doubling of the rate, it is suggested that the oxidative enzymes in the mitochondria are adequate but that their potentiality can only be demonstrated by eliminating the rate limiting factor, namely the availability of the phosphate acceptor.

The next experimental step is the substitution of a phosphate acceptor system for the nuclear fraction, as a supplement to the isolated mitochondria, and such studies have recently been reported by Lardy (32) (fig 4). Mitochondrial fractions obtained from rat liver were supplemented with purified hexokinase or with creatine phosphorylase and the respective phosphate acceptor systems. It was demonstrated that as much as 400 per cent stimulation of oxygen uptake could be obtained, under conditions that are comparable to what we employed in the studies

with mitochondria and nuclei (28, 31) Kielly and Kielly (33) and the Lipmann group (34) have also observed stimulation of oxygen uptake when mitochondria were supplemented with hexokinase and glucose. These studies leave little doubt that the mitochondria are not primarily limited with respect to oxidative enzymes and that their oxidative rate is in fact limited by the rate at which phosphate acceptors are made available. On the basis of these facts we suggest that similar considerations may apply to slices of certain normal tissues, in other words, that the oxidative rate of these normal tissue slices may also be limited by the rate at which phos-

like to devote the remainder of our time to a brief discussion of methods

METHODS

In a recent review Borsook (38) stated that the incorporation of amino acids into proteins does not necessarily depend upon the direct participation of the nucleus. While this statement may be true, it was based upon observations with mitochondria and microsomes that were almost certainly not free of nuclear material, owing to the fact that the cell fractions were obtained from material that had been subjected to the blades of a Waring blender for 1½ minutes. This device is not suitable for this type of study on the formed bodies that occur in cells because under the conditions employed it tends to disintegrate both nuclei and mitochondria with the result that fragments of each contaminate the subsequent fractions, as will be described. The criteria for identifying these cellular components are not arbitrary, and while we are constantly looking for better criteria, some progress has already been made, as Dr Hogeboom has shown today. It is our purpose to stress the use of at least *two* different types of analytical measurements for checking the homogeneity of cell fractions. We generally express the measurements on each cell fraction in terms of a percentage of the value for the total tissue sample. The two measurements are chosen to characterize the desired component and the chief contaminant, respectively.

Consider the isolation of nuclei. It is quite certain by now that all of the DNA of the cell is in the nucleus, and that the nuclei are sedimented at relatively low speeds in the centrifuge as the preceding speakers have emphasized. There are two points to be stressed in connection with recovery of DNA, which is the first objective in nuclear separations. When glass or lucite homogenizers are employed the recovery of DNA in the nuclear fraction is routinely near 100 per cent (1). However when the Waring blender is used, the nuclei are completely disintegrated within 30 seconds in a KCl medium, according to reports from D. E. Green's laboratory (39, 40). Direct analytical evidence for nuclear disintegration in these preparations was reported by Schneider and Potter (28) who found only 74 per cent of the tissue DNA in the sedimented fraction. That under these conditions nuclear fragments contaminate subsequent fractions is shown in a report by Keller (41) who reported the

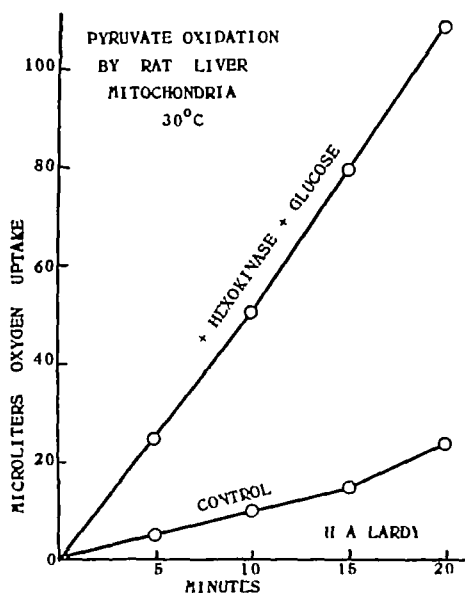


Fig 4 STIMULATION of pyruvate oxidation by the presence of a phosphate acceptor system (31)

phosphate acceptors are made available (cf 35, 26). This view implies that under appropriate conditions the oxidative rate of these normal tissue slices could be accelerated, and such increased rates of oxidation in tissue slices have of course been repeatedly observed in the presence of dinitrophenol and various other agents (cf 36, 37).

The experiments with isolated mitochondria, studied separately and in combination with other cell fractions or with a phosphate transferring system, provide models of the regulation of enzyme function in response to need, at the intracellular level. While a beginning has been made in this field, we feel that more rigorously defined preparations of cell fractions are needed for all studies of this type and therefore we would

isolation of 'microsome' fractions containing large quantities of DNA following the use of the blender. The second point is that the analytical recovery of 100 per cent of the DNA in the nuclear fraction is obtained with the use of trichloroacetic acid as in the original Schneider technique (42). Dr Recknagel recently found that when perchloric acid was used for the extraction (cf. 43) the cytoplasmic fraction gave a false test for DNA, which was easily recognized by taking the complete absorption spectrum.

The second objective in the evaluation of the nuclear fraction is to determine the extent of contamination by whole cells and by cytoplasmic fractions. This information is provided by the second measurement, which under ideal conditions would be an analysis for something completely absent from nuclei but abundant in cyto-

plasm or in sucrose of any molarity between 0.1 and 0.8 M occupy a large volume that is 5 or 6 times as great as the volume in isotonic KCl or in isotonic sucrose containing a small amount of electrolyte (0.01 M phosphate). In addition to the effect on the nuclear volume, the presence of the electrolyte increased the contamination by mitochondria as evidenced by the high succinoxidase content, which was 23 per cent of the total, and by the increased uptake of the mitochondrial stain, Janus Green B, as shown in the photograph.

We next turned our attention to a re-examination of procedures for the isolation of mitochondria, again using two types of measurements as a guide to homogeneity. For the present it has been assumed that succinoxidase activity can be used to characterize the mitochondria, while

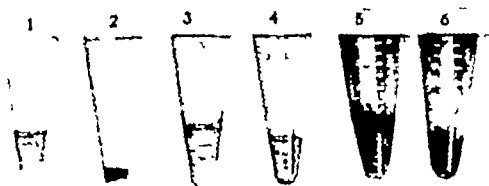


Fig 5 EFFECT OF CENTRIFUGATION MEDIUM ON volume of the nuclear fraction. Each tube contains the nuclei from 1 gm. of rat liver. Suspension media were as follows: *Tube 1*, H₂O, *tube 2*, isotonic KCl, *tube 3*, isotonic sucrose, *tube 4*, isotonic sucrose plus 0.1M potassium phosphate buffer, pH 7.6, *tube 5*, isotonic sucrose plus 50 γ Janus Green B, *tube 6*, isotonic sucrose plus 0.1M potassium phosphate buffer, pH 7.6, plus 50 γ Janus Green B. Centrifugation at 400 $\times g$ for 10 minutes.

plasm. Here we have chosen the determination of succinoxidase activity. It cannot be claimed on the basis of available evidence that the succinoxidase system is completely absent from nuclei, but it is the best test that we have seen for the quantitative detection of cytoplasmic contamination of nuclear fractions. By the use of pestle homogenizers, isotonic sucrose containing no electrolyte, and a low gravitational force of 400 g , we have obtained liver nuclei containing only 4 per cent of the total succinoxidase in the homogenate. For lower levels of contamination, only a careful examination with the microscope can assure complete absence of mitochondria.

The effect of the centrifugation medium is demonstrated in the next slide (fig. 5) which shows the packed nuclei from one gram portions of cells from perfused rat livers. Nuclei in water

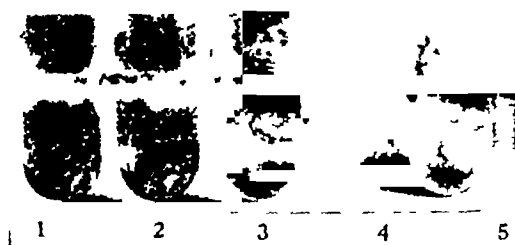


Fig 6 DIFFERENTIAL STAINING of mitochondrial and poorly sedimented layers. Centrifugation at 8000 $\times g$ for 10 minutes following removal of nuclei. *Tube 1*, unwashed particulate matter, *tube 2*, washed once, *tube 3*, 30 γ Janus Green B added, *tube 4*, 100 γ Janus Green B, then incubated at 38° C for 5 minutes. *Tube 5* shows incubated microsomes following addition of Janus Green B. The dark portion of the precipitate shown in *tube 4* was red.

PNA is being used to characterize the principal contaminant (microsomes). It is not implied that mitochondria contain no PNA, but it appears that they contain much less than the microsomes. Studies in the literature report rat liver mitochondria with PNA contents ranging from 5 to 40 per cent of the cellular PNA in normal rats on similar diets. The reason for this tremendous variation lies in the fact that the 'poorly sedimented' layer described by Schneider (1) has been removed by some investigators and left with the residue by others. It may be of interest to show a picture of this layer, and to show how a new application of the Janus Green stain has been employed by Dr Recknagel to differentiate the two layers. This stain is considered to be specific for mitochondria (44) and it has also

been known that the mitochondria reduce the dye to diethylsafranin, which is red (44). It was found that the differential staining could best be developed by including the dye in the medium prior to centrifugation and by incubating the packed particles at 38° for 5 minutes. In the presence of an excess of the dye, both the mitochondria and the poorly sedimented layer originally stain blue, but upon incubation only the mitochondria turn red so that a sharp line can be seen between the two layers (fig 6). The staining reaction and the reduction are optimum in isotonic sucrose, almost obliterated in 0.6 to 0.8 M sucrose, and weakened in hypotonic sucrose. In anaerobic suspension the reduction was stimulated by TPN but not by DPN.

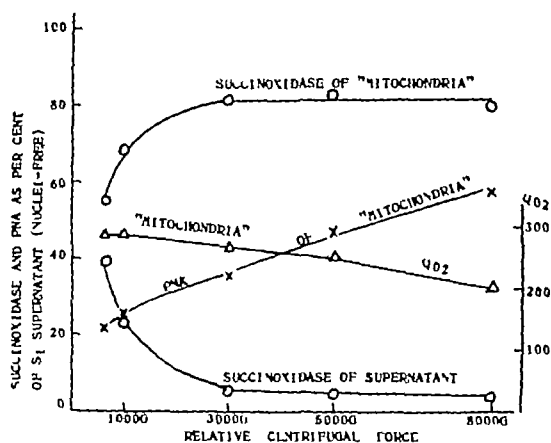


Fig 7 DISTRIBUTION of liver PNA and succinoxidase activity at different centrifugal forces. The medium was 0.25M sucrose containing 0.01M phosphate pH 7.6. The poorly sedimented layer was not removed from the mitochondrial layer.

The next slide (fig 7) shows succinoxidase and PNA measurements on the residues obtained at different centrifugal speeds, with the poorly sedimented layer left with the residue. It is clear that the succinoxidase recovery reached a maximum at relatively low speeds, while the PNA content and the amount of the poorly sedimented layer (observed visually) continued to rise as the gravitational force was increased.

On the basis of these findings, we concur in the opinion of Schneider and Hogeboom (1) that the particles in the poorly sedimented layer, at least in the case of liver, are probably not mitochondria, and that they are probably microsomes. Further studies on the microsomes and on other tissues using these and other criteria of homogeneity are needed.

The data in figure 7 may be compared with data employing the Waring blender in which it was reported (40) that after 30 seconds in the blender 45 per cent of the succinoxidase was in the 'microsome' fraction, and by 60 seconds the proportion occurring in the 'microsomes' had increased to as much as 73 per cent. Less than 30 seconds was insufficient time to break up the tissue. We interpret these data to mean that the blender is unsuitable for cytochemical studies involving the methods of differential centrifugation as a prelude to studies on enzyme systems since it comminutes both the nuclei and the mitochondria to the point where they occur in the microsome and supernatant fractions. Further evidence regarding the increased phosphatase activity (45) and decreased oxidative capacity (46) of the blenderized preparations has been reported in other laboratories. We object to the use of the word 'homogenate' for these blender preparations because it is clear that their properties differ considerably from the preparations to which we originally applied the word. The possible usefulness of the word homogenate has been destroyed by its indiscriminate use so that it can no longer be used without qualification. However, it was never an appropriate term for what is actually a heterogeneous suspension of cellular components, and its misuse is not too serious. The misuse of the words nuclei, mitochondria and microsomes is much more regrettable, although all of us are probably guilty in varying degrees. Moreover, the avoidance of the terms is undesirable if the data are to be useful in a cytochemical sense. The homogenate that we have used was merely a stage in the evolution of techniques for the study of enzymes at the intermediate level between the whole cell and the soluble protein (6). At this level, we feel that an effort should be made to relate enzymology to intracellular physiology, by maintaining the formed bodies of the cell in intact form insofar as may be possible, and by working with the separate components of the homogenate alone and in defined combinations.

The experiments we have described have been carried out with isotonic sucrose and with liver and kidney. We are advised that this medium may be unsuitable for tissues such as thymus and it should be emphasized that the methodology in this field is far from established.

SUMMARY

This discussion may best be concluded with a plea for more rigorously defined cell fractions and more careful definition of speeds, media, and homogenates. We predict that further attempts to isolate cell components with a minimum of

post-mortem changes and to duplicate the intracellular environment will add greatly to our knowledge of intracellular control mechanisms. We believe that the understanding of these control mechanisms is our most important goal, because with such understanding comes increased power to cope with the problems of disease.

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STRUCTURE AND FUNCTION OF NUCLEIC ACIDS AS CELL CONSTITUENTS

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IT is safe to say that living systems require the presence of both types of nucleic acid, or, in the case of parasitic systems, the presence of at least one. If, to use a designation of Schrodinger (1), one refers to the chromosomes as "the hereditary code-script," the great biological importance of all components of these nuclear structures, viz nucleic acids, proteins, and, perhaps, lipids, is obvious, unless one assumes that one or the other of these components has been added by Nature as a meaningless and purely decorative flourish. This is, however, not likely.

In animals and higher plants the desoxyribose nucleic acids (DNA) are exclusively or almost exclusively situated in the nucleus. Pentose nucleic acids (PNA) are present in the nucleoli and the various cytoplasmic elements, e.g. the mitochondria, submicroscopic particles, etc. Recent work with Elson (2) on the nucleotide composition of PNA in different fractions of rat liver cells has provided preliminary evidence of differences in composition between nuclear and cytoplasmic PNA. It is not yet known whether this will be generally true, but results, recently reported with Magasanik (3), seem to point to the presence in pig liver PNA of two differently composed fractions.

Whether DNA really is limited to the nucleus is not entirely certain, since the available cytochemical or cytophysical methods presumably require the presence of a compact mass of DNA and may not reveal its occurrence in a diffusely distributed form, as could be the case in the cytoplasm of egg cells (cf. 4). The bacterial cell represents a special case. Whether the microbial nucleus is the sole repository of DNA in microorganisms, cannot yet be decided with certainty.

BIOLOGICAL SIGNIFICANCE OF NUCLEIC ACIDS

The discussion of the biological significance of a ubiquitous cell constituent is, strictly speaking, superfluous. But there exist a few important instances pointing to a direct involvement of

nucleic acids, and some of them will be listed here briefly.

All virus preparations so far described are, or contain, nucleoproteins (cf. the recent survey of Davidson, 5). The same seems to be true of intracellular parasites, such as rickettsiae (6, 7) or paramycin (8).

Specific DNA preparations are known which are able to induce the transformation of bacterial types. This extremely important phenomenon, first discovered in pneumococci (9), has later been shown to operate also in *B. coli* (10) and in *Hemophilus influenzae* (11, cf. also 12). The possibility that reactions of this kind are of more general biological importance and not limited to the field of bacterial transformations cannot be rejected. What appears particularly remarkable is that it is here the free nucleic acid and not a nucleoprotein (as in the case of viruses) that is able to impose its own synthesis on the receptor cell, whereas in general nucleic acids seem to occur in cells only in the form of conjugated nucleoproteins. The mechanisms through which these transformations take place and the chemical features distinguishing these biologically active DNA specimens are completely obscure. There is, however, little doubt that it is the bacterial DNA itself, or a particular DNA fraction present in the transforming preparations, which is the carrier of activity. Recent work on the agent operative in the transformation of *H. influenzae* has shown that highly purified DNA preparations from two types are active in extremely low concentrations: 0.0004 γ of DNA per cc. in type b, 0.01 γ in type c (13).

The investigation of the relative efficiencies of different wave lengths of ultraviolet light has yielded curves closely resembling, but not entirely identical with, the UV absorption spectrum of nucleic acids. This work, mainly due to Stadler and Uber, Hollaender and Emmons, and Knapp and Schreiber, has been reviewed by Lea (14).

A certain degree of constancy, within the same species, of the DNA concentration per

diploid nucleus, and of roughly one-half this amount per haploid sperm nucleus, has been discovered by Boyin and his collaborators (15, 16, 4) and confirmed in other laboratories (17, 18)

DNA is in its composition identical in different tissues of the same species (19). Moreover, in the few cases where comparison was possible, no chemical differences have been observed between the composition of the DNA from the sperm cells and from differentiated tissues of the same species, in contrast to the very different composition of nuclear proteins in such instances.

DESOXYRIBONUCLEIC ACIDS

All DNA preparations that have been studied in detail have several features in common. They are asymmetric molecules of high molecular weight (around 10^6), yielding extremely viscous solutions. They appear to contain the same desoxy sugar, namely 2-desoxyribose. They contain two purines, adenine and guanine, and two or three pyrimidines, viz thymine and cytosine, and in several cases (20) also 5-methylcytosine. The desoxyribonucleotides released enzymatically from calf thymus DNA appear to be the 5-phosphates (21, 22).¹

The conclusions to which our work has led us have been summarized recently (19, 24) and I shall limit myself here only to the main points.

1 DNA is in its composition characteristic of the species from which it is derived. This can in many, but not in all, cases be demonstrated by determining the ratios in which the individual purines and pyrimidines occur. There will, how-

ever, be very many borderline cases in which such differences in composition are not sufficiently significant to permit their use as the sole criterion of differentiation. The most important question of the sequence in which, in a particular nucleic acid, the nucleotides follow each other has so far barely been approached. The elaboration of methods for sequence analysis is, perhaps, one of the most urgent problems in nucleic acid chemistry, since differences in nucleotide sequence may very well be among the determinants of chemical and biological specificity.

2 No differences in composition have so far been found in DNA from different tissues of the same species. This provisional conclusion refers only to the over-all composition. It is in this connection noteworthy that no chemical differences appear to exist between the composition of DNA from normal human tissue (25) and that of preparations from human cancer tissue (26).

3 The tetranucleotide hypothesis is incorrect.

4 There exist a number of regularities. Whether these are merely accidental cannot yet be decided. In almost all DNA preparations studied until now the ratio of total purines to total pyrimidines never was far from 1. Similarly the ratios of adenine to thymine and of guanine to cytosine were near 1.

5 There appear to exist two main groups of DNA, namely the 'AT type,' in which adenine and thymine predominate, and the 'GC type,' in which guanine and cytosine are the major constituents. The latter has so far been found only in certain microorganisms (27).

Data to support these conclusions have, in the main, been presented in previous publications and were summarized recently (19, 24). I should like to limit myself here to the discussion of a few as yet unpublished results. The study of the composition of the DNA of salmon sperm (28) has brought out some of the regularities mentioned before particularly clearly (tables 1 and 2). This substance belongs to the 'AT type'.

Another investigation, undertaken in collaboration with G. Braverman, deals with the DNA of wheat germ and the course of its degradation by crystalline pancreatic desoxyribonuclease. Previous work in our laboratory on the enzymatic disintegration of calf thymus DNA (29) had shown it to proceed according to a peculiar and complex pattern. The action of the enzyme resulted in the formation of dialyzable fragments and of a dialysis residue (core). The latter was

¹ In this connection mention may be made of experiments with D. Elson on the separation of the desoxyribonucleotides by filter paper chromatography. With neutral aqueous solutions of the nucleotides and the techniques described for the ribonucleotides (23) quantitative separation in the form of compact spots was obtained. With the buffered isobutyric acid-ammonium isobutyrate system (pH 3.6) the nucleotides were, in the order of increasing distance from the starting point, aligned as follows (with the distance of the adenine nucleotide arbitrarily taken as 100 and the relative distances of the other nucleotides placed in parentheses): 1) Desoxyguanylic acid (53), 2) thymidylic acid (66), 3) desoxycytidylic acid (80), 4) desoxyadenylic acid (100), 5) desoxy-5-methylcytidylic acid (137). We are indebted to Dr. W. E. Cohn for the nucleotides.

characterized by greatly increased ratios of adenine to guanine, thymine to cytosine, purines to pyrimidines, and by greater resistance to enzymatic attack. Preliminary results of studies of this type on wheat germ DNA are presented in table 3. The analytical findings on the intact DNA are in good agreement with analyses recently reported for the same nucleic acid from

TABLE 1 SALMON SPERM DNA, PROPORTIONS
(IN MOLES OF NITROGENOUS CONSTITUENT
PER MOLE OF P IN HYDROLYSATE)

CONSTITUENT	MEAN PROPORTION	STANDARD ERROR
Adenine	0.280	0.005
Guanine	0.196	0.004
Cytosine	0.192	0.006
Thymine	0.274	0.005

TABLE 2 SALMON SPERM DNA, MOLAR
RELATIONSHIPS

MOLAR RATIO	
Adenine to guanine	1.43
Thymine to cytosine	1.43
Adenine to thymine	1.02
Guanine to cytosine	1.02
Purines to pyrimidines	1.02
P accounted for as percentage of P in hydrolysate	95.8 (± 1.6)
Average no. of gm.-atoms N per mole constituent	3.7
Atomic N:P ratio in DNA prepa- rations	3.6, 3.7

two other laboratories (30, 31). The figures given here for the '19% core' and the '8% core' refer to the dialysis residues recovered when 81 and 92 per cent of the DNA respectively had been converted by the enzyme to dialyzable fragments. The trend of degradation appears similar to that observed with calf thymus DNA.

The molar ratios found in the DNA specimens studied in our laboratory are compared in table 4. The tendency toward certain regularities will be observed. The figures for hen DNA (chicken erythrocytes) and for the DNA from the K-12 strain of *B. coli* must be considered as preliminary. Both components were studied in collaboration with B. Gandelman. The DNA of *Hemophi-*

lus influenzae, type c, was studied by S. Zamenhof. A few points are noteworthy. In the case of wheat germ DNA, methylcytosine and cytosine apparently must be considered together, if the regular ratios, observed in most other instances, are to be obtained. Another remarkable fact is that the regularities are maintained even in the nucleic acids of the 'GC type' despite the complete inversion in individual ratios.

As was already mentioned before, it is almost impossible to decide at present whether these regularities are entirely fortuitous or whether they reflect the existence in all DNA preparations of certain common structural principles, irrespective of far-reaching differences in their indi-

TABLE 3 WHEAT GERM DNA, INTACT PREPARATION
AND ENZYMATICALLY PRODUCED CORES (IN MOLES
OF NITROGENOUS CONSTITUENT PER MOLE OF P
IN HYDROLYSATE)

CONSTITUENT	INTACT DNA	19% CORE	8% CORE
Adenine	0.27	0.33	0.35
Guanine	0.22	0.20	0.20
Cytosine	0.16	0.12	0.10
5-Methylcytosine	0.06	0.04	0.04
Thymine	0.27	0.26	0.23
Total purines	0.49	0.53	0.55
Total pyrimidines	0.49	0.42	0.37
Recovery	0.98	0.95	0.92

vidual composition and the absence of an easily recognizable periodicity. It may be assumed that the nucleic acids as we know them today, as for that matter also the proteins, are the result of an age-long selection process in the course of which many less suitable or less stable components must have been eliminated. One could speak of the survival of the fittest nucleic acids. Such macromolecules will exhibit diversity and uniformity at the same time, since they are called upon to perform, in diverse species, the same tasks. It is, therefore, perhaps not astonishing that the nucleic acids will share certain features that may be directly connected with their stability or their ability to form conjugated nucleoproteins. One property which is quite striking is the uniform absorption spectrum in the ultraviolet of all highly polymerized DNA specimens, both with respect to the position and the intensity of the

absorption maximum. The center of absorption fluctuates only between 257 and 261 m μ and the (ϵ)P is around 6600. Another surprising feature is the balance between amino groups and enolic hydroxyls in all DNA preparations examined by us (See last column of table 1.) Even in the core preparations, this ratio changed only very little.

But let us return for a moment to the other outstanding characteristic of nucleic acids, viz. their diversity. If we accept the evidence of the existence of species specific DNA, then there arise many new questions, both of a biological and chemical nature. DNA presumably is an important part of the chromosomes and may be

periodicity in a nucleic acid chain. Any simplified assumption with respect to periodicity has been disproved by the studies on the course of action of deoxyribonuclease on the DNA of calf thymus (29) and of wheat germ. The composition of both the dialyzable degradation products and the dialysis residues, the 'cores,' exhibited continuous and characteristic changes with respect to the distribution of purines and pyrimidines. One must conclude that the sequence is highly aperiodic and that it is not inconceivable that the same cellular DNA could give rise to many different nucleoproteins, depending upon the shape and configuration of the particular protein

TABLE 4. MOLAR RATIOS IN DNA PREPARATIONS OF DIFFERENT ORIGIN

SOURCE	ADENINE TO GUANINE	THYMINE TO CYTOSINE	ADENINE TO THYMINE	GUANINE TO CYTOSINE	PURINES TO PYRI- MIDINES	AMINO GROUPS TO ENOLIC HYDROXYLS
Ox	1.29	1.43	1.04	1.00	1.1	1.4
Man	1.56	1.75	1.00	1.00	1.0	1.3
Hen	1.45	1.29	1.06	0.91	0.99	1.5
Salmon	1.43	1.43	1.02	1.02	1.02	1.4
Wheat	1.22	1.18 ¹	1.00	0.97 ¹	0.99	1.4
Yeast	1.67	1.92	1.03	1.20	1.0	1.3
Hemophilus influenzae, type C	1.74	1.54	1.07	0.91	1.0	1.5
B. coli K-12	1.05	0.95	1.09	0.99	1.0	1.6
Avian tubercle bacillus	0.4	0.4	1.09	1.08	1.1	1.7
Serratia marcescens	0.7	0.7	0.95	0.86	0.9	1.6
Hydrogen organism Bacillus Schatz	0.7	0.6	1.12	0.89	1.0	1.7

¹ In these computations the sum of cytosine and methylcytosine was used. If cytosine alone is considered, the thymine to cytosine ratio is 1.62 and that of guanine to cytosine 1.33.

surmised to be involved in their biological functions. Does this mean that a cell contains as many different DNA individuals as it contains genes? Or can one and the same species-specific DNA form so many three-dimensional structures, in connection with the proteins to which it is attached, that the genic requirements are fulfilled? (For a more detailed discussion of some of these points, cf. 32.) This question, as so many others in this field, cannot yet be answered. No way has as yet been found to fractionate a family of very similar macromolecules which may differ in no more than the sequence of a few of their component nucleotides. But one could perhaps say that the more regular the arrangement of nucleotides is in a given DNA, the less the chance of its forming many different specific structures. And this brings us again to the very important question of nucleotide sequence and

It must, moreover, be understood that the recognition of periodicity, i.e. the presence of recurring units, will be particularly difficult in a macromolecule of the type of DNA. If, for instance, in a chain composed of 3000 nucleotides a particular sequence of 100 consecutive nucleotides were repeated 30 times, this periodicity could not be recognized, unless we had a method producing cleavage only at the points where these repeating units are joined. In other words, the perception of periodicity would require the proper distance for a bird's-eye view which will not be easy to attain.

Another approach to the problem of sequence analysis in DNA may be seen in the study of its controlled chemical degradation. That the purines can be detached from a nucleic acid with much greater ease than can the pyrimidines, has long been known, but the resulting end product,

thymic acid, was rather nondescript. In collaboration with M. E. Hodes and C. Tamm it has been possible to develop procedures, soon to be published, in which all purines could be cleaved from a DNA preparation, leaving behind a nondialyzable product in a yield of about 94 per cent that retained all the pyrimidines of the original DNA in unchanged proportions. We have designated preparations of this type as apurinic acid. These compounds may prove of interest for structural studies on DNA, since the position in the DNA chain of the initially present purine nucleotides is now marked by reactive aldehyde groups. The properties of a typical preparation of apurinic

TABLE 5 APURINIC ACID FROM CALF THYMUS DNA

	DNA	APURINIC ACID
N	14.9	6.0
P	9.1	10.7
N/P	3.6	1.2
Absorption maximum, $m\mu$ ¹	258.5	268
Absorption maximum, $\epsilon(P)$	6600	4600
Optical rotation ²	+102°	+50°
Adenine, moles per mole P	0.28	0
Guanine, moles per mole P	0.21	0
Cytosine, moles per mole P	0.21	0.18
Thymine, moles per mole P	0.26	0.24
Thymine to cytosine	1.3	1.3

¹ In M phosphate buffer, pH 7.1 ² In 0.1 M phosphate buffer, pH 7.1

acid are contrasted in table 5 with those of the calf thymus DNA specimen that served as the starting material.

PENTOSE NUCLEIC ACIDS

Time does not permit an adequate discussion of the chemistry of PNA. In the past few years, significant contributions to this field were made by Gulland, Kerr, Schmidt, Allen, Loring, Cohn, and many other workers. I should like to make brief mention of some recent work done by B. Magasanik in our laboratory (3) in which the course of action of crystalline ribonuclease on PNA from yeast and from pig liver was studied with the use of chromatographic and spectroscopic procedures. About 60 to 70 per cent, or in some cases somewhat more, of the nucleotides present in the initial substrates were liberated by enzymatic action as rapidly dialyzable nucleo-

tides of low molecular weight. This fraction consisted of free cytidylic and uridylic acids, which comprised a high proportion of the total pyrimidine nucleotides, and of combined purine and pyrimidine nucleotides. All PNA preparations yielded a nondialyzable residue, resistant to enzymatic attack, which was found to consist to about two thirds of guanylic acid and of varying amounts of the other nucleotides. A selection of these experiments is presented in table 6. These and other findings have led us to

TABLE 6 ACTION OF RIBONUCLEASE ON PNA

SOURCE	FRACTION	NUCLEOTIDE COMPOSITION (AS MOLES/100 M NUCLEOTIDE IN STARTING MATERIAL)				
		Guanylic acid	Adenylic acid	Cytidylic acid	Uridylic acid	Total
Yeast	Starting	30	21	23	26	100
	Dialyzable (total nucleotides)	18	15	19	26	78
	Dialyzable (mononucleotides)	0	0	14	19	33
	Core	66	18	8	8	
Pig liver	Starting	36	19	29	16	100
	Dialyzable (total nucleotides)	18	14	26	15	73
	Dialyzable (mononucleotides)	0	0	18	10	28
	Core	71	15	0	14	

certain conceptions concerning the specificity of ribonuclease and the structure of PNA, but reference must be made to a detailed article which is in press (3).

FINAL REMARKS

It is fitting to conclude this all too sketchy survey with a confession of ignorance. What our studies have taught us more than anything else is how little we know as yet about the chemistry of nucleic acids. The chemical specificity of macromolecules and the interactions between them through which the organization of the cell is maintained can only partly be understood in

terms of our present knowledge. In the approach to a scientific problem two principles are operative: generalization and simplification. Both are necessary and both dangerous. It is obvious that we can learn more geometry from the illustrations in a textbook on projective geometry than from the beautiful pictures in Sir D'Arcy Thompson's *On Growth and Form*. But it is difficult to say where the danger line lies beyond which over-simplification will produce a dogmatic ignorance.

Should we stress the multifariousness of Nature, which makes us forget the simplicity of its basic designs, or should the essential shape win over the accidental form? In Wycheley's *The Country Wife* a quack is addressed as follows: "Doctor, thou wilt never make a good chemist, thou art so incredulous and impatient." If patience and credulity were all the chemist needed, the problem of the nucleic acids—still so baffling and elusive—would have been solved a long time ago.

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AMERICAN INSTITUTE OF NUTRITION

Symposium on Utilization of Protein

Chairman A H SMITH

INFLUENCE OF NON-PROTEIN CALORIES ON PROTEIN METABOLISM¹

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THE PRESENCE of energy-producing foods in the diets of all peoples reflects a primary metabolic demand. Sources of energy must be provided for the work of the body—intrinsic and extrinsic—for the maintenance of body heat, and for the regulation of the myriad biochemical reactions governing the physiological economy. There is, however, an accumulating body of evidence indicating that both carbohydrate and fat play a role over and beyond the provision of calories. That the total energy balance and the nitrogen balance are inextricably interwoven is evident. However, a review of the information found between the covers of that repository of fact and interpretation, *The Biology of Human Starvation* (1), indicates that, in respect to the three basic nutrients, carbohydrate, fat and protein, the requirements of man as we meet him in his ordinary walks of life have not been defined clearly in terms of practical working recommendations that will take into account the many and varied situations in which he finds himself. As in the past, knowledge of the nutritional requirements of human beings will grow as facts accumulated in research laboratories of this and other countries are interpreted and applied. I have chosen, therefore, today to confine myself largely to the presentation of experimental studies that may have significance in solving the many problems that are met in the feeding of the human race, the world over, in times of peace and war.

The story of the dietary interrelation of proteins, carbohydrates, and fats in the maintenance

of nutrition had its beginnings in the laboratories of the great masters of physiology in Europe. As we, with our large fund of new nutrition knowledge and with research facilities of the highest order, look back to their achievements, our wonder ever grows that they could have accomplished so much. And certainly it is a challenge to the modern nutritionist to pick up the threads where these great men left off and to weave them into a finished tapestry.

Nitrogen balance is a tool that has been used by many workers in estimating the part played by non-protein calories in the establishment of nutritional state. I think we can say, in the main, that experimental studies show that nitrogen retention is a function in part of the caloric intake. However, there are many factors whose influence is not yet fully understood or appreciated that affect the metabolic interplay of these nutrients. What some of these factors are and how they work will be the theme of this discussion.

CALORIC RESTRICTION

It would seem that an understanding of caloric influence on the *endogenous* nitrogen metabolism characteristic of protein starvation should provide a base for fundamental first approximations. In figure 1A is shown the effect of systematic reduction of calories on the catabolism of 4 groups of rats as measured by the quantity of nitrogen excreted in the urine (2). The animals in each group had been protein-depleted under a standardized set of conditions and were catabolizing body tissue at a constant rate. The basal diet they received was protein-free, but otherwise adequate. When consumed *ad libitum*,

¹ Journal Paper, No. J-1958, Iowa Agricultural Experiment Station, Project 995

it provided approximately 19 Cal/rat/day. In this experiment, the ration was fed in quantities that supplied 100, 75, 50, or 25 per cent of the calories normally consumed by the rat. The data indicate that the first two reductions in caloric intake although inducing some increments in urinary nitrogen do not impose stresses to which the animals cannot make physiologic adaptation. It is only when the energy intake is reduced to one-fourth of the normal requirement that the deficit becomes critical and the animals no longer are able to maintain the integrity of their tissues. The increment in the output of urinary nitrogen is immediate and marked just as soon as the caloric deficit is imposed.

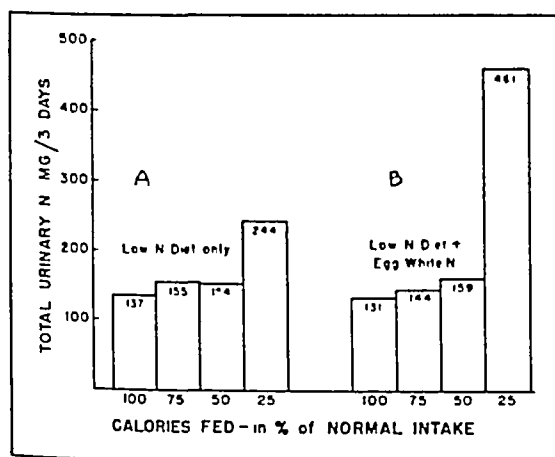


Fig 1 URINARY EXCRETION of nitrogen by rats fed rations of different caloric values

The way a protein-starved animal uses tissue protein under conditions of caloric restriction is reflected in its handling of protein derived from *exogenous* sources (fig 1B). To the diets of the animals in this group, egg white protein was added in a quantity that supported nitrogen equilibrium when the ration was of *adequate energy value*. It may be seen that there is a trend toward an increased excretion of nitrogen in the urine as the energy value of the diet drops to one-half of the normal intake. On the whole, the average rates of metabolism of the rats in each group are not very different than they were when the diet contained no nitrogen. But further reduction in caloric intake leaves a marked imprint, and the animal throws all of the nitrogen of its food protein away so that it may use the non-nitrogenous portion as well as similar moieties arising from the degradation of body tissue in satisfying its demand for energy. The increment in urinary nitrogen when the diet supplies

only one-fourth of the needed calories is approximately equal to the quantity of nitrogen ingested.

PHYSIOLOGICAL STATUS

The experiments just described are based on the response of rats undernourished in respect to protein. Of as great interest is the influence of the non-protein calories of the diet on the manner in which animals better nourished in this respect utilize protein provided in their food, a subject studied in detail by Dr. Allison's group at Rutgers University (3). Again, when dietary protein is administered—this time to the dog—in a quantity that supports nitrogen equilibrium at full caloric intake, there is a continuously augmented excretion of nitrogen in the urine as the energy value of the diet drops from a high to a low value. Relative increments are of a somewhat greater order than they are in standardized protein-starved rats. However, as early as 1946, Allison and his co-workers showed that despite these constantly increasing urinary losses the nitrogen balance index of the dietary protein fed is not altered until the energy provided by the ration is less than one-half the normal requirement of the animal (4).

When the diet supplies more than 50 per cent of the needed calories, the internal reserve that an animal in a reasonably good state of nutrition possesses in its hepatic and plasma proteins acts like a safety valve. These proteins when drawn upon in times of emergency may provide sufficient energy to satisfy caloric needs and thereby permit utilization of food nitrogen. If the period of stress is not too prolonged, the animal apparently suffers no injury over and beyond the diminution of tissue reserves for meeting future dietary crises.

The picture in general supports the views of Bosshardt and his co-workers (5) that an animal responds in two ways to a systematic reduction of the caloric value of the diet. A nominal limitation causes the animal to draw on existing reserves to get the energy its food is not supplying, then more drastic deprivation forces it to use both food and body nitrogen for energy purposes, and a marked breakdown of body tissue occurs.

BODY STORES

The significance and importance of these body stores of nitrogen have been stressed by the Rutgers group (3). In animals possessing such

tissue reserves, metabolism under conditions of reduced caloric intake does not remain static, but rather drifts into the state where nitrogen retention is markedly reduced. The speed at which the drift occurs and the character of the metabolic response depend on the size of the internal stores of protein. A dog, for example, with well-filled tissue reservoirs passes first into acute negative balance when a severe caloric restriction is imposed. Then it drifts toward nitrogen equilibrium, but before this state can be achieved, the impact of the caloric deficiency forces the animal into acute negative balance. On the other hand, with the first restriction of calories, an animal whose initial reserves are low in protein slips immediately into positive balance, but then suddenly and rather dramatically drops into negative balance—perhaps a full 25 days earlier than does the better nourished dog. These experiments point to the fact that situations exist where an animal may be in nitrogen balance but still losing body tissue. The function of tissue reserves in padding metabolic processes against the shock of dietary imbalance may explain in part why Keys and his co-workers (1) did not observe a trend toward a correlation of nitrogen retention and caloric intake until the men had lived on the semi-starvation diet for more than 12 weeks.

RESISTANCE TO CALORIC RESISTANCE

It is interesting also to note that Allison (3) sometimes encounters a marked resistance to the nitrogen-depleting effect of a restricted caloric intake in individual dogs. Some of these animals are able to utilize dietary protein at full efficiency for as long a time as 150 days. Exploration of the reason will yield information, I am sure, that will enlarge our comprehension of the general problem.

QUANTITY OF PROTEIN IN THE DIET

The quantity of protein in the diet also bears a major relation to the response that an experimental animal may make to caloric additions or restrictions. Increasingly higher planes of nitrogenous utilization are established in mice consuming constant quantities of protein by additive quantities of calories. These, however, are enhanced further by increments in the dietary protein. It has been found in human experiments (6, 7) that, while 1500 Calories will support retention on a low protein intake—approximately

the critical level—900 Calories are ample when the protein intake is doubled. On the other hand, we see in the 'Minnesota experiment' that in rehabilitation additions of protein to rations ranging in energy value from 2000 to 3000 Calories per day exert no favorable influence on the retention of nitrogen (1). It indeed will be of both theoretical and practical interest to learn the lowest combination of protein and non-protein calories that will sustain nitrogen equilibrium under a variety of conditions.

CALORIES AND THE 'TIME FACTOR'

The effect of the elapse of time that may occur between the feeding of the protein and carbohydrate moieties of the diet on the utilization of nitrogen has intrigued several groups of investigators for some time. There is Geiger's recent contribution (8), that in protein-depleted as well as in infantile rats (9), separate feeding of the carbohydrate and protein portions of the diet results in a diminished retention of nitrogen. In explanation, Geiger suggests that the simultaneous presence of carbohydrate and protein in the diet induces a continuous flow of the prerequisite essential and non-essential amino acids at a rate that is commensurate with the metabolic demands of repleting or growing tissue (10). Undoubtedly, Dr. Geiger will discuss the interesting phenomenon today. Indeed, his concept of the gastro-intestinal dynamics involved (11) may be a basis for explaining the extraordinary demand for calories in the attainment of nitrogen balance by young men ingesting pure amino acids and ammonium salts as their sole source of dietary protein (12).

In support of Geiger's views is Leverton's observation (13) of the protective influence of additional calories in alleviating the negative nitrogen balance induced in young women subjects by the exclusion of animal protein from one meal (14). This response occurs when the daily intake of protein is approximately 43 gm/person/day. Of interest is the fact that failure to include animal protein in each of the day's three meals is not associated with negative nitrogen balance when the daily intake of protein is raised to 63 gm, and that, with the inclusion of this much protein in the diet, the caloric intake may be as low as 1400 Cal/day with no adverse effects (15). So, quantity of dietary protein, the non-protein calories of the diet, the time factor, and the relative distribution of high quality protein in the

day's meal are intertwined together in the complex picture of protein utilization

DIETARY SOURCES OF NON-PROTEIN CALORIES

Fat is regarded often as being wholly interchangeable with carbohydrate on a metabolizable energy basis. Also, it is generally assumed that the protein sparing effect of carbohydrate is considerably greater than that of fat when subjects ingest the same quantity of protein. Furthermore, in complete protein starvation, it is believed that it is carbohydrate, and not fat, that has the property of sparing body protein (16). Under conditions of caloric restriction, however, we have found in our laboratory that fat exerts a profound body-sparing effect when included in protein-free diets fed to rats. Indeed,

The nitrogen retention of all animals in an experimental series, when consuming a ration poor in nitrogen but of full caloric value, is determined in a balance test beginning with the 19th day and lasting either 5 or 7 days. Then part of the animals are fed nitrogen-poor diets of reduced caloric value. Four days are allowed for dietary adjustments, after which nitrogen balance is determined again over an interval comparable in length of that of *period 1*. The remaining animals in the experimental series are continued on the full caloric diet and their balances redetermined.

Thus, the performance of the rats in any experimental group is controlled in two ways: i.e. by their own behavior in *period 1*, and by the performance of another set of animals cata-

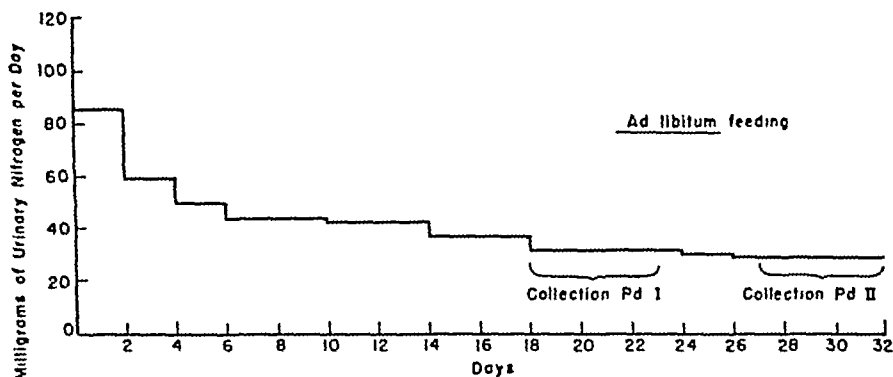


FIG 2 EXCRETION OF NITROGEN during successive intervals by rats fed a nitrogen-low diet.

the data suggest that fat has unique properties in regulating the course of the protein and perhaps the energy metabolism (17).

In these experiments, as in those previously described from this laboratory, the catabolism characteristic of protein-starved rats on a full caloric diet serves as the reference point in studying the influence of dietary modifications—in this case, the inclusion or omission of dietary fat in daily food quotas of descending caloric value. Male rats, 6 months old, are brought to an approximately steady state of nitrogen metabolism as judged by the quantity of the element excreted in the urine (fig 2). This condition with our particular stock animals prevails following maintenance for 18 days on a protein-free but otherwise adequate diet, and continues for another 14 to 16 days. All metabolic observations are made within this last interval of time. Two series of animals are prepared thus for study, the first group receiving a high fat ration during the period of depletion, the second a low fat diet

bolizing body tissue at the rate they would have been doing had no dietary modification been imposed.

The basal high fat diet used contains 10 per cent each of lard and butter fat, 38 per cent of the calories coming from fat (table 1). Fifty mg of vegetable oil supplement each diet. In the first experiments to be presented, the rations were offered *ad libitum* in *period 1*, and then held at this level of consumption in *period 2*. The rats so adjusted their intake of food that the average caloric value of the daily food quota of either the high fat or the low fat diet was approximately the same, i.e. 49 Calories. The vitamin mixture supplementing these basal diets contained all the recognized essentials.

In figure 3 are shown the nitrogen balances in *period 2* of rats fed the two basal diets—high fat and low fat—at four levels of caloric intake. The similarity in the rates of catabolism at normal and three-fourths normal caloric intakes suggests that the rats in the low fat group are not penalized

by the omission of the nutrient. Reducing the calories below this point, however, results in an intensification of the metabolic processes. Rats fed 12 Calories of the low fat diet, for example, lose tissue equivalent to 410 mg/nitrogen/100 gm body weight in 7 days, those given the high fat diet, 266. The findings have some counterpart in the observations of Pearson and Panzer (18), who report that the loss of the essential amino acids in the urine and stools of rats is considerably reduced when corn oil is a dietary component.

TABLE 1 COMPOSITION OF BASAL DIETS

DIETARY COMPONENTS	HIGH FAT DIET	LOW FAT DIET
	gm	gm
Dextrin	73	93
Lard	10	0
Butterfat	10	0
O & M salts	4	4
NaCl	1	1
Ruffex	2	2
Total	100	100
Cal/gm	4.8	3.8
Av intake in Cal/day	49	49
	(18 rats)	(36 rats)

In another series of experiments in which food intakes were controlled on the basis of *ad libitum* ingestion in period 1, the effect of varying the fat content in the diet was investigated (fig 4). The rations, isocaloric in value as fed, contained 20, 15, 10, 5 and 0 per cent of the butter fat-lard combination and were offered in quantities equivalent to 100, 75, 50, and 25 per cent of the normal energy requirement. The data depicted in the figure indicate that the nitrogenous metabolism of the animals is not altered until the dietary fat is reduced by 10 per cent, then the animals given the diet containing one-fourth the normal number of Calories go into a tailspin. The same condition prevails when the fat component is reduced further. In order to simplify comparisons, the nitrogen balances of all the groups whose caloric intake was held at one-fourth normal have been collected in figure 5. Seemingly, a ration should contain at least 15 per cent fat if it is to exert a protective action against the disintegration of body tissue induced by severe caloric restriction in a protein-starved rat.

We have obtained considerable evidence that

fats originating from different plant and animal sources vary in their capacity to protect against the body catabolism characteristic of the present experimental situation. Discussion of the relative merits of different fats does not seem warranted until results have been replicated and confirmed. Some related problems have been discussed recently by Barki, Collins, Elvehjem and Hart (19).

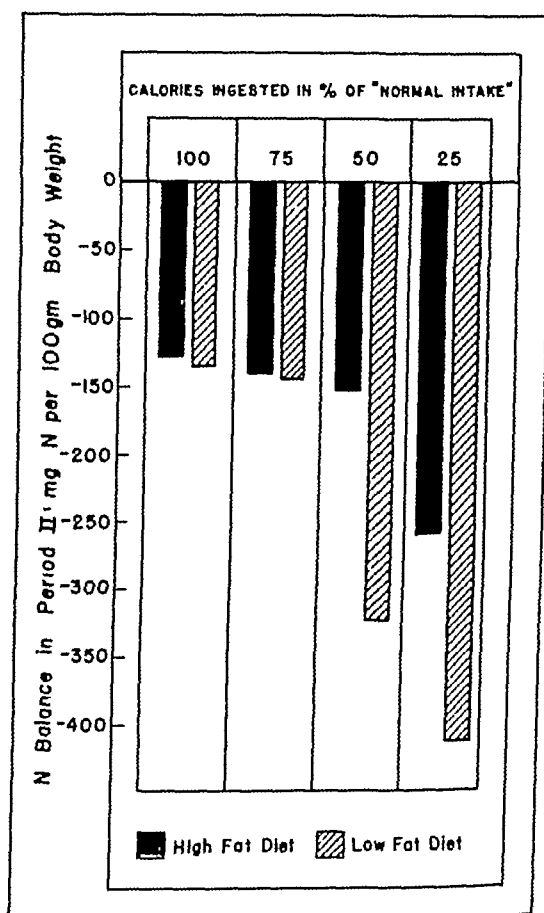


Fig 3 NITROGEN BALANCES induced by feeding protein-free diets high and low in fat and of graded caloric value

Inasmuch as rats fed rations deficient in protein involuntarily restrict their consumption of food, it seemed important to rule out the possible influence of inadequate food intake by more rigid control of this factor. Hoover, therefore, working in our laboratory, studied the response of animals to the force feeding of protein-free diets providing 100 per cent and 25 per cent of the daily energy requirement (20). The rations were made into slurries of the same caloric density, and the portions of each administered daily provided either 56 or 14 Calories/300-gm

rat (fig 6) Again, the presence or absence of fat in the diet is not reflected in any change in the total nitrogen balance when the animals are administered food supplying the full quota of necessary energy. Forced feeding, however, dramatically aggravates the rate of catabolism when only 14 Calories are given. Negative balance in these rats now is 1465 mg/300 gm rat/5 days, in contrast to 514 in the restricted group of fat-fed rats given the same number of calories in the form of the high fat diet.

madequate flood of nutrients from the gastrointestinal tract.

Nitrogen balance data presented thus far suggest that if the animal receives its full asset of calories, the removal of fat from the protein-free diet inflicts no handicap in the animal's adjustment to the absence of dietary protein. But before this conclusion is reached, let us examine other data. It was noted, for example, during the course of various force feeding experiments that a number of rats always died, and that this

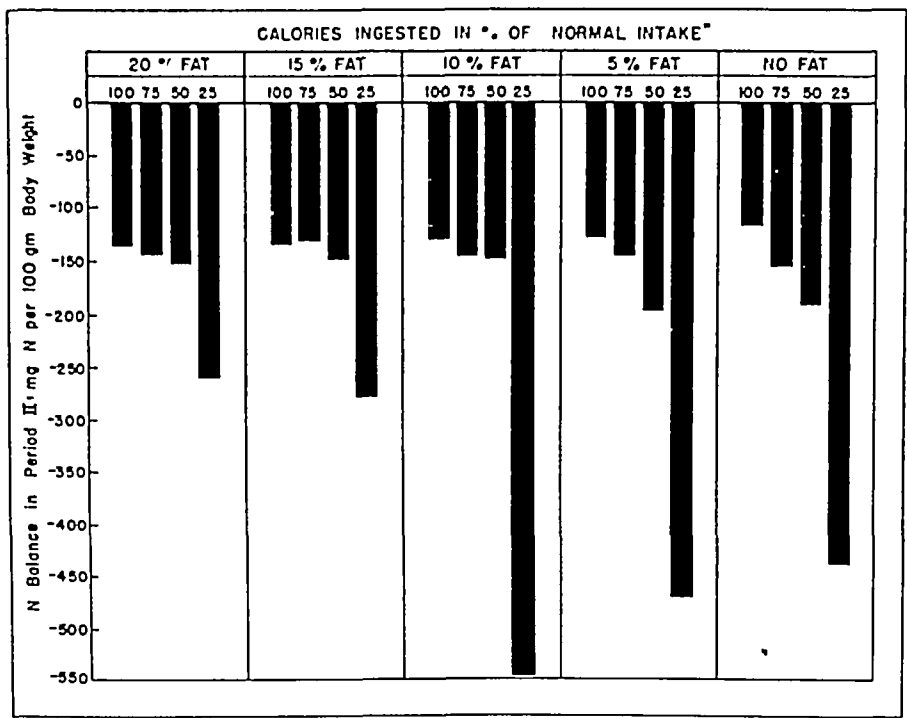


FIG 4 NITROGEN BALANCES induced by feeding protein-free rations of varying fat content and of graded caloric value

A comparison of the data recorded in table 2 would indicate that when *ad libitum* feeding is employed, deprivation of calories in the form of both carbohydrate and fat increases the catabolism about 1.8 times, in the form of carbohydrate alone, 3 times. On the other hand, when force feeding is employed, the latter ratio becomes 5.

Why force feeding aggravates the unfavorable response to the ingestion of the low fat diet is a moot question. It should be noted that the controlled feeding regime reduces variation within a group. Perhaps these data indicate that the loss of appetite associated with the ingestion of inadequate diets represents a protective physiological mechanism against metabolic obstacles encountered in the handling of a qualitatively

mortality occurred in the group receiving the full intake of the low fat diet (table 3). In the first 3 experiments listed, mortality in the group ranged from 30 to 50 per cent, whereas none succumbed in the groups fed the high fat ration. The response of group IV shows the effect of offering the daily quota of food in three feedings rather than two, and of equalizing the densities of the food slurries by the addition of ruffex to the high fat diet. It must be of significance that of the 207 rats observed deaths occurred only among the groups fed the fat-poor diet. Also it is noteworthy that no rats in either group maintained on restricted caloric intake died.

I am wondering here if we may not have evidence of an acute fat deficiency disease precipitated by the strain of metabolic adjustment to

a protein-free diet Decker and his co-workers (21) have written recently that chronic essential fatty acid deficiency may culminate as an acute syndrome by the imposition of various stresses and strains

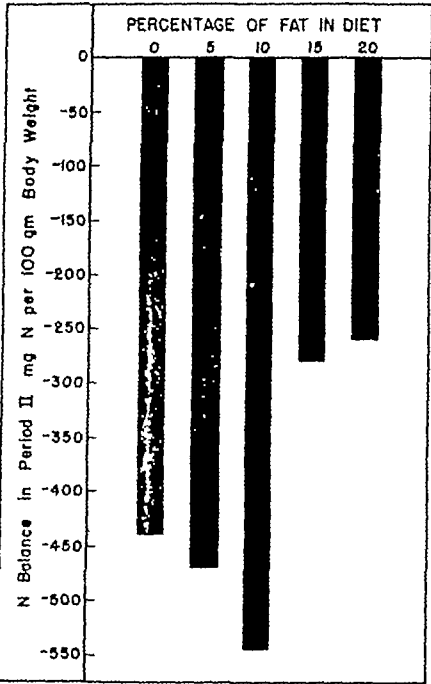


Fig 5 NITROGEN BALANCES when rats are fed protein-free diets of varying fat content and supplying only one fourth the needed calories

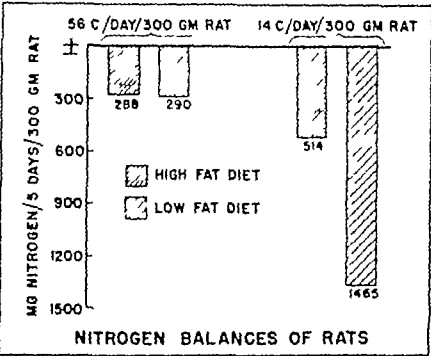


Fig 6 NITROGEN BALANCES induced by the force feeding of protein-free diets high and low in fat at two caloric levels

In studying further the effect of the removal of dietary fat, determinations of the partition of nitrogen in the urine (fig 7) disclosed an interesting alteration in the pathway of nitrogen metabolism when carbohydrate in restricted quantities served as the sole source of energy. It is not possible to present all data today, but it should

be indicated that a complete partition was made, and that in every experimental group the sum of the nitrogen present in the various components equalled the total excretion of nitrogen

Restricted caloric feeding induces a high excretion of urea in the fat-fed group. The increment is over two and one-half times greater, however, in the group receiving the low fat diet. But of greatest interest is the extremely large

TABLE 2 FIVE-DAY NITROGEN BALANCES WHEN CARBOHYDRATE CALORIES REPLACE FAT CALORIES IN THE DIET

REPLICATIONS OF EXPERIMENT	METHOD OF FEEDING	LOW FAT DIET		HIGH FAT DIET	
		Full caloric intake	Restricted caloric intake	Full caloric intake	Restricted caloric intake
year		mg/300 gm rat	mg/300 gm rat	mg/300 gm rat	mg/300 gm rat
1946	Ad lib ¹	-201	-724	-231	-418
1948	Ad lib ¹	-225	-725	-235	-412
1949	Force	-290	-1465	-288	-514

¹ In period 1 and held at this intake in period 2

TABLE 3 MORTALITY OF RATS FORCE-FED PROTEIN-FREE RATIONS

GROUP NO	TIME	LOW FAT RATION		HIGH FAT RATION	
		No of rats	No of rats dying, %	No of rats	No of rats dying, %
		56 Cal/day/300 gm rat		56 Cal/day/300 gm rat	
I	Spring 1949	48	31	48	0
II	Summer 1949	27	48	12	0
III	Fall 1949	12	50	12	0
IV	Winter 1949	12 ¹	25	12 ¹	0
	Winter 1949	12	17	12 ²	0

¹ Three feedings per day ²Ruffex added

excretion of ammonia nitrogen in the urines of this group of rats

Examination of these data, in connection with the concentrations of urea nitrogen and amino nitrogen in the blood, may be of interest (table 4). Feeding the low fat ration at full caloric intake results in an increment in the relative concentration of amino nitrogen in the blood over that in the blood of rats receiving adequate calories and fat in their ration. Some of this apparently spills over into the urine, and chromatographic determinations of amino acids therein disclose their presence in largest quantities in

the urines of rats fed unrestricted amounts of the low fat diet

The response of the rats in the low caloric-low fat group permits interesting speculation. What initiates the tremendous outpouring of nitrogen when this ration is fed in the first instance, and why does the animal receiving fat in its diet seemingly do a better job in adjusting to the stress of caloric restriction in protein starvation than its carbohydrate fed control? Perhaps the speeding up of the catabolic processes may reflect

suggests that the animal, in the absence of fat, is unable to take acidic intermediaries arising in the catabolic freeing of amino acids through the metabolic transformations required for their utilization as energy, and the kidney is forced to manufacture ammonia to care for the excretion of these intermediaries

There are other evidences of an impaired carbohydrate metabolism. Inability to convert the non-nitrogenous portions of the degraded protein molecule into glycogen is shown by an extremely low reserve of glycogen in the livers of the rats fed the restricted low fat diet, i.e. 0.7 per cent vs 2.0 per cent in the fat-fed groups. The glucose tolerance curves of the four groups

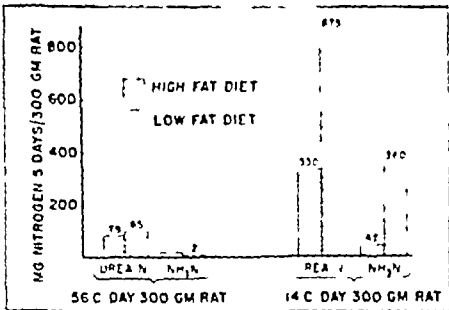


Fig 7 UREA NITROGEN and ammonia nitrogen in urines of rats fed protein-free diets high and low in fat

TABLE 4 CERTAIN NITROGENOUS CONSTITUENTS IN URINE AND BLOOD

ENERGY VALUE OF DIET PER 300-GM RAT	RATION	URINE			BLOOD	
		Urea N	NH ₃ N	-NH ₂ N	Urea N	-NH ₂ N
		mg	mg	mg	mg %	mg %
Calories/day						
56	High fat	79	5	4	6	26
56	Low fat	85	2	12	2	47
14	High fat	330	42	9	6	34
14	Low fat	875	349	4	31	18

the increased specific dynamic effect of a simultaneously metabolizing pool of carbohydrate and protein in relation to one of fat and protein (22). But I do not believe that this is all of the answer.

Possibly, here, there is a block in the course of the carbohydrate metabolism. Normal rats on restricted caloric intakes and receiving the same quantity of nitrogen in their food protein as the rats in this experiment liberate from tissue sources can transform the nitrogen of their food amino acids into urea, thereby presumably deriving full energy value from the non-nitrogenous portion. The high proportion of ammonia nitrogen to urea nitrogen in the urine of a calorie-starved rat

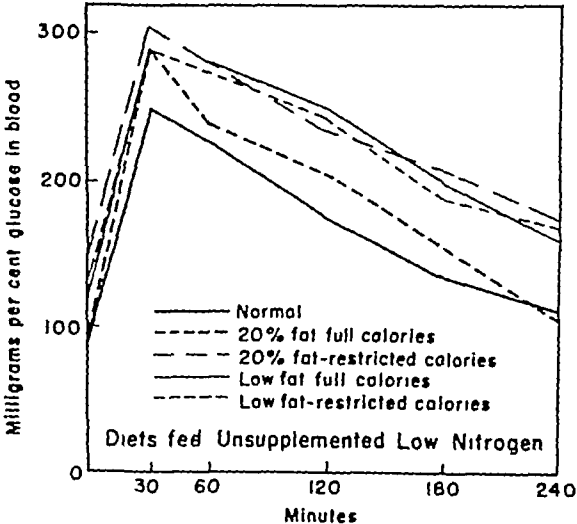


Fig 8 GLUCOSE TOLERANCE curves of rats fed the stock ration and various modifications of a low nitrogen diet

in relation to that of normal stock animals are also interesting in this connection (fig 8). The tolerance of the rat receiving its full caloric quota of the high fat diet most nearly approaches the normal. Inanition may explain the lower tolerances of the rats fed restricted number of calories—either high fat or low fat—but it is significant that the average tolerance curve of the animals with a *luxus* consumption of carbohydrate calories falls in the same range as the tolerances of the rats restricted as to their caloric intakes. On the whole, the animals maintained on the low fat diets have many characteristics that are diabetic-like in nature. Others have noted that rats subsisting on a low fat diet cannot tolerate the increment in metabolic rate induced by the feeding of thyroid (23).

Very pertinent in this connection is the sug-

gestion from the work of Swift and his co-workers at the Pennsylvania State College, that rats fed diets containing fat use energy derived from dietary sources more efficiently than animals on a low fat dietary regime (22). In the words of these workers, the presence of fat in the diet confers "economy of utilization of food energy." Supporting this hypothesis that fat is needed for the maximal use of energy are the observations of several groups of workers showing that, under differing experimental conditions, inclusion of fat in the diet is associated with an increased capacity for work (24, 25).

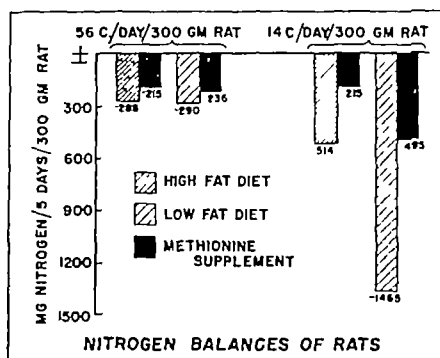


Fig 9 NITROGEN BALANCES of rats fed protein-free diets high and low in fat when methionine is added as a dietary supplement

Possibly our own observations that methionine seems to play a role in these metabolic conversions will provide a clue in the final unraveling of the problem (fig 9).

Its addition to the protein-free diet in quantities equivalent to 4 mg/nitrogen/day results in an immediate reduction in the total nitrogen excreted by all experimental groups, but particularly by the low fat-low calorie rats, and thence to a decrease in the negativity of the nitrogen balance. The concentrations of urea nitrogen in the urines of both groups fed 14 Calories drop concomitantly (fig 10). There is a fall in the

excretion of ammonia when methionine supplements the low fat diet, but not when it is added to the rations of rats receiving the restricted amount of the high fat diet. That despite the marked decrement in total urinary nitrogen the relative proportion of ammonia to urea nitrogen in the urines of the rats fed 14 Calories of the low fat diet remains the same as it was before the methionine was administered, suggests that methionine plays its part in regulating the process, whatever it is, that catalyzes the catastrophic breakdown of body tissue.

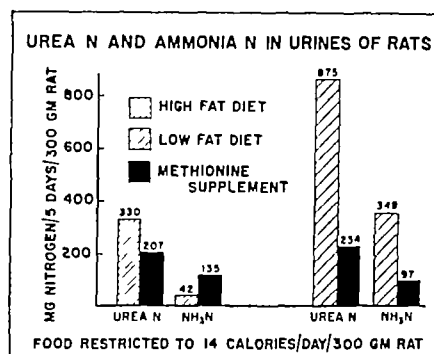


Fig 10 EXCRETION of urea and ammonia by rats fed protein-free diets supplemented with methionine and providing 14 Cal/day/300-gm rat

On the basis of the discussion today, nitrogen retention represents the interaction of many factors, i.e. caloric value of the diet, source of non-protein calories in the ration, physiological state, body stores, individual resistance, quantity of dietary protein particularly in relation to the energy value of the diet, the time factor, and the distribution of animal protein over the day's meals. Only when the influence of these and probably other factors in the establishment of nutritional state in man is fully understood will it be possible to state his protein requirements in terms of practical recommendations.

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EXTRA CALORIC FUNCTION OF DIETARY COMPONENTS IN RELATION TO PROTEIN UTILIZATION¹

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"Interpretations based on single factor relations do not adequately take into account the multiplicity of other factors, both in the organism and in the experimental diet, which obviously condition the results of the experiment"

J and TH GILLMAN²

A NECESSARY requirement for the utilization of dietary proteins and amino acids in growth and maintenance is the availability of all essential nutrients in the body. Deficiencies of vitamins, minerals, or any other essential food elements usually result in cessation of growth, loss of weight and a negative nitrogen balance. Since all the 'essential' food elements are normally supplied by the diets, a discussion of the effect of dietary composition on amino acid utilization would comprise practically the whole territory of intermediary metabolism, which is beyond the scope of this paper.

We will rather discuss more specifically the effect of those food ingredients which are fed simultaneously or within a short interval of time before or after consumption of the proteins.

The sparing effect of dietary fat and carbohydrate on protein utilization was recognized early and was explained by the concept of *isodynamism*. The idea that dietary carbohydrate may have a specific effect on protein utilization in addition to its calorogenic action was often hinted in the early literature concerning the dietotherapy of diabetes. Rose (1), in his fundamental paper on essential amino acids, stressed as early as 1937 the point that, "factors such as the proportion of fat and carbohydrate in the ration may play important roles in determining the

minimum level of a given component," i.e., essential amino acid.

Since that time several investigators indicated the existence of a specific effect of dietary components on the utilization of amino acids and proteins.

In recent studies on the development and possible nutritional improvement of meal patterns, we investigated the importance of time relations in the consumption of different food ingredients. In confirmation of earlier experiments (2), we found that the nitrogen-sparing effect of carbohydrate depended on the time which had elapsed between the feeding of the protein moiety and the carbohydrate moiety of the diet. When sugar had been fed more than four hours before or after the protein meal, no nitrogen-sparing effect could be observed.

In well-fed *adult* rats, the nitrogen-sparing effect could be demonstrated during the first few days only, after which time the nitrogen balance, in spite of subsequent simultaneous feeding of sugar, returned to its original level, probably because of the inability of the adult organism to store abnormally high amounts of protein. Further investigations on *growing* rats as well as on *protein-depleted adult* animals in which increased and prolonged protein demand existed demonstrated that both the growth response in infant animals and the repletion in depleted adults was superior when sugar was given simultaneously with protein, as compared with control animals which received sugar and protein separately, the total caloric intake being identical in both instances. In these latter experiments the nitrogen-sparing action of sugar lasted not only for 2 to 3 days, but for the duration of the experiments, i.e., 14 to 21 days (3). These results indicate that dietary nitrogen-containing

¹ The unpublished experiments discussed in this paper were performed in collaboration with I. El Rawi, E. B. Hagerty and L. E. Geiger. The experiments have been supported by grants from the National Vitamin Foundation, the Williams Waterman Fund of the Research Corporation and by the Van Camp Sea Food Co., Inc.

² In *Human Malnutrition*. New York: Grune and Stratton, 1951.

compounds when fed simultaneously with carbohydrates are retained and utilized for growth and repletion, i.e., they do participate in normal protein metabolism.

Such simultaneously fed sugar or any other dietary ingredient may *a priori* influence the protein utilization at one or all of the three following phases: in the gastrointestinal phase, in the phase of body protein formation and in the metabolic phase.

in growth promotion, had to be investigated. Consequently, *adult* male rats were induced to eat spontaneously definite quantities of either skim milk or a standard casein powder or one of the strained meats. Two, four and six hours later the nitrogen in the stomach and intestinal content was determined. We were surprised to find that the milk protein disappeared much more slowly from the digestive tract than any of the other proteins tested. Our thinking has been

TABLE 1. GASTROINTESTINAL ABSORPTION OF NITROGEN-CONTAINING COMPOUNDS

MATERIAL FED	CONSUMED NITROGEN ABSORBED AFTER				RESIDUE AFTER TWO HOURS	
	2 hr	4 hr	6 hr	8 hr	In stomach	Intestines
	%	%	%	%	%	%
Skim milk, spray dried	18.3	36.9	59.3	79.2	91.5	8.5
Whole milk, spray dried	22.0	53.3	70.3		94.0	6.0
Fish ¹	55.7	71.2	92.2	93.6	80.4	19.6
Pork	36.5	63.6	85.6	92.0	79.7	20.3
Casein	56.6	81.3	99.0		86.0	14.0
Lactalbumin	31.8	66.7	83.8		60.0	40.0
Fish ¹ plus 0.66 gm glucose	27.4		79.0		69.3	30.7
Fish ¹ plus 0.66 gm cellulose	55.7				74.5	25.5
Casein plus 0.6 gm glucose	37.5	73.1	99.0		81.0	19.0
Casein plus 0.8 gm lactose	32.0				93.0	7.0
Casein plus 0.6 gm cottonseed oil	15.8		54.6		78.0	22.0
Casein plus 0.6 gm sucrose	43.0		97.0		88.5	11.5
Casein digest ²	89.4	93.0			1.0	99.0
Casein digest ² plus 0.6 gm glucose	81.6				29.0	71.0
Casein digest ² plus 0.4 gm cottonseed oil	71.4				73.0	27.0
Zein	40.0		46.0		2.0	98.0
Gelatin	72.0				5.0	95.0

The different proteins were fed in quantities containing approximately 100 mg nitrogen to adult male rats, after 24 hours' starvation. Experiments with casein, zein and gelatin indicate that gastric emptying time is dependent on some characteristics of the protein.

¹ Strained tuna

² Spray dried acid hydrolysate fortified with tryptophan

The importance of the gastrointestinal phase in this respect was suggested by some experiments in which the nutritive value of commercial 'strained' meat products was investigated, using as a standard spray-dried skim milk. We found that diets containing skim milk as the only protein source had the strongest growth-promoting effect on infantile rats, the next best effect was obtained with diets containing strained tuna, while strained beef, pork, veal and lamb proved to be less effective.

Since the amino acid inventory of these proteins is nearly identical, the possibility that some differences in the digestibility of the proteins used could account for the observed differences

conditioned by the rather unsubstantiated claim that 'easily digested, easily absorbed' proteins are nutritionally superior to the others. Therefore, the result of this investigation, indicating that the protein source which promoted growth best disappeared most slowly from the digestive tract, was unexpected (see table 1).

We should consider, however, that the growth rate is determined by inherent regulatory factors and is not accelerated by superoptimal supply of the building stones. Tissue protein synthesis, and hence growth, requires the *simultaneous* presence of all the amino acids. Evidently the dietary proteins are best utilized for growth when the rate of supply from the digestive tract corre-

sponds closely with the growth requirements. When the rate is too fast, the utilization will suffer because no excess of amino acids can be stored normally for later use, the surplus is either excreted or irreversibly metabolized (4, 5). Therefore, a delay in the protein digestion and absorption should tend to improve the utilization. Such a mechanism is suggested also by the investigation of Leverton and Gram, as well as Wu and Wu (6, 7). Their experiments on adult human beings indicate that the utilization of dietary protein improves when intake and resorption are evenly distributed throughout the day by consuming more frequent protein meals.

We recently found that lactalbumin, which supports growth to a higher degree than casein, disappears more slowly from the intestinal tract than does casein. This observation seems to favor the outlined ideas.

Experiments prompted by such considerations revealed that simultaneous consumption of sugar does delay the disappearance of protein and absorption of its cleavage products from the digestive tract, suggesting that the delay in the intestinal resorption may well be involved in the nitrogen-sparing action of sugar fed simultaneously.

Further experiments have shown that in accordance with the classic observations of Ewald and Boas (see 8) on gastrointestinal motility, simultaneously fed fat, even more than carbohydrate, considerably delayed the disappearance of protein from the digestive tract.

A similar effect of fat on resorption of another nutrient has been reported by Nief and Deuel (9), who found that the rate of intestinal absorption of galactose varies inversely with the percentage of fat present in the diet, and that fat secures a better utilization of this sugar by preventing 'galactose-flooding' of the organism.

Based on these results, the effect of simultaneous fat feeding on growth has been investigated. One group of infantile male rats received casein from 7 P.M. to 11 P.M. and a protein-free, but otherwise complete, diet from 7 A.M. to 3 P.M. Another group received the fat moiety of the diet mixed to the protein meal. It was found that the rats receiving fat with the protein grew markedly better than the controls, although the total caloric and protein intakes were identical (see fig. 1).

The proper balance between rate of supply and rate of utilization seems to be even more critical in feeding amino acid mixtures than in feeding

protein. Accordingly, the growth-promoting action of carbohydrate and fat is more pronounced when fed simultaneously with a tryptophan supplemented casein hydrolysate.

The experiments discussed so far are in complete agreement with many earlier data. For example, it was reported that rats fed equicaloric diets grew better if the fat content of the diet was increased (10, 11). The protein digestion was improved, the nitrogen retention increased, and the fecal and urinary excretion of some essential amino acids, such as valine, lysine and methionine, was less on diets containing corn oil than on a fat-free diet.

These effects could be explained by the observation that simultaneously fed fat generally delays the intestinal passage and thus improves

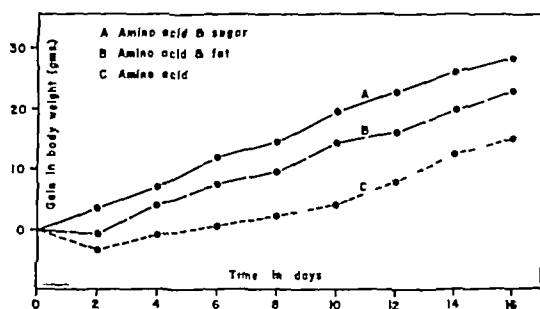


Fig. 1 EXPERIMENTS in which the protein moiety of the diet was fed either separately or together with sugar or fat. Infantile male rats of the Wistar strain were used. Each group contained 8 rats with a starting weight of 50 to 55 gm.

digestion of the protein and the absorption and utilization of amino acids.

This explanation is, however, at variance with the results of some other authors (12, 13), who found that an increase of the fat content of the diets which were kept isocaloric with the control diet promotes growth without affecting nitrogen retention.

Munro has shown, furthermore, that fat, unlike sugar, when fed simultaneously with protein, fails to increase nitrogen retention in adult rats. In view of our experiments with carbohydrate, we investigated the effect of simultaneously fed fat on the repletion of protein-depleted rats and found that, contrary to the effect of sugar, simultaneously fed fat did not increase nitrogen retention.

However, it should be emphasized that our results cannot be compared directly with those of some earlier authors since the daily fat intake in our experiments was not changed—the same

amount of fat was fed either apart from or together with the protein moiety of the diet.

We believe that in addition to its action on gastrointestinal resorption, fat also exerts an effect on protein utilization at some other level. It has been reported (10, 11) that animals kept on protein-free, isocaloric diets, lose less weight if the fat intake is kept high. Evidently the high fat intake decreases also the catabolic destruction of body tissue. Furthermore it was reported that the damaging effects of excessive methionine doses can be antagonized by increasing the fat content of the diet.

These experiments would indicate that although simultaneous fat intake improves protein utilization by retarding the rate of amino acid supply from the digestive tract, its total effect must be much more complex.

Returning to the protein-sparing action of carbohydrates, it was found that although the retardation of absorption is smaller than in the case of fat, the growth promotion and the nitrogen-sparing effect are much more pronounced when carbohydrate is fed simultaneously with protein instead of fat. This indicates that the effect on absorption is only a minor factor involved in the protein-sparing action of sugar.

Following the fate of food constituents after transfer from the digestive tract to the body proper, they may affect the utilization of the absorbed amino acids in the process of tissue protein synthesis. Since protein synthesis requires the simultaneous presence of all the constituent amino acids, the most obvious effect of sugar seems to be the provision of precursors for the formation of missing nonessential amino acids. This possibility was investigated in collaboration with Dr. Arne Wick (14) by feeding C^{14} -labeled sugar and ammonium phosphate to growing or protein-depleted rats, together with or apart from Rose's mixture of essential amino acids. It was to be expected that if the sugar is utilized for amino acid formation and thus supports the protein utilization, more labeled material will be taken up by newly formed or repleted tissue. The results obtained on normal adult rats do not substantiate this hypothesis, although the error of the procedure may still mask possible differences. Experiments are now in progress in which the uptake of labeled carbon by the protein formed during liver or plasma regeneration is investigated. These recent studies show that protein isolated from the regenerating liver tissue contains considerably more C^{14} when

sugar and essential amino acids are fed together. This supports the hypothesis that simultaneously fed sugar promotes protein synthesis by providing the necessary precursors for the synthesis of nonessential amino acids.

Recently published experiments (15) in which more nitrogen was retained by the body if ammonium salts were fed together with sugar than without sugar also seem to indicate that simultaneously fed sugar may expedite the utilization of nitrogen used for amino acid synthesis.

The conditions are still more complicated in growth where the formation of new tissue requires probably, besides the necessary amino acids, also the simultaneous availability of other cell constituents. How far a fast-growing organism can draw on reserves of such factors as minerals, etc., if they are missing from the diet has not yet been investigated.

That some of the nutrients such as vitamins once deposited in the tissues are no longer easily available can be demonstrated by feeding to vitamin-depleted rats tissues of their equally depleted litter mates. The result is usually a dramatic improvement. We therefore investigated whether growth of rats is influenced when the water-soluble vitamins are fed simultaneously with or apart from the protein-containing diet and found that in some experiments the growth was definitely improved when the vitamins were fed simultaneously with the diet, while in others there was no apparent difference. However, the rats receiving the vitamins separately from the diet never grew better than the others. For all practical purposes the growth was the same on meals containing vitamins or with later vitamin supplementation.

That the feeding of some minerals in proper concentration may improve the utilization of dietary proteins has been recently proposed by McQuarrie and Ziegler (16). These authors reported that by balancing the phosphorus and calcium content of the diet the biological value of meat products can be raised to that of milk. We repeated the latter experiments, adjusting the calcium and phosphorus content of all the diets to values equal to the milk diet. In spite of this, the efficacy of the diets was the same as in the control experiments. The growth on milk was best, then strained tuna, beef, followed in order (fig. 2).

We are still at a loss to explain the reason for the differences between the results of the Minneapolis group and ourselves. However, our experi-

ments show that equalizing the P, Ca, Mg and K content in itself does not raise the biological value of the meat diets to that of the diet containing milk protein

How the intake of precursors or of substances promoting the formation of cell constituents such as lipids, certain carbohydrates and nucleoproteins influences the amino acid utilization has yet to be investigated. One perhaps may object that the organism with intact lipo- and glyconeogenic faculties is able to form such compounds itself and does not depend on dietary supply. Such objections usually result from a misinterpretation of Schoenheimer's fundamental concept of 'dynamic equilibrium' and from the

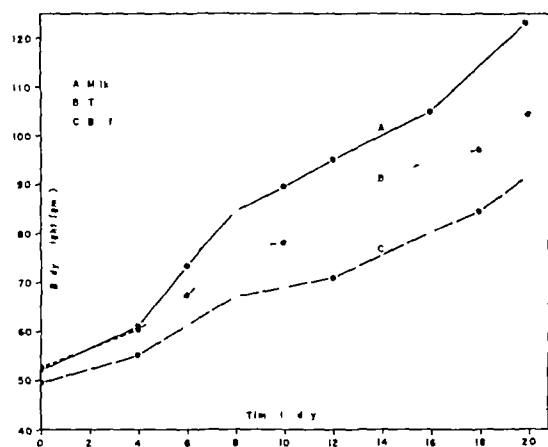


Fig 2 GROWTH EXPERIMENTS with diets containing equal amounts of calcium, phosphorus, magnesium and potassium. Each group contained 6 male rats of the Long Evans strain

indiscriminate use of the term 'metabolic pool'. It has been, therefore, assumed erroneously that it is irrelevant whether substances like sugar have been produced in the intermediary metabolism or were provided with the diet. In the first place, it is questionable whether or not such substances produced in the body can be supplied at a rate optimal for tissue synthesis.

Evidently a substance carried in high concentration by the portal system from the digestive tract to the liver has a different effect and fate than when produced in the tissues. For instance, absorption of dietary sugar increases the insulin production and also activates the anterior pituitary gland (17, 18). It has been shown that both hormones improve protein utilization (19). We investigated whether such increased hormone production could be held responsible for the nitrogen-sparing effect of simul-

taneously fed sugar. In these experiments diabetic as well as hypophysectomized animals reacted like normal rats so that the Langerhans islands and the anterior pituitary play no major role in the nitrogen-sparing action of sugar.

The third point at which dietary constituents may exert their influence on protein utilization is the 'metabolic phase,' comprising formation, destruction and transformation of amino acids.

A specific action of fat on the utilization of cystine was reported by Salmon (20) who also found that the nicotinic acid requirement is decreased in fat-rich diets. This latter effect may spare some tryptophan and divert it to other metabolic pathways.

The problem in which we are particularly interested at the present time is how sugar may exert its action on amino acid utilization in this metabolic phase. One possibility under investigation is that the simultaneous resorption of sugar protects the circulating amino acids from premature deamination in the liver. This possibility is supported by the experiments of Krebs (21) who found that the deamination by liver slices *in vitro* is decreased in the presence of easily oxidizable material such as lactate, pyruvate, etc. The decreased specific dynamic action of protein when fed simultaneously with sugar also suggests protection against catabolic changes.

It is likewise possible that some preliminary steps for protein synthesis, such as acetylation or phosphorylation is promoted by simultaneously fed sugar. This hypothesis is supported by Miller's (22) experiments in which it was found that the uptake of labeled lysine during liver perfusion is promoted by sugar.

SUMMARY

We tried to demonstrate that dietary substances fed together with proteins do promote growth and utilization of the dietary amino acids, quite apart from their calorogenic sparing action. Of course, some of our conclusions are still open to objections and will have to be supported by further experiments. Especially, the effect of parenteral administration of fat and sugar will have to be investigated.

We believe that besides the effect on intermediary metabolism, special attention should be given to the effect of food ingredients on gastrointestinal dynamics, including motility, gastric emptying time, as well as the effect on the production of bile and other digestive juices.

and, finally, on their influence on rate of digestion and absorption

We have discussed mainly factors which promote protein utilization but it is known that some ingredients such as cocoa (23) may interfere with protein digestion and with the efficiency of its utilization

The possibility should not be overlooked either that some effect may be produced by the food ingredients tested indirectly by influencing the bacterial flora (24)

We did not discuss the mutual effect of different dietary proteins and amino acids on their

utilization, although the phenomena of supplementation and amino acid imbalance may also be influenced by the presence of other factors in the meal

It is unknown how far the reported findings on animals are applicable to human nutrition. Therefore we refrain from drawing practical conclusions at the present time. We are convinced, however, that the indicated lines of study may eventually lead to a scientific planning of meal patterns with an optimum utilization of available protein resources

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INTERPRETATION OF NITROGEN BALANCE DATA

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RECENTLY, Mitchell (1) has presented an excellent outline of protein metabolism which is based on Folin's (2) concept of two distinct types of metabolism but includes later developments, particularly those of Whipple (3) and Schoenheimer (4) and their associates. This outline accepts the principle that endogenous metabolism represents irreversible reactions involving tissue proteins and other nitrogenous constituents as typified by the dehydration of creatin to creatinine. Exogenous catabolism could be defined as catabolism of nitrogenous constituents in amounts exceeding the needs of the animal for endogenous maintenance and growth. Dietary and body nitrogen may be said to meet in a common metabolic pool of blood and tissue proteins, a pool that can be drawn upon for either exogenous or endogenous purposes. Exogenous catabolism could include, therefore, the utilization of dispensable stores of body proteins, stores which have been described as being raided for specific amino acids not supplied in the diet (3, 5, 6).

The depletion of body protein stores is reflected by a decrease in excretion of urinary nitrogen. When an animal is placed on a protein-free diet, urinary nitrogen excretion decreases, rapidly at first, then more and more slowly. This initial rapid loss of nitrogen represents the catabolism in part of labile protein stores. After these stores are depleted the loss of nitrogen is less rapid, urinary nitrogen excretion approaching a low and constant value (fig. 1). It is interesting to note that, during regeneration, accomplished by feeding protein, those nitrogen stores that are depleted last are probably refilled first. These results suggest that the stores depleted last are more essential and are replenished before those which are dynamic and dispensable (7).

A better understanding of nitrogen stores can be obtained, however, from nitrogen balance than from urinary nitrogen excretion. Nitrogen balance (B) is the difference between dietary nitrogen intake (I) and nitrogen excreted in the feces (F) and urine (U) so that

$$B = I - (F + U) \quad (1)$$

The animal is gaining nitrogen when B is positive, it is losing nitrogen when B is negative, and it is in nitrogen equilibrium when B is zero.

The relationship between nitrogen balance and nitrogen stores is illustrated in figure 2. These results were obtained by feeding alternately a protein-free diet and one containing protein to a dog (8). The white bars record the nitrogen balance during the protein-free period, while the bars with slanted lines represent the balance while the animal was receiving a constant nitrogen intake. The magnitude of the protein stores of this dog was average, resulting in a negative nitrogen balance of -2.4 gm of nitrogen/day/m² of body surface area (first white bar). Protein was added to the diet, but not sufficient protein to put the dog in nitrogen equilibrium. Continued negative nitrogen balance resulted in further reduction in the catabolism of body stores so that, when the animal was returned to the protein-free diet (second white bar), the urinary nitrogen excretion was reduced still further and the nitrogen balance was less negative. Continuation of this type of alternate feeding resulted eventually in depletion of the protein stores to such an extent that the amount of nitrogen being fed produced a positive balance. Thus, nitrogen balance is a variable, changing with time as the protein stores are increased or decreased.

The relationship between nitrogen intake and balance is illustrated by the curves in figure 3. It is assumed that the data for these curves were determined over a short period of time, so that nitrogen balance was not altered significantly by continued feeding of nitrogen. Curve A was obtained by feeding whole egg protein to a dog depleted in protein stores. The low stores are demonstrated by the relatively low negative balance of -1 gm of nitrogen/day/m² of body surface area. A small amount of dietary nitrogen was required to maintain nitrogen equilibrium in this animal. The curve extends far into the region of positive balance, showing marked capability on the part of the animal to fill depleted stores. A maximum was reached at approximately

+6 gm of nitrogen/day/m² surface area. Curve C illustrates data obtained while feeding the same protein to a dog with full protein stores. The fullness of the stores is reflected by the balance of -4 gm/day/m² surface area and by the relatively large quantity of dietary nitrogen necessary to maintain nitrogen equilibrium. Since the stores of this dog are essentially full, only a slight positive nitrogen balance was produced. The degree of depletion of the stores in an animal can be estimated by the magnitude of positive balance which is produced by a given nitrogen intake.

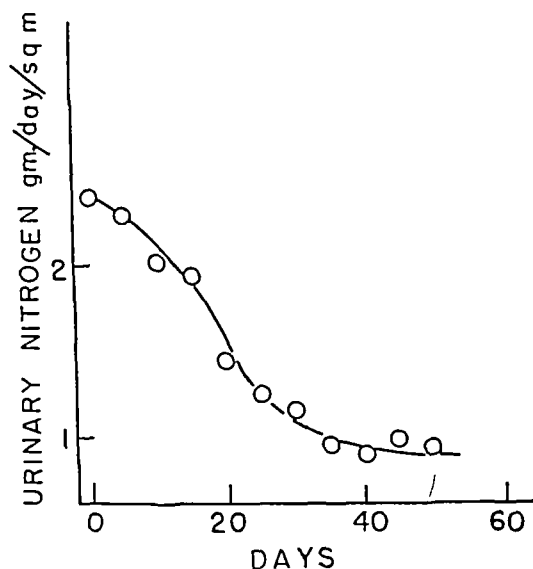


Fig 1 URINARY NITROGEN EXCRETION (gm/day/m² body surface area) in a dog fed a protein-free diet for 50 days

Curves B and D represent data obtained while feeding wheat gluten instead of whole egg protein. The rate of increase of nitrogen balance with nitrogen intake was less with wheat gluten than with whole egg in the diet, and the maximum positive balance was also less. The nutritive value of the dietary proteins can be measured in terms of the slopes of the lines in figure 3, and by the maximum positive balance which can be produced.

The curves in figure 3 are essentially linear until the point of maximum utilization is reached. The relationship, therefore, between nitrogen balance (B) and absorbed nitrogen (I) can be expressed by the following equation

$$B = KI + B_0 \quad (2)$$

where K is the slope of the line and B_0 is the

balance when the nitrogen intake is zero. This equation may be rewritten to eliminate nitrogen balance as follows

$$U = (I - K) I + U_0 \quad (3)$$

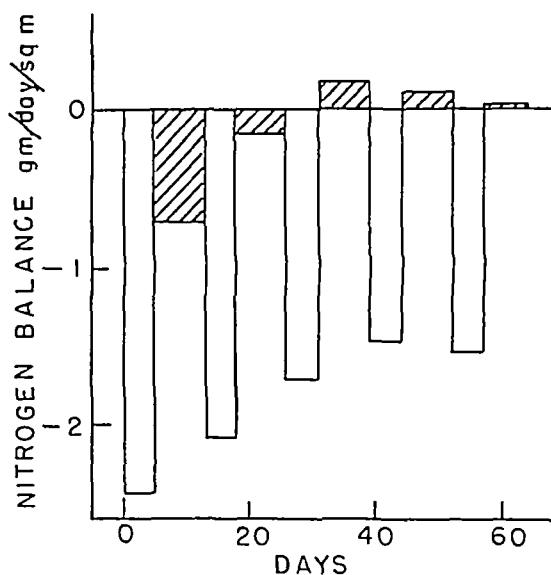


Fig 2 NITROGEN BALANCE (gm/day/m² body surface area) in a dog fed a protein-free diet (white bars) alternately with diet containing 0.9 gm of egg white nitrogen/day/kg body weight (bars with slanted lines)

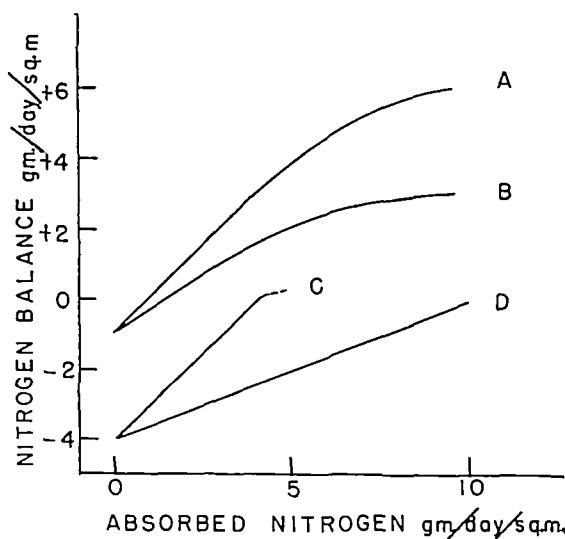


Fig 3 NITROGEN BALANCE vs absorbed nitrogen in a protein-depleted dog fed whole egg protein (A), in a protein-depleted dog fed wheat gluten (B), in a normal dog fed whole egg (C) and in a normal dog fed wheat gluten (D)

where U is the excretion of urinary nitrogen during nitrogen feeding and U_0 is the excretion of urinary nitrogen on a protein-free diet (9). It

has been proven (9, 10) that K in these equations is the fraction of absorbed nitrogen retained in the body of the animal, provided the excretion of body nitrogen (B_0 or U_0) is constant over the whole range of nitrogen intakes. The fraction of absorbed nitrogen retained in the body is by definition the 'biological value' (11) of the protein. The slope of *line D* is 0.4. If body nitrogen excretion is constant, then 40 per cent of the wheat gluten nitrogen was retained in the body of the animal. It was pointed out in the introduction, however, that dietary and body nitrogen may enter a common pool which is available for catabolism as well as anabolism. It is not surprising, therefore, to find evidence that feeding nitrogen may decrease or increase the contribution of body nitrogen to catabolism.

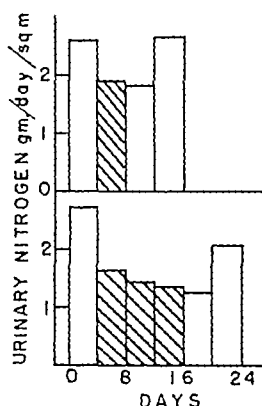


Fig 4 AVERAGE DATA obtained on dogs fed protein-free diet (*white bars*) and protein-free diet plus 1 gm of *dl*-methionine per day per dog (*bars with slanted lines*)

The conservation of body nitrogen by dietary nitrogen has been demonstrated by feeding methionine to dogs (12-14) and to rats (15). The excretion of urinary nitrogen in the dog is decreased by adding a small amount of methionine to a protein-free diet (fig 4). Less body nitrogen is contributed to the catabolic pool in the presence than in the absence of methionine. It has been suggested (16) that the demands for methionine in the dog are relatively great so that body protein reserves are utilized to supply this amino acid, the excess catabolism of nitrogenous constituents thereby contributing to an increased excretion of urinary nitrogen. Mitchell (1) has suggested that the extra demand for methionine is the result of the growth of hair at the expense of other tissues of the body, the hair demanding large amounts of sulfur amino acids. Addition of

an optimal amount of methionine to some proteins such as casein can increase the value of K in *equation 2* to values over unity. Values over unity would be impossible if B_0 in the equation represented the excretion of body nitrogen at all nitrogen intakes. Values over unity can be interpreted to mean that feeding nitrogen reduces catabolism of the nitrogenous constituents in the metabolic pool. Swanson and coworkers (14) have given several other examples of the nitrogen-sparing action of dietary nitrogen in the rat.

Dietary nitrogen may also increase the utilization of the nitrogen in the body pool. A large amount of methionine (5%) in a diet containing casein when fed to the rat increases the catabolism of these stores (17). The data suggest, too, that the metabolism of excess methionine requires the simultaneous catabolism of fat (18) and of the utilization of the amino acid glycine (19). Excess methionine may draw on the nitrogen pool for glycine, thereby causing increased catabolism of other amino acids in the pool. Addition of glycine together with methionine reduces this excess catabolism. The beneficial effects of glycine may be due to the participation of this amino acid in the catabolism of methionine, glycine being the precursor of serine. One pathway for the metabolism of methionine is by the way of a combination of homocysteine and serine to form cystathionine (20-22). Other examples of raiding of nitrogen stores have been suggested by the work of Whipple and associates (5, 6). They have found that blood proteins can take priority over other tissue proteins, that depleted dogs will continue to produce much plasma protein and hemoglobin for many weeks while being fed a low nitrogen diet. An inadequate protein-like globin, or an inadequate mixture of amino acids can result in formation of blood proteins, other tissue proteins possibly being depleted to supply the deficient amino acids (23).

Since K in *equations 2* and *3* need not be equal to 'biological value,' this constant has been called the nitrogen balance index of the dietary protein (24). The index is a function of the fraction of nitrogen retained in the body of the animal. It is more specifically the rate of change of nitrogen balance with respect to nitrogen intake, and it is a relative measure of the amount of nitrogen necessary to maintain equilibrium, a measure of the over-all utilization of nitrogen by the animal.

Table 1 records nitrogen balance indexes for six standard proteins or protein foods in adult dogs. These data were obtained from experiments which were a part of a cooperative study of protein evaluation sponsored by the Bureau of Biological Research of Rutgers University. The effects of supplementing casein with methionine and wheat gluten with lysine on the nitrogen balance indexes are illustrated in the table.

The index for whole egg protein in adult dogs is usually found to be equal to or better than egg white. The relatively low index for whole egg protein recorded in table 1 is believed to be associated with changes produced during processing and drying of this particular whole egg preparation. It is interesting to compare these indexes

with those obtained in other studies. Variations in technique and in the physiological state of the animal. Some of these variations will be mentioned in the following discussion.

The index for raw egg white was found to be abnormally low in one so-called normal dog. This low value was associated with poor digestibility which could be attributed to the antitryptic factor in raw egg white. Poor digestibility of raw egg white was found also in most dogs depleted in protein stores (7). Morgan *et al* (25, 26) have found the dog, particularly the puppy, to be vulnerable to this antitryptic activity of egg white.

Nitrogen balance indexes all tend to increase when the protein stores of the dog are depleted markedly. Indexes, for example, in protein-de-

TABLE 1 AVERAGE DATA OBTAINED ON SIX DOGS

PROTEIN SOURCE	ABSORBED NITROGEN I	URINARY NITROGEN U	PROTEIN EFF. URINARY NITROGEN U ₀	NITROGEN BALANCE INDEX E
	<i>gm/day/m²</i>			
Casein + methionine	3.3	2.02	2.60	1.17 ¹
Egg white	1.79	1.69	1.94	1.14
Whole egg	1.73	2.53	2.30	0.87
Beef muscle	3.02	2.75	2.05	0.77
Casein	3.10	2.74	1.90	0.73
Wheat gluten + lysine	2.04	3.02	2.44	0.73(7)
Peanut flour	4.22	3.74	1.89	0.56
Wheat gluten	3.01	3.09	1.11	0.44

The nitrogen balance indexes were calculated from equation 3 in the text.

¹ Average on 3 dogs.

with some determined several years ago on different samples. These earlier determinations yielded the following indexes: casein plus methionine, 1.2; egg white, 1.0; beef, 0.85; casein, 0.80; and wheat gluten, 0.44 (8). The casein with the index of 0.80 was a cruder sample than the one with the index of 0.73 (table 1) and probably had a higher methionine content. The beef sample with the index of 0.85 was selected from the best cuts of meat while the sample listed in table 1 was representative of all types of beef protein. These indexes for beef in the adult dog are much higher than values reported by Morgan *et al* (25), a difference which emphasizes the susceptibility of the nitrogen balance method to varia-

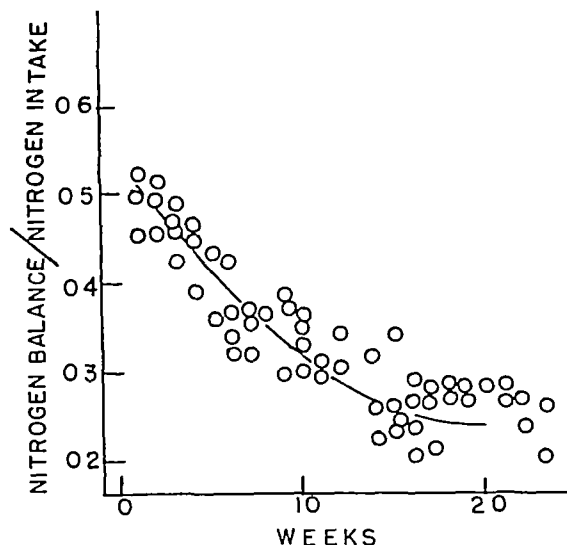


Fig. 5 NITROGEN BALANCE/nitrogen intake in puppies fed 25 per cent casein diet beginning at time of weaning (8 weeks).

pleted dogs were found to be as follows: egg white, 1.12; whole egg, 1.06; casein, 0.84; and wheat gluten, 0.70 (7). In general, however, indexes are essentially constant and independent of the magnitude of the protein stores until those stores have been depleted markedly. Nitrogen balances, on the other hand, vary with time, the animal always drifting toward equilibrium if the balance is either negative or positive. In this way the stores are depleted or repleted to a point of equilibrium if the balance is either negative or positive. In a growing animal, fed an adequate protein, the positive nitrogen balance per gram of nitrogen intake decreases rapidly as the animal approaches adulthood, a decrease which is illustrated in figure 5. Nitrogen balances per gram

of nitrogen intake have been found to have greater significance as measures of protein utilization in the growing dog than the customary protein efficiency (grams gain in weight/gram of nitrogen intake) The data in table 2 compare nitrogen balance with body weight gain data for the evaluation of dietary proteins in beagle puppies These data were calculated over a period of

TABLE 2 AVERAGE INCREASE IN BODY WEIGHT PER GRAM OF NITROGEN INTAKE BW/I AND AVERAGE POSITIVE NITROGEN BALANCE PER GRAM OF NITROGEN INTAKE I/B IN THREE BEAGLE PUPPIES DURING FAST-GROWING PERIODS (15)

PROTEIN SOURCE	$\frac{B}{I}$	$\frac{I}{B}$
Whole egg	9.0	0.50
Casein	9.8	0.43
Wheat gluten	8.8	0.18

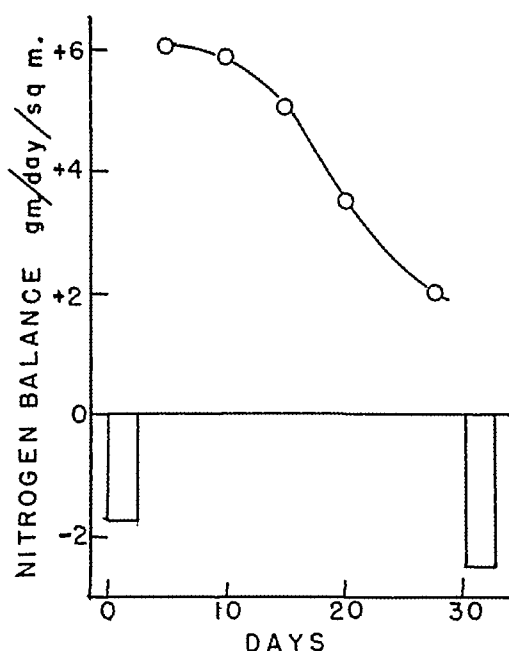


Fig 6 White bars represent nitrogen balances in a dog fed a protein-free diet before and after repletion. Circles represent positive nitrogen balances produced while repleting with a constant intake of whole egg protein

several weeks of the fast-growing period of the animal

These data demonstrate that weight gains are not as accurate a measure of the utilization of a protein as nitrogen balance. The puppies fed wheat gluten increased their caloric intakes above those receiving whole egg. They became fat and soft, while those fed whole egg became lean and hard, but both sets of dogs weighed about the

same after 70 days on the respective diets. Growth of the protein stores, however, was much greater in the dogs fed whole egg than in those receiving wheat gluten.

Positive nitrogen balances decrease in depleted adult dogs fed a constant nitrogen intake. This gradual drop in nitrogen balance is illustrated in figure 6. The first bar in negative balance records the excretion of nitrogen on a protein-free diet. The gradual drop in positive balance during the protein-feeding period can be interpreted to be a reflection of increasing protein stores. The increased catabolism of these stores is illustrated by the greater negative balance obtained while feeding a protein-free diet following the repletion period (second white bar). Similar experiments using wheat gluten have demonstrated that, even after prolonged feeding of this protein, the balance on a protein-free diet is often not made more negative, but may even be more positive. These results suggest that wheat gluten may deplete some protein stores to aid in the repletion of others (7).

The proteins of the body and nitrogen balance can be effected also by the caloric intake (27). Recent experiments in dogs demonstrate that the nitrogen balance decreases regularly as the caloric intake decreases. Data in table 3 were obtained on dogs receiving a constant nitrogen intake of 3.82 gm of casein nitrogen/day/m² of body surface area. As the caloric intake was reduced, the nitrogen balance decreased from +0.68 to -0.74. Measurements of nitrogen balance were taken over a short period of time, so that the protein stores of the body were not markedly altered by prolonged feeding. Other experiments (26) demonstrated that the nitrogen balance index was not altered initially by the decrease in calories. Curves such as those illustrated in figure 3 were shifted downward farther into the region of negative balance but without altering their slopes. The values for B_0 in table 3 illustrate this shift into negative balance. These values were calculated using an average index of 0.73 for casein determined over short feeding periods for all caloric intakes except the very lowest of 95 calories/day/m². These results suggest that restriction in calories increases the catabolism of exogenous reserves of both body and dietary nitrogen. The increased catabolism, however, does not alter at first the mechanism of filling these stores as measured by the nitrogen balance index. When the more labile body stores are re-

duced, however, the index decreases rapidly, essential tissues are depleted, and irreversible damage may be done if the caloric restriction is continued. It has been demonstrated that animals with adequate body protein reserves can resist the depleting effects of a caloric restriction over long periods of time while those with inadequate stores rapidly deteriorate. The rapid deterioration is illustrated by *curve A* in figure 7. *Curve A* represents data obtained on a dog partially depleted in protein so that 3.82 gm of casein nitrogen/day/m² and 480 calories/day/m² produced a positive nitrogen balance. In the absence of abundant labile protein stores, the index was immediately reduced and the ani-

was always associated with the drift into negative balance following a restriction in calories. It should be emphasized that these data were obtained on dogs being fed a low fat diet containing a quantity of casein which maintains the average dog on an adequate caloric intake in nitrogen equilibrium. The response to a caloric restriction is a function of the balance between dietary constituents as well as of the physiological state of the animal. Further evidence for the importance of the balance between calories and proteins is found in the work of Schwimmer and McGavack (28), who demonstrated that young men fed a low nitrogen intake (3

TABLE 3 AVERAGE VALUES FOR CALORIC INTAKE AND NITROGEN BALANCE (B) FOR THREE DOGS FED 3.82 GM CASEIN NITROGEN/DAY/M² OF BODY SURFACE AREA

CALORIES/DAY/M ²	NITROGEN BALANCE B GM/DAY/M ²	PROTEIN FREE NITROGEN BALANCE (CALCULATED) B ₀ GM/DAY/M ²
3190	+0.68	2.10
2860	+0.18	2.60
2290	+0.13	2.65
1910	-0.10	2.88
480	-0.22	3.00
95	-0.74	

Protein-free nitrogen balances (B₀) were calculated assuming an index of 0.73 (27).

mal drifted rapidly into negative balance. Nitrogen integrity of the body could not be maintained in this animal at a low caloric intake even with nitrogen in the diet. *Curve B* represents data obtained on another dog with large protein stores, so that 3.82 gm of casein nitrogen/day/m² and 480 calories/day/m² were not sufficient to put this animal into positive balance. Prolonged feeding depleted these stores, the dog approached nitrogen equilibrium, but because of the low caloric intake eventually drifted into a period of rapid loss of nitrogen. Thus *dog B* resisted the damaging effects of caloric restriction over a longer period of time than *dog A*. Indeed, until *dog B* began to drift rapidly into negative balance, this animal was alert and seemed to be in good clinical condition. Loss of interest in daily routine, irritability and marked weakness

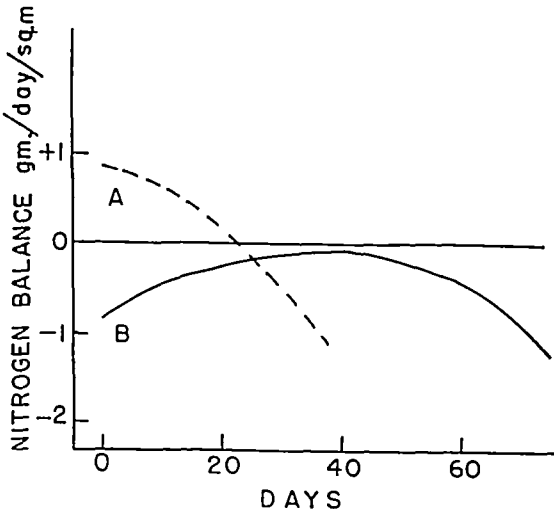


Fig 7 NITROGEN BALANCE (gm/day/m²) vs days of feeding a reduced caloric intake (480 calories/day/m²) and a constant nitrogen intake of 3.82 gm casein nitrogen/day/m². *Dog A* was partially depleted in protein stores at the beginning of the experiment, *dog B* had adequate stores.

gm egg white nitrogen/day) did not retain dietary nitrogen until the caloric intake had been increased to 1500 calories. If the intake was increased to 6 gm/day, the nitrogen was retained at a much lower caloric level (900 calories). Bosshardt *et al* (29) demonstrated that an increase in calories at a constant protein level improved the utilization of protein, which was enhanced still further by the addition of more protein. The authors suggest that protein is the most important limiting factor in many states of semi-starvation.

Data in the literature suggest that fat in the diet exerts a role over and beyond the provision of calories, possibly through an effect on protein metabolism. Swanson and collaborators (30),

studying the effect of nitrogen excretion in low nitrogen diets, reported that "the catabolism of rats, ingesting isocaloric quantities of high fat and low fat rations, proceeded at essentially the same rate. However, upon adjustment of the energy value of the diet to one-fourth of the customary caloric consumption, the elimination of fat doubled the destruction of body tissue." Schwimmer and McGavack (28) found that a diet of 900 calories, containing 30 per cent fat and 6.0 gm of nitrogen daily, decreased the urinary excretion of nitrogen in men below the excretion when the diets provided 10 and 20 per cent fat. They believed that "the nitrogen-sparing effect of 30 per cent fat was not due to increased calories, but rather to something intrinsic in the higher fat per se."

The balance between protein and calories is very important for the maintenance of normal tissues. Diets, for example, which are relatively high in calories but low in quantity and quality of protein will deplete the dispensable protein reserves of the animal, produce a fatty liver and fill the fat depots of the body. Rats, for example, fed 120 calories of a protein-free diet per kg body wt developed livers with an average of 1.1 gm of protein and 57 per cent fat. Littermates fed the same caloric intake but with 10 per cent protein developed livers with an average of 1.86 gm of protein and 28 per cent fat. Increasing the calories in the absence of protein may indeed have little conserving effect upon

body proteins, but it can have a marked conserving effect in the presence of protein. The total liver protein was increased from 1.27 to 2.30 gm as the calories were increased from 50 to 200 gm/kg body wt in animals fed a constant protein intake.

Depletion of the protein stores of the body, either through lack of calories or lack of protein or both, results in an imbalance of tissue proteins and enzyme systems which can alter the physiology of the animal markedly. The plasma albumin, for example, is depleted rapidly, but plasma alpha globulins are not (31). The succinoxidase system of the tricarboxylic acid cycle is reduced in activity, while the cytochrome oxidase system is not (32). Thus, there can be a differential depletion and repletion of many types of protein systems depending upon the physiological state of the animal and the diet. The nitrogen balance method, however, does not describe these shifts between the various protein compartments of the body, but rather measures the overall status of nitrogen retention in the animal. Nitrogen balance is the sum of the gains and losses of nitrogen from the various compartments of the body. It is possible for an animal to be in positive nitrogen balance and yet be depleting some labile stores in protein. Nitrogen balance, like body weight or growth, is the summation of many variables and the significance of the balance becomes more meaningful as those variables are evaluated.

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FEDERATION PLACEMENT SERVICE

IN THE portion of the *History of the American Physiological Society*, written by Dr Charles W Greene and published in 1938 on the occasion of the semicentennial of the Society, it is recorded that as early as 1919 there was "ever-increasing discussion among Federation membership concerning the difficulties of young scientists in securing permanent positions" and reference is made to a proposed solution offered by Dr Samuel F Meltzer, a former President of the Society, to organize a "scientific information service" for which he contributed \$300 of personal funds for the expense involved for the ensuing year. This proposal was then referred to the Federation, where it was adopted as a project, the Treasurer of the Physiological Society was made custodian of the funds and Dr E E Brown of the University of Minnesota was appointed Director. As far as can be learned, Dr Brown remained in charge of the Placement Service until sometime during the 1920's, when Dr C W Edmonds assumed the duties. In 1931 Dr H B Lewis of the Department of Biochemistry of the University of Michigan became the Director, giving generously of his time and interest to the project for over fifteen years. Small sums were made available each year by assessments on the constituent Societies to cover postage and stationery, and the use of a room at the Annual Federation Meeting was arranged in 1941. Dr Lewis continued to serve until 1946, when he asked to be relieved but agreed to serve another year.

In 1947 a committee headed by Dr Philip Dow made a study of the Placement Service and recommended that 1) it be continued as a function of the Federation, 2) it be operated by the Office of the Federation Secretary, and 3) an annual fee of one dollar be paid by applicants, with the expectation that this would pay a secretary one-fourth time. This sum was soon inadequate to meet the expenses, for by 1948 they were already estimated at \$1,300 and the Federation continued to provide the funds. When Dr Milton O Lee became Federation Secretary in 1947 the records of the Placement Service were moved to Washington to the new permanent office, and all subsequent activities have been under his direction.

From these available records it is evident that the original Placement Service of the Federation of American Societies for Experimental Biology was an outgrowth of the informal placement activity of every teacher of graduate students, to whom employers and colleagues frequently write seeking likely persons to fill available positions. For more senior positions those searching for the best available candidate may gather the opinions and recommendations from a number of individuals already leaders in that particular discipline. Such methods remain extremely satisfactory where they can function effectively, but since the field of experimental biology has become extensive, the employers of experimental biologists diverse and numerous, and the number of academic departments contributing to it so large, a central source for exchange of information under the auspices of the professional societies in that field has come to meet a real need that can no longer be effectively met by unorganized efforts.

In addition, it has been convenient for candidates for positions and employers seeking personnel to meet at the Annual Spring Meetings of the Federation. Hence the Placement Service has attempted to facilitate and expedite activities already occurring, by providing a central source of information for both employers and candidates to be available at all times, and a scheduled meeting place for them at the Annual Meetings, with information of the preparation, experience, and interests of each candidate present, in a brief and standardized form which is available to all employers.

During the last few years these activities have expanded to meet the increasing needs as they have become evident. Its main function has continued to be to serve as a clearing house of factual information for both candidates and employers, with no attempt to handle references or to recommend or evaluate candidates or positions. Originally, annual lists and monthly supplements of applicants with a brief description of their qualifications and experience were sent to all employers who registered positions or who wished to be kept on the mailing list, and interviews were arranged at the annual meetings by the Director, still on a rather informal basis with very few facilities. In 1950

at the Atlantic City Meeting a new system of scheduling interviews was used with a generous floor space allotted to the Service for offices and interview booths and a staff of about twelve assistants. This proved very satisfactory and was successfully repeated in 1951 at Cleveland with a few modifications and a slightly enlarged staff.

Meanwhile, as the number of applicants increased, the method of issuing the lists of available personnel was revised. In the summer of 1950 a questionnaire was sent to all members of the American Physiological Society asking the kind of information they wished in these lists and the intervals at which it was desirable to receive them. From the returns to this questionnaire it appeared that a schedule of quarterly lists of available personnel, semiannual lists of available positions, and an annual list of available fellowships was considered by the majority to be the most useful. Such a schedule has been adopted for this year with the quarterly lists of candidates prepared in February, May, August, and November, and the lists of available positions issued in March and September. The list of fellowships was issued in April of this year but probably would best be issued in the autumn on an annual basis. There have been many requests for these new lists of available positions and fellowships.

Detailed application forms of all applicants on the list are available for study upon request by an interested employer. In addition, there are on file the application forms of a few individuals, usually with some years of additional experience, who are available but do not wish their names circulated. Obviously as there are no facilities for work with individuals, the mechanism of the circularized lists is of no help in these instances, but the information so filed has been of considerable aid to the Director in making suggestions when he has been consulted in the filling of positions requiring this additional background. In the same way a number of positions are described for the files with the request that they not be included in the publicized list. These employers prefer to retain the initiative in approaching candidates. These procedures can be retained within the framework of the present activities, but it is to be emphasized that the Service can function most effectively where there is a free interchange of information.

During the past year an addressograph-plate mailing list has been assembled of heads of

academic departments and laboratories hiring and/or training personnel in the fields of the member Societies and the circularized lists have routinely been sent to them, permitting the availability of both positions and personnel to reach a wide audience among potential candidates and employers even if they have not registered with the Service.

Such an activity becomes more expensive as its responsibilities increase and, while its value and standards can best be continued and developed under the direction of the Federation, it seems equitable that the cost should be shared by those who use it. As a step to accomplishing this, at the Cleveland Meeting in April 1951 the Executive Committee of the Federation set up a new schedule of rates, consisting of a three-

TABLE 1. APPLICANTS AND POSITIONS REGISTERED, MARCH 1950-MARCH 1951

	APPLI CANTS	% TOTAL	POSI TIONS	% TOTAL
Physiology	173	38.3	177	34.8
Biochemistry	214	47.3	186	36.6
Pharmacology	38	8.4	85	16.7
Pathology	7	1.6	13	2.4
Nutrition	10	2.2	21	4.2
Immunology	2	.4	24	4.7
Miscellaneous	8	1.8	3	.6
Totals	452		509	

dollar annual registration fee for candidates, and a ten-dollar subscription fee for all lists, with a five-dollar fee for any single copy. To be eligible to use the interview service at the Annual Spring Meeting an employer must be an annual subscriber to the lists or to the then current February list of applicants. It is estimated that this will provide an income to meet approximately half the cost of the Service, with the Federation supplying the remainder from its general funds. Thus the Federation will continue to direct the Service, which would be available to its membership and to non-members on much the same basis as in the past. From an over-all viewpoint the importance of this service to non-members should not be overlooked, for while it is recognized that the Service is most useful to young people just receiving their doctorate or possibly with a few years' experience, most of whom are not yet eligible for membership in the constituent Societies, the opportunity

these candidates have for performing significant research in the following few years may well determine whether or not they will become useful members of the Federation. It follows then that the Federation may thus perform a very worthwhile function not only for the individuals concerned, but ultimately for the member Societies and for the fields of scientific endeavor they represent.

A review of the records of the Placement Service especially for the past year is of interest in indicating its present and possible future usefulness, and a summary of the registrations of the year ending March 1951 are presented in table 1.

TABLE 2 APPLICANTS AND POSITIONS REGISTERED
AT CLEVELAND MEETING, APRIL 1951

	NO APPLI CANTS REGIS- TERED	NO APPLI CANTS INTER- VIEWED	NO INTER- VIEWS	POSI TIONS REGIS- TERED
Physiology	52	41	160	86
Biochemistry	124	91	294	90
Pharmacology	18	16	62	35
Pathology	0	0	0	4
Nutrition	12	6	14	7
Immunology	3	2	2	4
Totals	209	156	532	226

In a period of expanding activity such as the present there are more positions than candidates to fill them, with accompanying competition in bidding for the services of well-prepared applicants and a consequent raising of all salary requirements and a careful selection on the part of candidates as to the opportunities presented by the position. This trend has become marked as more of the defense positions become available.

More pertinent and timely perhaps is table 2 showing the number of candidates registered at Cleveland divided into their fields of major interest. It must be pointed out that this division is arbitrary in some cases where candidates have more than one major interest or where the major interest is not included directly in the field of a member Society. In addition to the total registration, the number having interviews is recorded in column 2.

Of the 53 applicants not having interviews, about half did not register as present at Cleve-

land until the third day of registration, hence their application forms were not open to most employers when they first studied the forms. This points to a definite advantage to candidates of early attendance at the meetings.

It is interesting that a large number of biochemists, both candidates and employers, use the Service. This is not in proportion to the number of members of the Federation in that field, for while the registrations in biochemistry approximate half of the total in all six fields, the American Society of Biological Chemists has only about 70 per cent of the membership of the American Physiological Society and twice that of the American Society of Pharmacology and Experimental Therapeutics. Perhaps this is due in part to the general familiarity of all chemists with the very successful employment service of the American Chemical Society. The well-known scarcity of pharmacologists and immunologists is reflected very clearly in this table.

The positions were registered by a total of 161 employers present. Compared to the number of applicants registered, the relative number of positions does not seem to differ much from that in table 1, but a greater proportion of candidates than employers currently registered as active were present in Cleveland. Many non-member employers do not attend the Annual Meetings, while candidates seem to attend whether members or not.

To learn the qualifications of candidates which were most sought by employers at Cleveland, those receiving interviews were broken down by age groups and by degrees held. The following tables include only those for whom regularly scheduled interviews were arranged through the Placement Service.

These figures show the desire of employers for younger applicants, which is a well-recognized trend. The large number of interviews for the few applicants holding only a bachelor's degree shows a demand for trained technical assistants by these employers even though the applications of individuals not having advanced degrees have been discouraged, keeping to the policy that the Service should function chiefly on the professional level. It may also indicate that the present increase in all salary levels has forced some employers to lower their degree requirements if their funds are insufficient to meet the current range offered to candidates with advanced degrees.

Thus it would seem that the Placement Service

is meeting in a measure at least the needs of the professional group of employers is outlined above. These needs vary somewhat from the point of view of the employer and candidate.

The employer wishes objective information concerning the applicant's education and previous experience, with a chronologically complete record of his activities from the time he received his bachelor's degree, a brief account of investigative work participated in with the names of individuals under whom he may have worked, a bibliography

TABLE 3 APPLICANTS INTERVIEWED AT
CLEVELAND
Breakdown by Age Groups

AGE GROUP	NO APPLICANTS	NO INTERVIEWS	INTERVIEWS/PERSON
20-25	15	61	1.1
26-30	79	321	4.1
31-35	38	95	2.5
36-40	19	46	2.4
41-45	3	6	2.0
Over 45	2	3	1.5
Totals	156	532	

TABLE 4 APPLICANTS INTERVIEWED AT CLEVELAND
Breakdown by Degrees Held

DEGREE HELD	NO APPLICANTS	NO INTERVIEWS	INTERVIEWS/PERSON
A.B. or B.S.	5	22	4.4
M.A. or M.S.	16	42	2.6
Ph.D., D.Sc., or M.D.	130	438	3.4
Ph.D. and M.D.	5	30	6.0
Totals	156	532	

of published work, a list of honors received and society memberships, and the salary he expects. The employer ordinarily prefers to obtain personal references directly and to evaluate the other information himself, for employers filling positions with experimental biologists are with few exceptions prepared by experience, association and knowledge of the field to do this best.

As mentioned before, the greatest number of candidates are recent graduates and are seeking an opportunity to continue experimental work, with or without teaching responsibilities, and at a salary adequate to meet the now constantly rising cost of living. Unless the degree was ob-

tained under a department head who has few other administrative duties, many current contacts, few students and an aggressive program for placing his students, the recent graduate may not know where to look for the position he has spent so long preparing for. Once the original contact is made the candidate may not feel able to evaluate the opportunities of the position. Since it is impossible for the Placement Service to assume this function it is necessary that he seek this assistance from his older and more experienced associates.

Not infrequently older, more experienced candidates, confronted by changed conditions or by changed interests, may wish to be considered for other positions. Unfortunately in the past there has been some stigma attached to the scientist having to seek employment. The world was expected to beat a path to his door. This is not a realistic attitude in these times and it is hoped that the short factual announcements as now prepared by the Placement Service of the Federation, which is interested only in maintaining and improving the standards within the profession, can remove the reasons for this attitude and the consequent reluctance by some to utilize its service.

When positions are plentiful as now, the Service is most useful to employers. In times of scarcity of positions its usefulness to candidates is increased. At all times the more widely this information is disseminated the more opportunity there is for an applicant to accept a post for which his training and interests prepare him. Such exchange of information also tends to equalize opportunities, conditions and salaries for positions of equal rank and responsibility, requiring similar training. The list of fellowships brings together in one place the essential facts about the fellowships available in the field of experimental biology, such as place and date of application, place and conditions of tenure, requirements and stipend for the consideration of both candidates and department heads when considering their own or their students' future plans.

Since it is desired to increase the usefulness of the Placement Service in accordance with the changing needs of the individuals using it, suggestions are welcomed.

LETHA K. ANDERVONT
Assistant to the Director
Federation Placement Service

CORRECTIONS TO MARCH ISSUE

Page 167 BROQUIST, STOKSTAD AND JUKES, *title*
Delete word "crystalline "

Page 171 CILCOTE AND O'DEA *Name of first author* should read, "MAX E CHILCOTE "

Page 177 DOUNCE, SIMMONS AND KAY, *line 3*
Change "sodium dodecyl sulfonate" to read
"sodium dodecyl sulfate "

Page 189 GHOSH, WOODBURY, SAYERS AND SMITH, *line 12* Change pH 8.0" to read "pH 1.0 "

Page 227 NAJJAR, *line 25* (last line on page)
Insert " μ g " after "200"

Page 237 RIGGS, CHRISTENSEN AND RAJ, *line 9*
Sentence should read "When duck erythrocytes were suspended in saline medium containing elevated levels of glycine, glycine was taken up by the cells against the concentration gradient "

Page 400 ALTMAN AND EDSALL, *lines 14-15* Instead of "Twenty-five of the remainder," read "Twenty-five per cent of the remainder "

AAAS PHILADELPHIA MEETING

December 26-31, 1951

THE 118th Annual Meeting of the American Association for the Advancement of Science will include programs of all 18 of the Association's sections and of about 15 participating societies. The focus of the approximately 225 sessions will be Convention Hall, adjacent to the University of Pennsylvania's School of Medicine and its associated hospitals; there will be some meetings in the downtown hotels, especially the Bellevue-Stratford (headquarters) and the Benjamin Franklin (zoologists). Programs of particular interest to those in medicine and experimental biology include

SECTIONAL PROGRAMS

AAAS Section C—Chemistry Eleven sessions, including one on Medicinal Chemistry, *Dec 28*, two on Forensic Sciences, *Dec 29*, two on Reaction Mechanisms, *Dec 26, 27*

AAAS Section F—Zoological Sciences A symposium, "Estuarine Ecology," jointly sponsored by the American Society of Limnology and Oceanography, American Society of Zoologists, and the Ecological Society of America, *Dec 30*, a two-session symposium, "Sex in Microorganisms," jointly sponsored by AAAS Section G, the American Society of Protozoologists, American Society of Zoologists, Botanical Society of America, and the Genetics Society of America, *Dec 30*

AAAS Subsection Nm—Medicine Four-session symposium, "Aging" (with special reference to cholesterol and arteriosclerosis), *Dec 29, 30*

AAAS Subsection Nd—Dentistry Three sessions including a symposium, "Fluoridation as a Public Health Measure," *Dec 29*

AAAS Subsection Np—Pharmacy Six sessions, co-sponsored by the American Association of Colleges of Pharmacy, American College of Apothecaries, American Society of Hospital Pharmacists, and the Scientific Section, American Pharmaceutical Association, *Dec 27-29*

AAAS Section O—Agriculture Four-session symposium, "Mineral Nutrition in Relation to Plant, Animal, and Human Health," *Dec 27, 28*

PROGRAMS OF SOCIETIES

Oak Ridge Institute of Nuclear Studies Two-session symposium, "Radioisotopes in Medicine," and luncheon, *Dec 28*, a series of lectures either *Dec 27 or 29*

American Society of Protozoologists Eight sessions of papers, a luncheon, and a business meeting, *Dec 27-29*

American Society of Zoologists Fourteen sessions of papers, *Dec 28, 29*, also symposium, *Dec 27*, demonstrations, *Dec 28*, zoologists' dinner and Biologists' Smoker, evening of *Dec 29*, symposia, *Dec 30*

Herpetologists League Conference session, *Dec 28*

Society of Systematic Zoology Five sessions including breakfast, business meeting, and symposium, "Classification of Animals," *Dec 28*, two paper-reading sessions, *Dec 29*

American Microscopical Society Three sessions, *Dec 27-29*, including a symposium, "Modern Methods in Microscopy"

Biometric Society, Eastern North American Region Six sessions of papers on Biometry, *Dec 27-29*

Society for Research in Child Development and AAAS Section N Four sessions including a symposium, "Biochemistry of Nutrition in Human Growth," *Dec 27*

Alpha Epsilon Delta Premedical Honor Society Luncheon and afternoon program, *Dec 28*

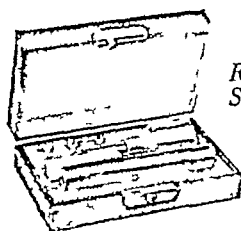
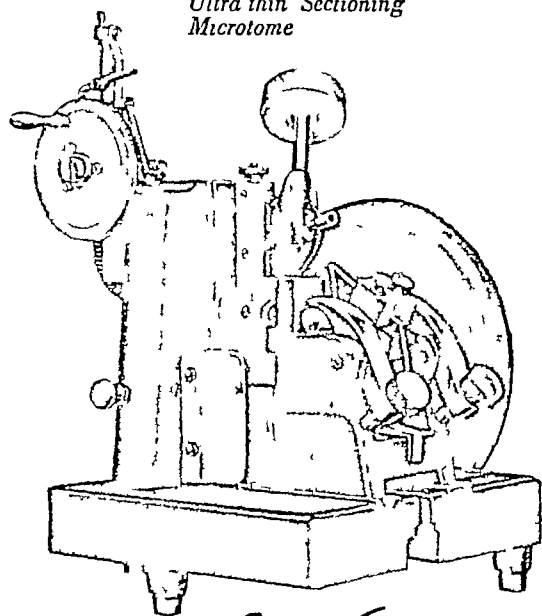
American Industrial Hygiene Association Special program in connection with inauguration of the new AAAS Section P—Industrial Science

SPECIAL SESSIONS

AAAS Annual Exposition of Science and Industry The 1951 edition of the Association's Exposition again will total about 150 booths and will fill the arena of Philadelphia's Convention Hall. In addition to exhibits by leading publishers, instrument makers, and suppliers of scientific materials, there will be technical exhibits by prominent manufacturers of pharmaceuticals and biologicals. In or near the exhibit area will be the Fifth Annual International Photography-in-Science Salon and the AAAS Science Theater which, almost continuously, will show the latest foreign and domestic scientific films.

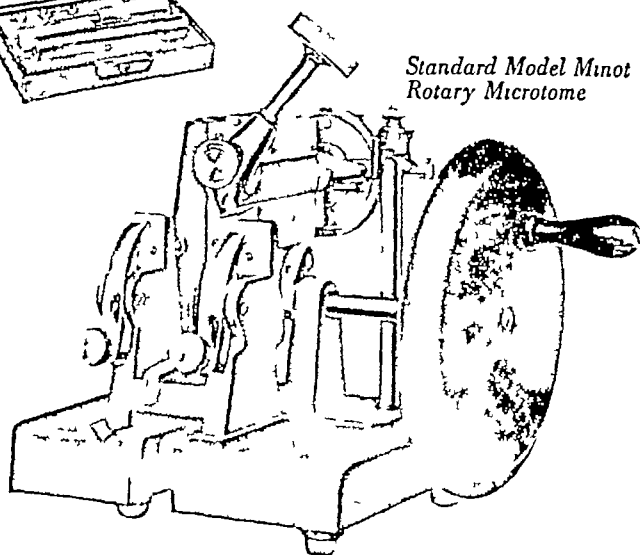
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American Society of Biological Chemists

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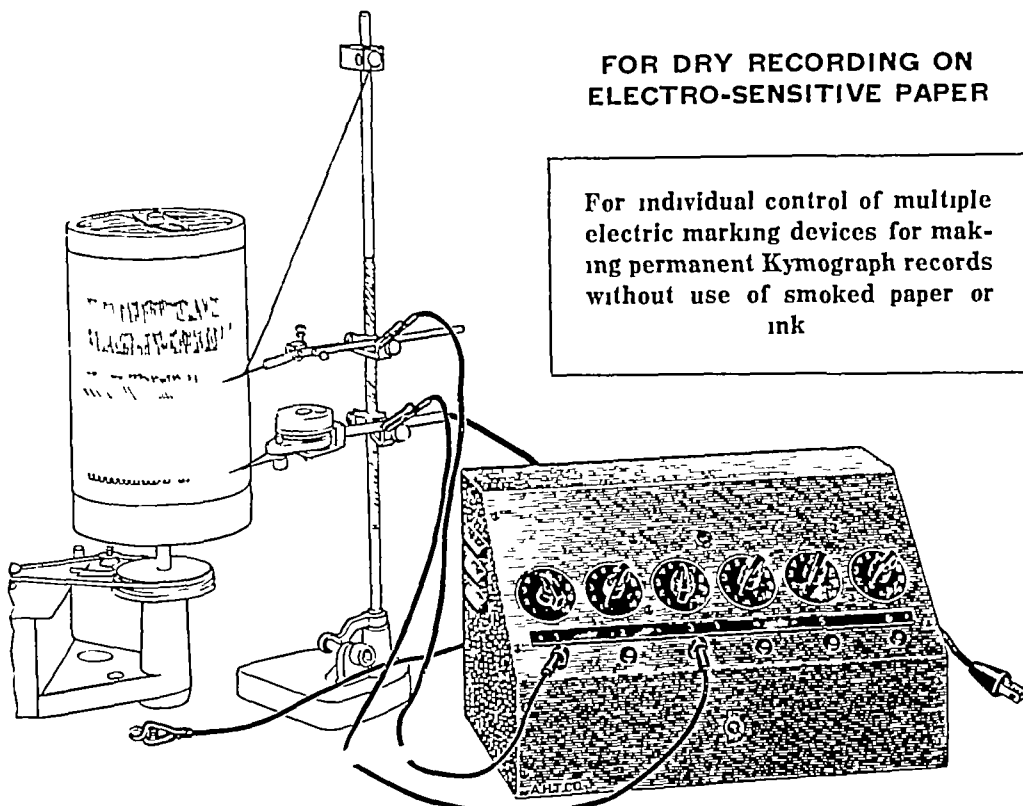
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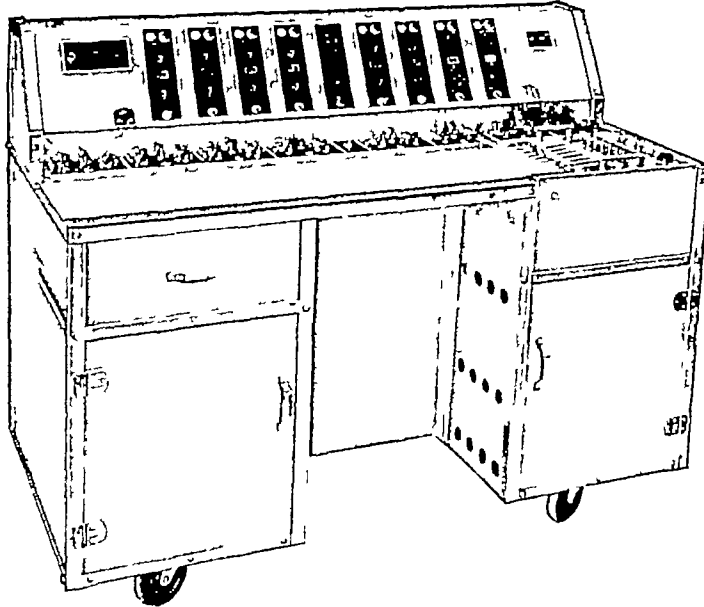
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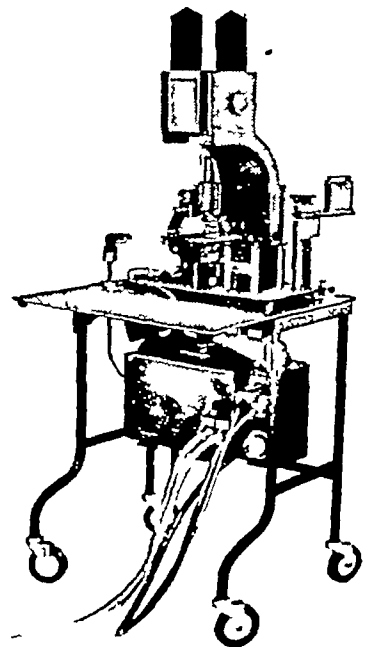
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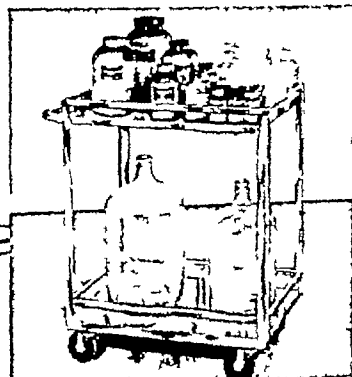
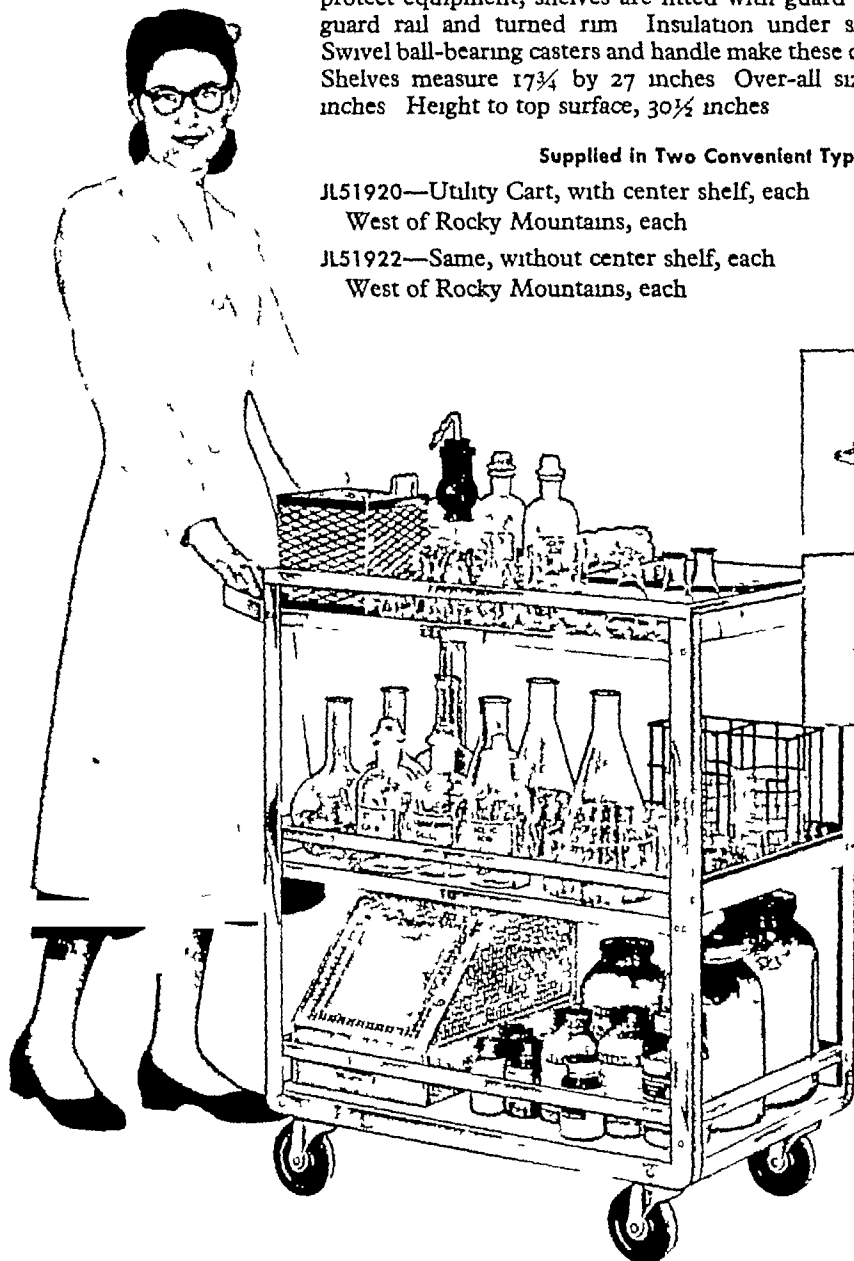
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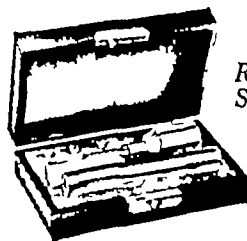
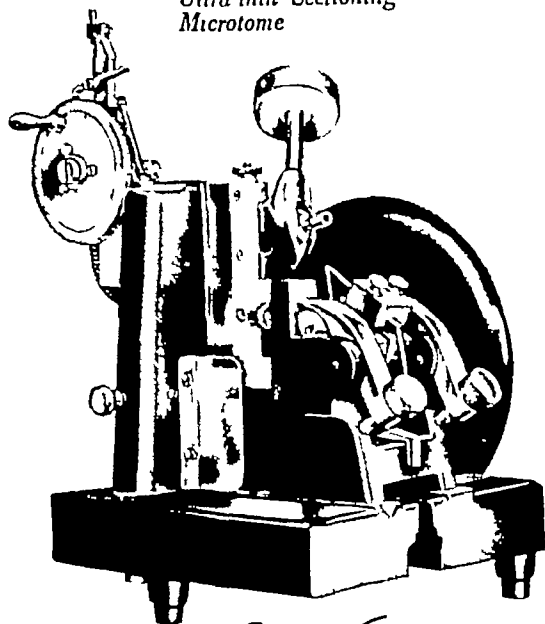
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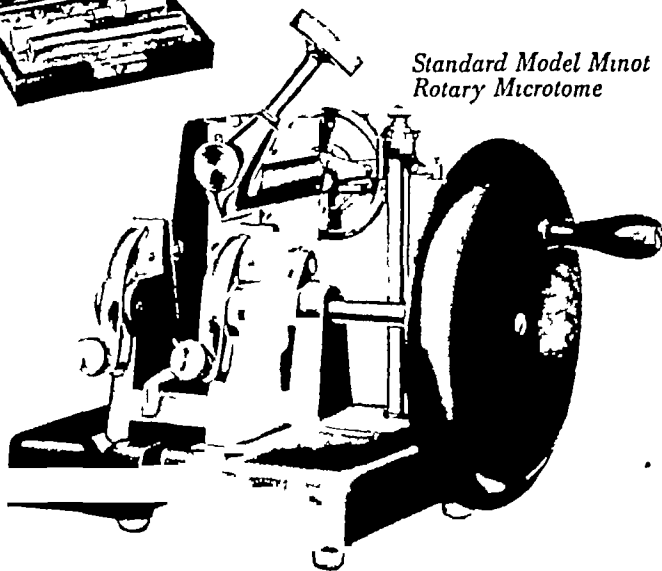


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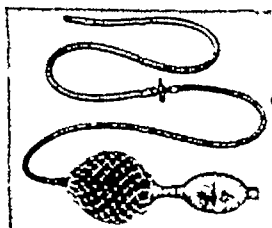
Pure polyethylene plate is finding increased use in cranioplasty. Indications are that it will eventually replace other materials, metal, plastics, and bone now in use, report Drs. Eben Alexander and Peter H. Dillard in *Journal of Neurosurgery*, VII, 6, pp 492-498, 1950.

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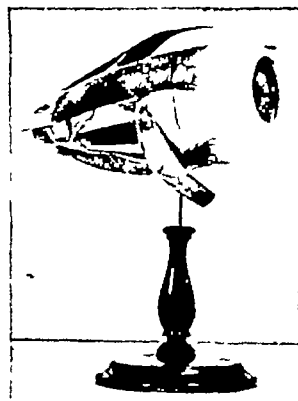
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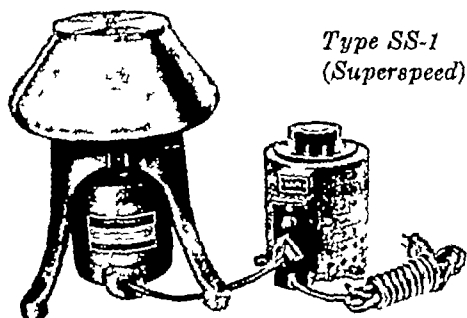
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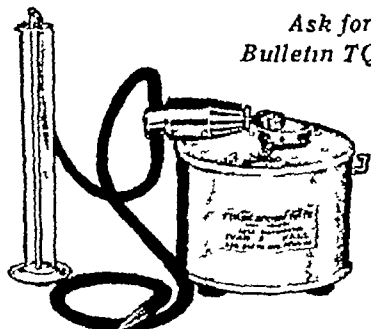
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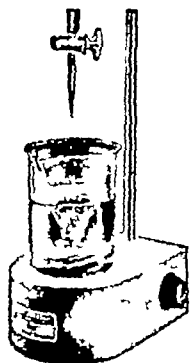
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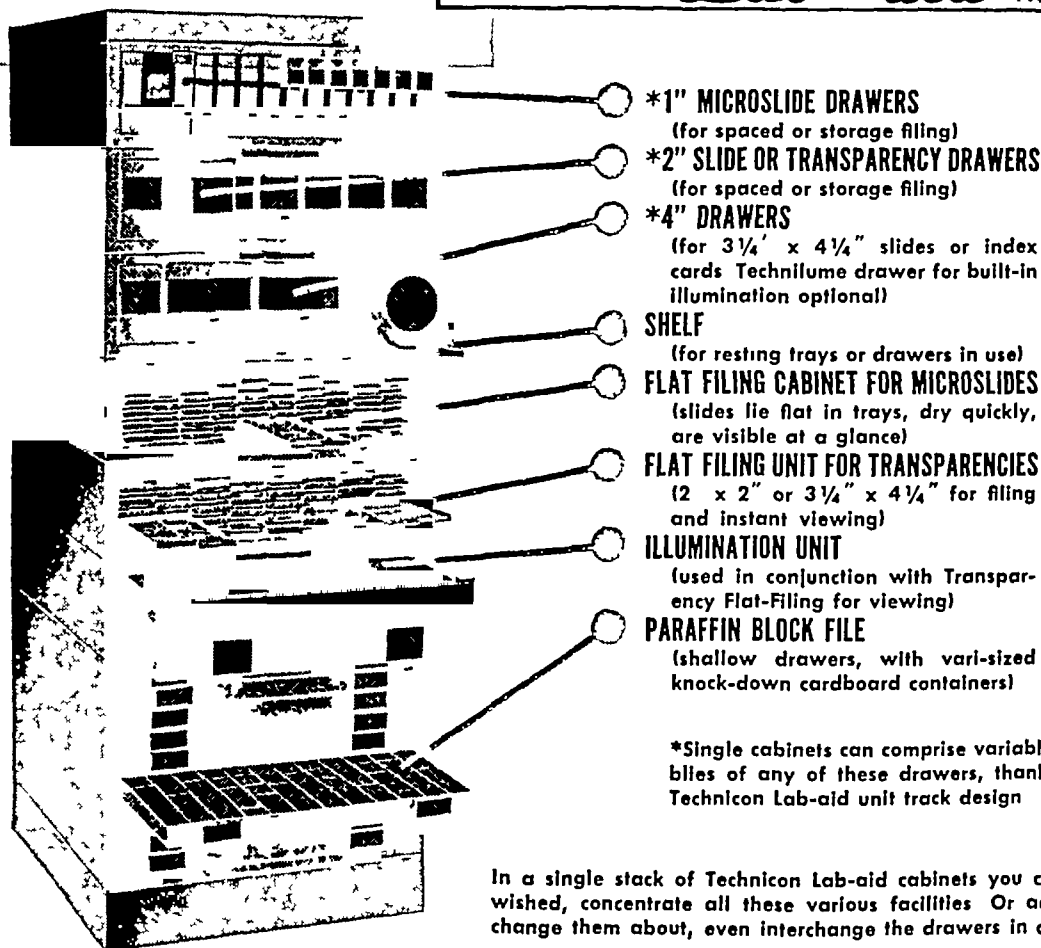
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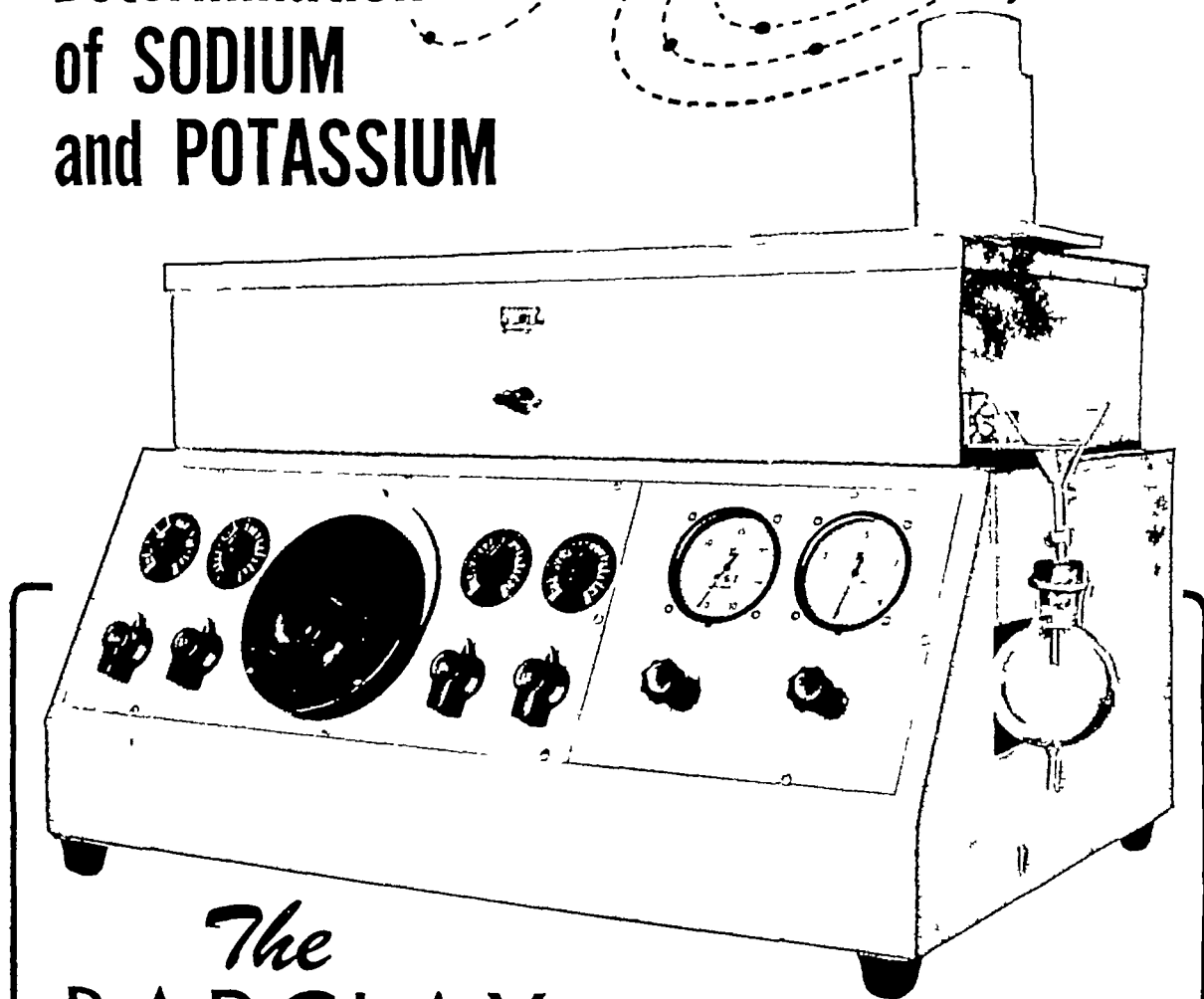
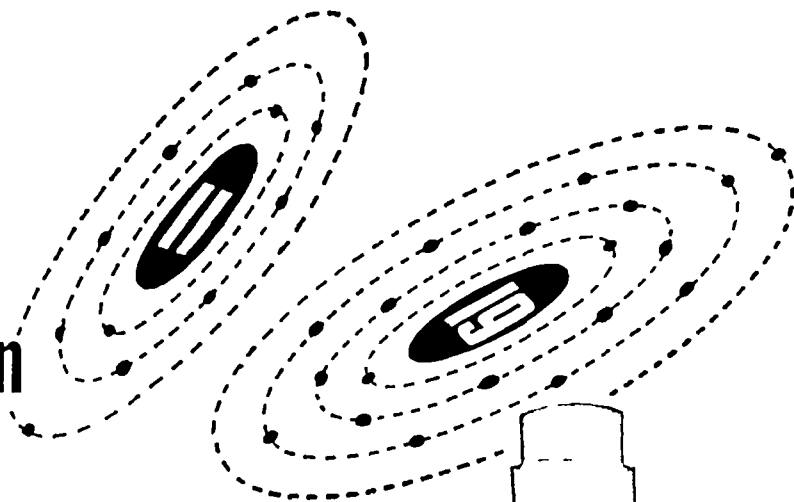
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THE XIIth INTERNATIONAL CONGRESS OF PURE AND APPLIED CHEMISTRY will be held in New York City, U.S.A., in September 1951, in connection with the 75th Anniversary Meeting of the American Chemical Society. The Section of Bio'ogical Chemistry will welcome research papers on biochemistry (including fermentation, leather, and microbiology) from all research workers who plan to attend the New York Meetings.

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Federation Proceedings

VOLUME 10

June 1951

NUMBER 2

NOTES ON THE THIRTY-FIFTH ANNUAL MEETING OF THE FEDERATION

Cleveland, Ohio, April 29-May 3, 1951

THE THIRTY-FIFTH ANNUAL MEETING of the Federation was held at Cleveland, Ohio, April 29-May 3. The Hotels Statler and Cleveland served as headquarters, scientific sessions were in the Cleveland Public Auditorium. Dr. Geoffrey Edsall, President of the American Association of Immunologists, was Chairman of the Executive Committee of the Federation and presided at the Joint Session held in the Public Auditorium on Monday evening.

Scientific sessions of the constituent Societies began Monday, April 30, and continued through Thursday afternoon, May 3. One hundred and twenty-six scientific sessions were held, at which 1298 papers were presented and 175 were read by title. Motion pictures were shown at a special session Tuesday evening. There was a Mixer and informal reception, arranged by the Local Committee, immediately following the Joint Session on Monday evening. Symposia were presented by the American Physiological Society, the American Society of Biological Chemists, the American Institute of Nutrition and the American Association of Immunologists. Three Societies had dinner meetings and various other groups arranged special functions such as business meetings, dinners and luncheons. The total registered attendance was 4787. Forty-nine industrial exhibits and 13 exhibits by members of the Federation were shown.

FEDERATION ACTIONS

The following actions of general interest were taken by the Executive Committee of the Federation:

1. Action on the proposed Constitution printed in the December 1950 issue of the *Proceedings* was deferred. By unanimous vote, ratified at the business meetings of the member Societies, Federation By-Laws 1, 2 and 8 were changed to provide for an 18-man Executive Committee with the Chairmanship passing in rotation among the Past-Presidents of the member Societies. A new committee will be appointed to consider further

suggestions for a Federation Constitution and By-Laws.

2. The Federation assessment for the year July 1, 1951 through June 30, 1952 was set at \$3 per member of each constituent Society.

3. Dr. M. O. Lee was reappointed Federation Secretary.

4. Plans were confirmed to hold the 1952 meeting in New York City April 14-18, and the 1953 meeting in Chicago April 6-10. The meeting will be held in Atlantic City in 1954 and on alternate years thereafter.

5. The American Physiological Society will be host Society for the 1952 meeting and will arrange the program for the Joint Session. Dr. D. B. Dill, as Past-President of the Physiology Society, will serve as Chairman of the Executive Committee beginning July 1, 1951.

6. A meeting of the Executive Committee of the Federation will be held in January 1952.

7. The subscription price of *Federation Proceedings* to non-members was raised to \$6 per year, effective with Volume 11, 1952.

MILITARY UTILIZATION OF SCIENTISTS

The Federation is interested in the proper and best utilization by the armed forces of the scientific skills of its members who are serving temporarily. It is requested that members who enter active military service by enlistment, commission or from the reserves notify either the Secretary of their Society or the Federation Secretary for purposes of record. The Federation will attempt to obtain the correction of gross misassignment, or misutilization of skills of any members who request its help.

PLACEMENT SERVICE

Beginning with the February quarterly list of applicants, the lists of the Placement Service will be supplied on a subscription basis. Information as to rates will be distributed to those receiving this service. The registration fee for applicants will be increased to \$3 annually.

CORRECTIONS AND ADDITIONS TO MARCH ISSUE

Page 34 DERN AND PULLMAN, *5th line from end of abstract* The phrase " $P = 0.1-0.02$ " should read " $P = 0.01-0.02$ "

Page 52 The following abstract was omitted

Studies on synergism of several anti-neoplastic agents ABRAHAM GOLDIN, EZRA N GREENSPAN AND EMANUEL B SCHOENBACH *Clin Research Unit of Natl Cancer Inst, U S Public Health Service, U S Marine Hosp, and Dept of Preventive Medicine, Johns Hopkins Univ School of Medicine, Baltimore, Md*

Combinations of anti-neoplastic agents which had been shown to be effective when employed individually, were studied with respect to host survival time, inhibition of local tumor growth, and peripheral leukemic manifestations in dba mice inoculated with a transplantable acute-stem-cell leukemia (Lymphoma 1210). The combinations studied included the following agents: 5-amino-7-hydroxy-1H-v-triazolo [d] pyrimidine (guanazolo, 8-azaguanine), 4-amino-pteroylglutamic acid (aminopterin), methyl bis (B-chloroethyl) amine (HN2), alpha-peltatin, and 2,4,6-triethylenimino-s-triazine (TEM). Combined treatment with guanazolo plus aminopterin, guanazolo plus alpha-peltatin and aminopterin plus alpha-peltatin appeared to exert an additive inhibitory effect on the tumor without proportionate increase in host toxicity. Survival time was more prolonged than was observed when the drugs were employed individually. The other combinations of agents also affected the local growth and peripheral leukemic manifestations of the tumor to a greater degree, but the resultant host toxicity was increased to such extent that no prolongation of survival time was apparent.

Page 58 HAFT AND MIRSKY, *table* The term "slide" should be changed to read "side"

Page 112 ROSTORFER, GEBER AND ONYETT, *line 16* The sentence beginning in this line should read, "The nitrite-treated avian and mammalian cells reduced methemoglobin at the rates of 1.57 and 1.34 gm %/hour, respectively." Delete the rest of the printed sentence.

Page 225 MORGAN AND GUEHRING, *line 1* Change "pathogenic" to read "pantothenic"

Page 258 TAUBER, MCLEOD, GARSON AND MAGNUSON, *line 25* The sentence beginning in this line should read, "The spirocheticidal substances were present in Precipitate III—1,2 associated with "

Page 280 BEUTNER AND WILLIAMS, *line 10* The word "invariably" should read "mostly"

Page 523 The following entry was omitted from the list of member exhibits

Experimental radiographic visualization of the gallbladder J O Hoppe, J C Seed* and J W Hart,* *Sterling-Winthrop Research Institute, Rensselaer, N Y* Radiopaque substances are used as diagnostic aids for visualizing soft tissue structures. A brief outline of some of the steps involved in the search for an improved cholecystographic medium as well as myelography and urography are illustrated. See also HOPPE, J O and S ARCHER *Federation Proc* 10:310, 1951

Retraction The findings on holophosphorylase reported by Irwin Feigin, Jerome Fredrick and Abner Wolf in *Federation Proceedings*, Volume 9, page 170, 1950 and Volume 10, pages 181 and 184, 1951, are hereby withdrawn at the request of Irwin Feigin and Abner Wolf, since the results described cannot be duplicated.

JOINT SESSION OF THE FEDERATION

Cleveland, Ohio, April 30, 1951

Chairman GEOFFREY EDSALL

PHYSIOLOGICAL ADAPTATION TO COLD IN ARCTIC AND TROPIC ANIMALS

LAURENCE IRVING

From the Arctic Health Research Center, Anchorage, Alaska

DURING recent years, and with the assistance of numerous colleagues, I have been carrying on studies of adaptations to arctic cold which might be described in physiological terms. As a result of these studies, we have developed an outline of the physiological economy of heat which appears useful as a guide for further investigation. Certain physiological systems are arranged in arctic animals for the conservation of heat, thus the metabolic costs of living in low temperature are apparently held down to about the same basal level of energy expenditure as is found in warmer climates. Observation of free wild arctic animals does not indicate that they expend more time feeding than do their relatives in warmer areas. In consequence they have time for the social, mating, play and explorational activities which are equally essential in any climate for the preservation of a race.

I do not hesitate to call adaptive those devices possessed by arctic animals, and deficient in tropic forms, which obviously serve to conserve heat. Certain other physiological characters concerned with heat appear to be common to all climates, these are nonadaptive to cold and part of the common heritage of the warm-blooded condition.

We have recently measured the body temperature of arctic reindeer and dogs near the village of Barrow during a period in which the air temperature remained below -45°C for a week. We have measured the rectal temperature of porcupines in the Talkeetna Mountains at temperatures around -30°C . These animals were living freely under natural conditions. In order to obviate the effects of disturbance during observation, the measurements were confirmed upon

animals which were shot while at rest. We have gone to some trouble to ascertain that in the free condition the body temperature of arctic animals is, as reported for a few captives (1), characteristic of the warm-blooded state rather than of the climate in which they dwell. Body temperature is therefore not adapted to the conservation of heat.

In earlier studies (2) we reported that the resting metabolic rate of some arctic and tropic animals was proportional to their size, as is true among animals of temperate regions. The metabolic rates of all arctic and tropic animals examined fit closely upon the curve set by the equation

$$\text{Calories per day} = k \times \text{kg}^{\frac{1}{4}}$$

The rates thus conform to the famous statement by Benedict of the relative metabolic rates of animals varying in size from the mouse to the elephant. We have found no indication that the basal metabolic rate is adaptable to cold, although I must reserve for future study some uncertainty about small arctic birds.

The length of the arctic animal's fur is conspicuous and its protective value against cold is shown by its effective use in Eskimo clothing. We have measured the insulation afforded by the fur of some arctic and tropic animals (3). In general, the insulating effect varies with the thickness of the fur. Among arctic animals the fur thickness increases with size up to that of the arctic white fox. In larger animals, such as the caribou and polar bear, no marked increase in thickness of fur is found. What further significance there may be in this interesting relation awaits study.

Knowing the factors of temperature and metabolic heat production we can test the applica-

tion of the law of cooling, using a simple expression

$$\Delta TC = K \times I \times E$$

in which ΔTC is the largest temperature difference between the animal's body and the air which can be maintained at the basal metabolic rate, K is a constant for the units used, I is insulation and E is metabolic heat production. Plotting E against ΔTC gives lines representing $I \times E$ by their slope when all basal rates are referred to 100 per cent

Applicable data are available for 9 arctic mammals and birds, 8 tropic mammals and birds along with comparable observations from other authors upon some 20 animals of more temperate regions. A plot of these observations brings out the basic system of animal heat conservation in all climates as a function of basal rate and insulation (1)

Insulation of adult arctic animals larger than the arctic fox is sufficient to enable them to maintain normal body temperature down to -30°C , the lowest attainable in these experiments. By theoretical indications and from observation of the animals' disregard for natural temperature around -50°C , it appears that arctic animals larger than the fox need not increase basal metabolic heat production until temperatures fall lower than -50°C , and then at only a small rate of increase

Insulation is so meager among animals in the tropics that even the small diurnal and microclimatic changes often embarrass these animals with cold. But well-adjusted arctic animals do not shiver in any winter weather. In the wide range of temperature tolerable to them they seem to be in a better position with reference to the temperature of their climate than animals in the tropics

This adaptive factor, insulation, is characterized by its physiological variability, for it may be suited to air temperatures of -50°C or $+30^{\circ}\text{C}$. It may promptly be changed to permit rapid dissipation of heat through the metabolism of violent exercise. It may obviously be altered morphologically during the seasons, although we have not yet measured this very apparent process

It is also interesting to note that all three physiological functions—insulation, metabolic rate and body temperature—which are related in the application of the law of cooling as if they were single factors, are nevertheless obviously

compounded of a variety of conditions which exist discretely at any one time in the animal body. Their formulation together then expresses the organization of the animal in administering the economy of metabolic heat

We have recently made some measurements of the body skin temperature of arctic mammals and birds. At Barrow, we reported temperature of the body skin of 4 dogs in air at -3°C to be from 33°C to 37°C . In air at -19°C the temperature of one dog's skin was 30°C .

This winter we were fortunate in being at Barrow Village during a period of low winter temperature. In air temperatures around -30°C to -50°C , the rectal temperature of active or sleeping dogs was around 38°C . The surface skin temperature was around 30°C , with the immediately subcutaneous temperature a few degrees higher. Contrary to some views, these powerful arctic sled dogs are unusually stable toward those accustomed to their ways. We were able to make rectal and subcutaneous temperature measurements by inserting fine needles containing thermocouples even under the skin of the paws and nose without disturbing the animals

The subcutaneous temperature of the bare foot pads was found variously to be at from $+10^{\circ}\text{C}$ to $+20^{\circ}\text{C}$ in air temperatures ranging from -30°C to -45°C . Snow did not melt on the feet, but measurements on bare skin by surface contact are uncertain in such gradients

Conditions for surface contact measurements on the bare muzzle are better and indicated low temperature. However, subcutaneous measurements on noses 2 or 3 mm below the skin surface near the hair line at the muzzle registered only a little higher than those on the surface and were surprisingly low, indicating temperatures of 10°C to 15°C . From the muzzle toward the eyes the subcutaneous temperature rose rapidly, reaching the body level on the head just back of the eyes

With the assistance of Mr. Arnold Brower, we traveled by dog team to the location of a reindeer herd at Tapogaruik, about 70 miles south of Barrow. During the week the air temperature remained below -46°C . We were able to confirm that the temperature in the hooves and lower legs of both living and freshly shot deer remained regularly at 10°C or even lower

These observations epitomize, in our opinion, many others which we have made under less conclusive conditions upon the fleshy portions

of the exposed feet of arctic animals and birds, showing that they are often lastingly at low temperature. We have long been convinced that the skin of seals and other arctic marine mammals, and the feet of ducks and gulls must persistently remain at low temperature. We regard our measurements in the arctic only as a final demonstration of an *a priori* view that the exposed uninsulated tissues of many northern mammals and birds must for long periods exist at temperatures far below the level of the mammalian body.

That this low temperature will spare expenditure of heat is apparent, but it might be argued that as an unavoidable consequence of exposure, the condition is not in adaptation. We have usually considered that the properties of biological substances are set to operate well at normal mammalian body temperature levels. However, the cold state of some mammalian tissues as found in these studies imposes some possible difficulties in this concept. As substan-

tiation of this, we have obtained, through the aid of Dr. Norman Abrahamsen, measurements of the melting point of fat from the marrow of the hind leg of a caribou shot near the Meade River in November. In the distal metatarsal bones the fat was soft, melting at 15° C, while in the upper femur, well within the body, it was 47° C. There are other indications that the fats in tissues exposed to low temperature have low melting points (4), and thus afford the apparent advantage of preserving flexibility in the cold.

I cannot forego the suggestion that certain tissues of warm-blooded animals in the arctic must have in this and other respects a sort of chemical adaptation of their substances to suit them for operation at temperatures lower than that of the mammalian body. In accepting this view, if it is reasonable, we will considerably modify the further experimentation by which we will search for physiological adaptations enabling animals to live successfully in arctic climates.

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BIOCHEMICAL MECHANISMS IN INSECT GROWTH AND METAMORPHOSIS

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Each of us at the outset of his or her individual life story is macroscopic and one sole cell. By that cell's multiplication and by its descendants' coherence, each of us attains final form and size. The doings of this cell-assembly are those of a being which is a unity. Yet each of its constituent cells is a life centered in itself—managing itself, feeding and breathing for itself, separately born and destined separately to die. Further, it is a life helped by and in its turn helping the whole assembly, which latter is the corporate individual.

SIR CHARLES SHERRINGTON (1)

FOLLOWING Sherrington, we can recognize the growing organism as an expanding community of cellular lives, each centering in itself and yet each presupposing the whole. The growth and differentiation of this community takes place in a coordinated manner and according to a detailed blueprint inherited from the preceding generation. Indeed, genetics tells us that each cell is equipped with such a blueprint in its nucleus. A central problem of our science is to understand how the cells, as 'servants of the genes,' execute the hereditary plan of the final organism.

The phenomena here encountered are obviously not peculiar to the higher and more pretentious organisms such as the human species. In the tiger, as in the tiger beetle, growth, differentiation, and cellular coordination present substantially the same triad of problems.

The riddle of cellular differentiation seems no less baffling today than it did to preceding generations of biologists. We can only hope that the geneticist and the experimental embryologist, approaching the problem from opposite ends, may ultimately break through to one another. Most happily, however, significant progress has been made in understanding the two other aspects of the matter, namely, the mechanisms of cellular growth and intercellular coordination. Biochemistry with its new emphasis on synthetic processes is at long last gaining access to growth phenomena at the molecular level. Likewise, the physiologist is finding cellular events to be coor-

minated and controlled by chemical agents conveyed by blood or released from nerve.

It is from these biochemical and endocrinological points of view that I propose to consider what we have learned during the past few years about the coordination of growth and the growth process itself. The studies to be considered represent the joint effort of a group of investigators, chiefly graduate students, working at the Harvard Biological Laboratories. Insects were chosen as the subject of our study since the metamorphosis which punctuates their life history gives expression to morphogenic events which in most other animals are reserved to the early stages of embryonic development.

CECROPIA SILKWORM

Consider, for example, the *Cecropia* silkworm—an insect I shall use as a text for physiological thinking about the phenomenon of metamorphosis. Postembryonic development is here partitioned into three epochs: the growing larva, the quiescent pupa, and the reproducing adult. The transition between these successive stages is called metamorphosis.

The larva is fundamentally a digestive tract equipped with caterpillar tread. From the vegetation upon which it feeds, it acquires a stock pile of fat and protein which is stored away in a special tissue called 'fat body.' In a very real sense, the accumulation of this reservoir and stock pile is the biochemical objective of larval life. Thus the pupa, which forms within the larva during the pre-pupal period, is a closed system, save for the gradual evaporation of water and the interchange of respiratory gases. Likewise the moth, which forms within the pupa during the

¹ A number of studies considered in the present communication have been supported, in part, by the Lalor Foundation, the American Cancer Society, Inc., and the Public Health Service.

period of adult development, must also be built from the assets which the caterpillar has accumulated. For though the moth possesses a normal-looking digestive tract, it cannot be used because mouth parts are lacking at its anterior end. This paradox is answered by the finding that the 'normal-looking' digestive tract is devoid of digestive enzymes and therefore incapable of functioning. (2) So, notwithstanding the fact that the larval, pupal, and adult insects are organized according to different plans, the three stages are, biochemically speaking, a reworking of the same old atoms and molecules.

ANATOMY OF METAMORPHOSIS

Metamorphosis begins at the end of larval life during the so-called prepupal period. The specialized structures of the caterpillar are at this time dismantled to make way for the equally specialized structures of the pupa. Dismantling of the one and construction of the other proceed side by side as the organism reshapes itself according to a new plan. These events are camouflaged by the old caterpillar skin which, like a loose sack, continues to cover the insect during the prepupal period. The shedding of this skin at the time of the pupal moult reveals the pupa itself.

Now the pupa is a stage of transition between larva and adult. It represents the first great stride in the metamorphosis of the species—a metamorphosis so profound that it amounts to the fabrication from the caterpillar of an essentially new organism, the adult moth.

If we look inside such a pupa, we find it filled with a great paired mass of fat body, the biochemical inheritance from the caterpillar. A more detailed search reveals the presence of a number of longitudinal structures: a dorsal heart and gonads, a ventral nervous system, and a central gut. Only in the skin of the pupa do we find any indication of the form of the future adult moth. Here the epithelium, but a cell's depth in thickness, shows a simple mapping-out of the moth's head, thorax, and abdomen. From this modest beginning within the pupa there is destined to develop the final adult insect, whose internal organization is no less complicated than that of a human being.

PUPAL DIAPAUSE

In the Cecropia silkworm as in many other insects, adult development does not proceed

promptly after the pupa is formed. To the contrary, the rapid tempo of cellular events, which during a period of seven weeks has transformed the egg into the pupa, now comes to an abrupt halt. During the months that follow, the pupa persists in a state of developmental standstill, the pupal diapause.

For the insect in nature, the period of pupal dormancy provides for the overwintering of the species. Though the pupa makes no morphological advance during this period, the months of exposure to winter's low temperature are not 'time out'. By its direct action within the pupa, low temperature promotes certain endocrinological changes which assure the termination of diapause the following spring. Such a chilled pupa, after a few days of exposure to room temperature, shows an abrupt initiation of adult development signalling the termination of diapause. The construction of the moth within the pupa then proceeds with clock-like precision so that, at a temperature of 25°C, the adult insect emerges 21 days thereafter. It has taken ten months to develop that moth from the initial egg. And of the ten months, eight were assigned to the pupal diapause.

ENDOCRINE CONTROL OF DIAPAUSE

From the biochemical point of view, the most interesting aspect of metamorphosis is the pupal diapause. Here the biochemical mechanisms underlying normal growth and differentiation are mirrored in the abrupt transitions from growth to diapause and, with the latter's termination, back to growth. Since this sequence can be controlled at the convenience of the investigator, the matter becomes accessible to experimental analysis.

In view of the synchronization of morphogenic events at the cellular level, one fact is self-evident: there must be some overall control of the genesis and the termination of diapause. And it is no surprise to find this controlling mechanism to be endocrinological in character.

This fact is perhaps best illustrated by the behavior of the male sex cells of the dormant pupa when cultured *in vitro* (3, 4). If one removes the testis from a diapausing pupa and tears it open in a few drops of the insect's blood, an enormous number of little hollow cysts are released. The walls of each cyst are formed by a simple layer of undifferentiated cells, the primary spermatocytes. At the beginning of adult development each

of these cells undergoes two divisions (meiosis), followed by rapid differentiation into the spermatozoa of the moth

When the dormant cysts of the pupal testis are placed in a simple hanging drop of the insect's blood, their behavior is remarkably conditioned by the status of the animal which donated that drop. In the blood of a diapausing pupa, the cysts show no significant development, though they survive for approximately a week (fig 1A). In contrast, if one uses a drop of blood from a pupa that has just initiated adult development, the cells undergo prompt meiosis followed by differentiation into normal spermatids (fig 1B).

The behavior of the spermatocytes in the hanging drop is therefore a kind of scale model of what goes on in the intact insect. During diapause the blood lacks a growth hormone which is necessary for the growth and differentiation of the testicular cells and of all other tissues. The secretion of this hormone in adequate titer terminates diapause and substitutes exuberant growth for developmental standstill. It seems proper to term this important growth factor the "growth and differentiation hormone" (5).

Time does not suffice to consider the role of this hormone in detail. Suffice it to say that growth at all stages in the life history seems to require its presence. It appears to be necessary for the growth and recurrent moulting of the larval insect, for the transformation of the larva into the pupa, and for the fabrication of the adult moth within the pupa. And, as we have seen, its absence following pupation results in the deficiency syndrome which we recognize as the pupal diapause.

As a growth factor of no mean proportions, the properties of this growth and differentiation hormone are of obvious interest. At long last this problem has become accessible by virtue of the assay method now provided by the tissue-culture technique. When tested in this manner, the hormone is found to behave as a protein or a smaller molecule tightly bound to a protein. It is non-dialyzable, stable when heated to 75°C for 5 minutes, but precipitated by exposure to 80°C for 5 minutes (4). It has been impossible to mimic its action by any vertebrate hormone, vitamin, or biologically active substance.

The first visualization of the growth and differentiation hormone is presented in figure 2 (6). Six components are encountered in the electrophoretic pattern of active blood obtained from post-diapausing pupae. Figure 2A shows the still-

active blood after exposure to 75°C. Figure 2B shows the inactive blood after heating to 80°C. We suspect that the small protein peak, present in A but absent in B, is attributable, in part, to the hormone itself.

In the intact insect the source of this growth and differentiation hormone has been identified. It is secreted by a pair of endocrine organs within the thorax, the 'prothoracic glands'. Curiously enough, these glands are able to secrete the hormone only under the tropic stimulation of a further hormone arising in 26 neurosecretory cells within the brain itself (7-12).

So within the insect we recognize the presence of a miniature endocrinological system—the prothoracic glands secreting the growth and differentiation hormone but only when triggered to do so by the brain hormone (fig 3). Morphogenesis in this indirect manner is brought under the control of the brain, a rational device, since the brain then becomes the meeting place of the two great systems of integration, the nervous system and the endocrine system. Though the pupal diapause of the *Cecropia* silkworm results from the absence of the growth and differentiation hormone, it is noteworthy that the primary defect occurs at the level of brain function—the failure of the pupal brain to evoke adult development by triggering the function of the prothoracic glands (9, 13). The brain regains its secretory powers when exposed to the low temperatures of winter and, thus, the insect's life history is synchronized with the seasons (fig 3).

ENZYMOLGY OF METAMORPHOSIS

But the most interesting problem is still to be considered. Hormones are merely reagents. The reactants are the target organs, in this case the various tissues of the pupal insect. Between the hormonal reagent and the tissue reactants, a reaction must occur. And there is no reason why such a reaction should not be understandable in biochemical terms.

Now the simplest approach to the problem is diagrammed in figure 3. Let us assume that a biochemical defect is present in the cells of the diapausing pupa which prevents their metabolism from coupling to morphogenesis. This view would require the growth and differentiation hormone to promote some synthetic process which repairs the biochemical defect. I now propose to consider what evidence has been obtained as to the existence and identity of this hypothetical defect in the cells of the diapausing insect.

The first useful piece of information is the insensitivity of the diapausing pupa to cyanide. Though cyanide is one of our best insecticides, killing the caterpillar or the adult moth promptly, for the pupal insect it is not even a poison. Indeed, in our surgical maneuvers on pupae, we routinely use a Ringer's solution containing 0.01 M potassium cyanide. One can implant crystals of cyanide into a diapausing pupa without any ill effects. At the pH of the insect the agent is rapidly excreted through the tracheal system in the form of HCN. But before this occurs, its only

components, the pupal heart, for example, continues to beat normally when isolated in cyanide Ringer's. In the adult moth the situation is radically different. Here we find a well-developed cytochrome system in the vast majority of the insect's tissues. Evidently a remarkable synthesis of the cytochrome system takes place during adult development.

In order to appraise the matter in quantitative terms, cytochrome *c* was extracted from post-diapausing individuals and assayed spectrophotometrically (10, 12). Only a trace was found prior

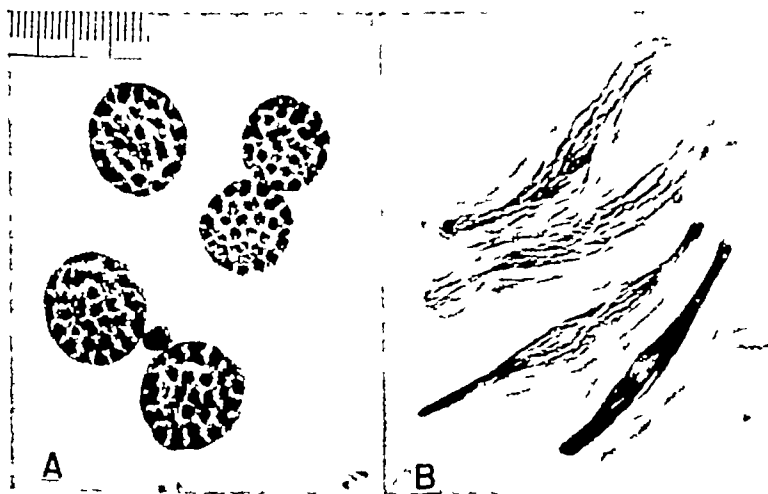
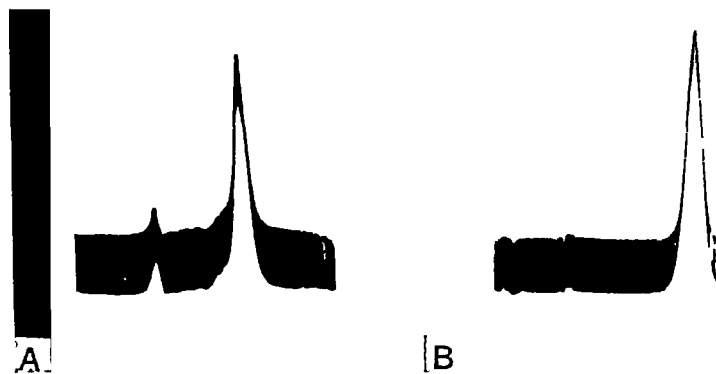


Fig 1 CYSTS OF DORMANT PUPAL spermatocytes in hanging-drop cultures. A The cysts have been cultured in inactive blood from a diapausing pupa and show no development. B The cysts have been cultured in a drop of active blood from a postdiapausing insect. The growth and differentiation hormone has caused each spermatocyte to undergo meiosis and prompt differentiation. Each cyst is thereby transformed into an elongate bundle of spermatids. Smallest division on scale equals 10 μ .

Fig 2 ELECTROPHORETIC patterns of dialyzed blood from post-diapausing pupae. A The still-active blood after heating for 5 minutes at 75°C. B Blood inactivated by 5 minutes' exposure to 80°C. The small peak, present in A and absent in B, is thought to consist in part of the growth and differentiation hormone.



obvious effect is to paralyze the intersegmental muscles of the abdomen. Attention therefore focusses on the status within the pupal insect of enzymes containing heavy metals and, more particularly, the iron-containing enzymes of the cytochrome system.

The curious insensitivity of the pupa to cyanide becomes intelligible when its tissues are studied spectroscopically. Under the Zeiss microspectroscope only the intersegmental muscles of the abdomen show the absorption bands of the three cytochromes, *b*, *c*, and *a* + *a*₃. Lacking these

to the actual initiation of adult development. The termination of diapause and the progress of adult development were found to be accompanied by a rapid and progressive synthesis of cytochrome *c*.

Recognizing the difficulty of extracting the enzyme quantitatively, the study was repeated by manometric assay of cytochrome *c* in the brei of insects at various stages of development (14-16). The results were essentially identical with those previously demonstrated by the spectrophotometric method. There can be little doubt that the synthesis of cytochrome *c* begins its

synchrony with the termination of diapause and continues in step with the progress of adult development

Cytochrome *b* and cytochrome oxidase were found to behave somewhat differently. Indeed the initiation of adult development is accompanied by a precipitous *decrease* in the activities of these enzymes. In the case of cytochrome oxidase, the initial decrease is followed by a rapid synthesis which begins on approximately the second day of development (12, 14). To the contrary, the low activity of succindehydrogenase presumably mirroring the function of cytochrome *b*, persists throughout the first half of adult development and only thereafter undergoes rapid increase (17). These changes in cytochrome *b* resemble in all respects the behavior of the two dehydrogenases we have studied, namely, malic dehydrogenase and α -glycerophosphate dehydro-

From these enzymatic studies we learn that the diapausing pupa, with the exception of its abdominal muscles, is characterized by a cyanide-stable flavoprotein functioning as terminal oxidase. At the outset of adult development this system begins to break down and is replaced by a cytochrome system containing an increasing titer of cytochrome *c* and a temporarily decreasing titer of cytochrome oxidase. Thus the termination of diapause is accompanied by profound alterations in two of the three great electron transmitters—flavoprotein and cytochrome. In the absence of the cytochrome mechanism, the flavoprotein system can apparently provide for maintenance, but not for mitotic activity or that forward progress which we recognize as growth. The coupling of growth to metabolism appears to be synchronized with the synthesis of the cytochrome mechanism.

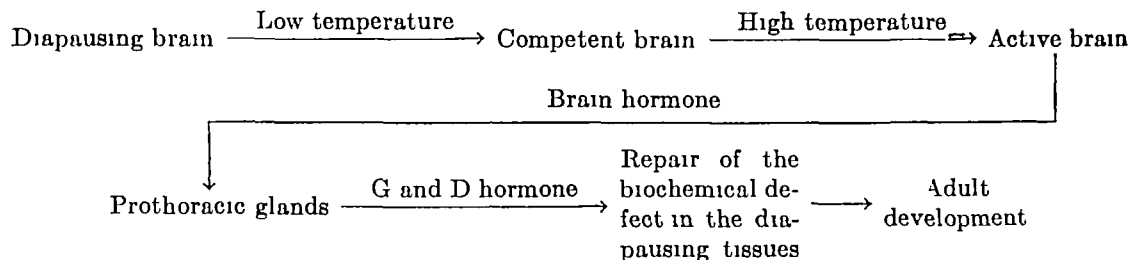


Fig 3 DIAGRAM of endocrine control of adult development by the brain and prothoracic glands. For explanation, see text.

genase. A resemblance is also evident between these results and those obtained by Agrell (18, 19).

Consequently, the activation of the growth mechanism at the outset of adult development and the sudden release of mitotic activity show a positive correlation with the behavior of cytochrome *c* and a negative correlation with the changes occurring in cytochrome *b*, cytochrome oxidase, and the two dehydrogenases that we have studied.

In the absence of the cytochrome system, the pupal insect is still able to consume oxygen apparently by the use of a flavoprotein as terminal oxidase. Biological assay of protein-bound riboflavin demonstrates a maximal titer of flavoprotein during diapause and a progressive decrease following the onset of adult development (14, 16). Since the autoxidation of flavoprotein produces hydrogen peroxide rather than water, it is noteworthy that the changes which it undergoes are paralleled by those occurring in catalase.

Our search for a biochemical defect in the diapausing insect has therefore revealed at least one such defect in terms of the absence of a functional cytochrome system. Is it possible that the cytochrome mechanism plays some special role in morphogenesis and that the absence of this system is the fundamental biochemical defect in the diapausing cells? I shall finally consider evidence that tends to answer this question in the affirmative.

If the function of the cytochrome system is prerequisite for morphogenesis, then growth should be brought to a standstill by inactivation of this enzyme system. Fortunately, in an animal such as an insect, where hemoglobin is absent and where environmental gases pass directly to the tissues through the tracheal system, it is possible to block the function of the cytochrome system by a highly specific agent, carbon monoxide. Only one difficulty is encountered: the combination of cytochrome oxidase with carbon monoxide is reversed by oxygen. The ratio between the ten-

sions of carbon monoxide and oxygen must therefore be high, in any case not less than about 20 to 1.

We have used two means to accomplish this end. In the first method the animals were placed in air-filled chambers and compressed with 3 to 10 atmospheres of carbon monoxide. In this manner the oxygen tension was held constant and the carbon monoxide tension and total pressure increased. In the second method the experiment was performed at a total pressure of one atmosphere, the animals being placed in a chamber and exposed to a mixture of 5 per cent oxygen and 95 per cent carbon monoxide. Thus the total pressure was held constant and the oxygen and carbon monoxide tensions varied.

Substantially the same results were obtained by the two methods (17, 20). Diapausing pupae were unaffected. Pupae just prior to the initiation of development remained alive but underwent no development during the experimental period of 21 days. In the case of animals that had already escaped from diapause, the carbon monoxide caused a complete or almost complete cessation of development and enforced an artificial diapause which persisted during the experimental period of 21 days. When returned to air such animals usually recovered promptly and resumed development where they had left off.

Results of this character consistently observed during the past three years, strongly suggest that the cytochrome system plays a crucial role in the metabolism of morphogenesis (21). We find this to be true, not only in the *Cecropia* silkworm, but also in the several other insects which we have examined, for example in the adult development of the blowfly *Phormia* and the parasitic wasp *Mormoniella*. Indeed, by exploiting the transparency of *Mormoniella*, it has been possible to demonstrate a partial reversibility by light of carbon monoxide's inhibition of growth (17). This result confirms a previous finding on *Drosophila* (22) and gives assurance that carbon monoxide's power to dissociate growth from maintenance results from its combination with cytochrome oxidase.

Among several hundred chemicals which we have tested, selective blockade of the growth mechanism has been accomplished by two further agents—diphtheria toxin and certain imidazole derivatives (23).

Injection of 0.1 to 1 γ of diphtheria toxin into mature caterpillars or post-diapausing pupae causes prompt cessation of development followed

by delayed death about a week thereafter. Diapausing pupae, however, survive 1000 times this dose of toxin. Its only visible effect is to render the intersegmental muscles of the abdomen flaccid and uncontractile.

These results become intelligible in terms of Pappenheimer's (24) previous studies where diphtheria toxin was found to correspond apparently to the free protein moiety of the diphtheria bacillus' cytochrome *b*. Its astonishing toxicity for the mammal is thought to result from its functional derangement of the cytochrome system.

Consequently, within the diapausing insect, the toxin finds no mechanism of action, save in the abdominal muscles, the only tissue possessing an intact cytochrome system. However, in the growing larva and developing adult, the reaction between the toxin and the cytochrome system causes a prompt cessation of growth.

It is possible to accomplish this same end by the use of imidazole and certain of its derivatives, namely, pilocarpine, dimethyl imidazole, and methyl imidazole (23). These substances are distinguished by their remarkable affinity for oxidized heme (hematin) with which they combine to form a stable hemichrome (25). Evidently, within the living insect they act as scavengers of hematin and render this important molecule unavailable for the synthesis of cytochrome. Though the pupal insect continues to live for a year or longer after the injection of pilocarpine, the onset of adult development is permanently prevented. In insects that have already terminated diapause and initiated the synthesis of the cytochrome system, pilocarpine is still capable of blocking development, though the threshold is now twenty times higher than that of the pupa. Developing animals blocked in this manner undergo delayed death several weeks following injection.

Summing up the information available at the present time, we find that the growth mechanism can be dissociated from the metabolism of maintenance by the action of several substances whose biochemical target within the living cell is the cytochrome system. As Needham (26) has prophetically remarked, "It almost looks, therefore, as if non-ferrous respiration cannot be geared to morphogenesis."

Our initial objective of understanding the biochemistry of insect growth and metamorphosis has therefore led us far afield—to a meeting place between energy and synthetic mechanism at the subcellular level. But as far as our initial and

humbler objective is concerned we feel assured that the synthesis of the cytochrome system from its hemochromogen precursors is causally related to the termination of pupal diapause. We are therefore persuaded to regard the absence of the cytochrome system as a basic biochemical defect

in the cells of the diapausing insect. Since the repair of this biochemical defect, as we have seen, is brought to pass by the 'growth and differentiation hormone,' the way seems open to an analysis of this endocrinological reaction in biochemical terms.

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FATE OF I¹³¹ LABELLED BOVINE GAMMA GLOBULIN IN RABBITS*

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THE TECHNIQUE of labelling and tracing protein antigens with radioactive iodine is being used increasingly in immunologic studies. Mention of a few of the more important chemical, physiological, and immunological features of this technique will indicate both its possibilities and its limitations. I¹³¹ can be added to almost any protein in traceable amounts without altering the protein immunologically. Accurate quantitation of I¹³¹ in blood, tissues, or immunologic precipitates is possible with a Geiger counter, using proper absorption and coincidence corrections. These radioactivity measurements indicate the concentration of antigen in the material tested. In addition, approximate histologic localization of the I¹³¹ antigen can be obtained with autoradiographs, in which ordinary histologic sections are mounted on special photographic emulsions and, in effect, take a picture of their own radioactivity. Autoradiographs do not, however, permit the detailed histologic localization possible with fluorescent antibody (1) or dye-labelled proteins (2, 3).

Protein antigens labelled with I¹³¹ appear to retain the I¹³¹ label *in vitro* (4) and *in vivo* (5) as long as they are immunologically active. The studies of Knox and Endicott (5), as well as our own observations, show close agreement between quantitative precipitin and radioactivity determinations of antigen.

Catabolism of I¹³¹ protein antigens liberates non-protein-bound I¹³¹, and in animals pre-

ferred ordinary iodide, this liberated I¹³¹ is rapidly eliminated, primarily in the urine with lesser amounts in breath and feces. Following injection of an I¹³¹ antigen, the radioactivity in blood and tissues can be separated into protein-bound and non-protein-bound fractions, the protein-bound activity represents residual antigen while the non-protein-bound I¹³¹ present in the blood and tissues, plus that excreted in the urine, provides a reliable index of the rate of antigen catabolism. This technique, therefore, gives an excellent quantitative measure of antigen concentration in blood and tissues and also indicates rate of antigen catabolism.

Before discussing our observations on the rabbit's handling of the antigen, I¹³¹-labelled bovine gamma globulin (I*-BGG), let us look first at the rabbit's metabolism of his own plasma globulin. Madden and Whipple (6) have shown that plasma proteins exist in dynamic equilibrium in vascular and extravascular fluids and serve to supply, in part, the nutritional needs of the body's tissues. Our findings using homologous globulin labelled with I¹³¹ (I*-RG) concurred with their's. Figure 1, which is a semilogarithmic plot of the labelled globulin content of blood against time in days, shows that within one day after intravenous injection there was a sharp drop in the I*-RG blood content to approximately one-third the amount injected. This drop represents the equilibration of I*-RG between vascular and extravascular components of the plasma protein pool and indicates twice as much globulin in extravascular as in vascular fluid. Once the equilibrium was established, the I*-RG disappeared at a constant rate with a half life of about 2 days,

¹ May 1, 1951, Cleveland, Ohio

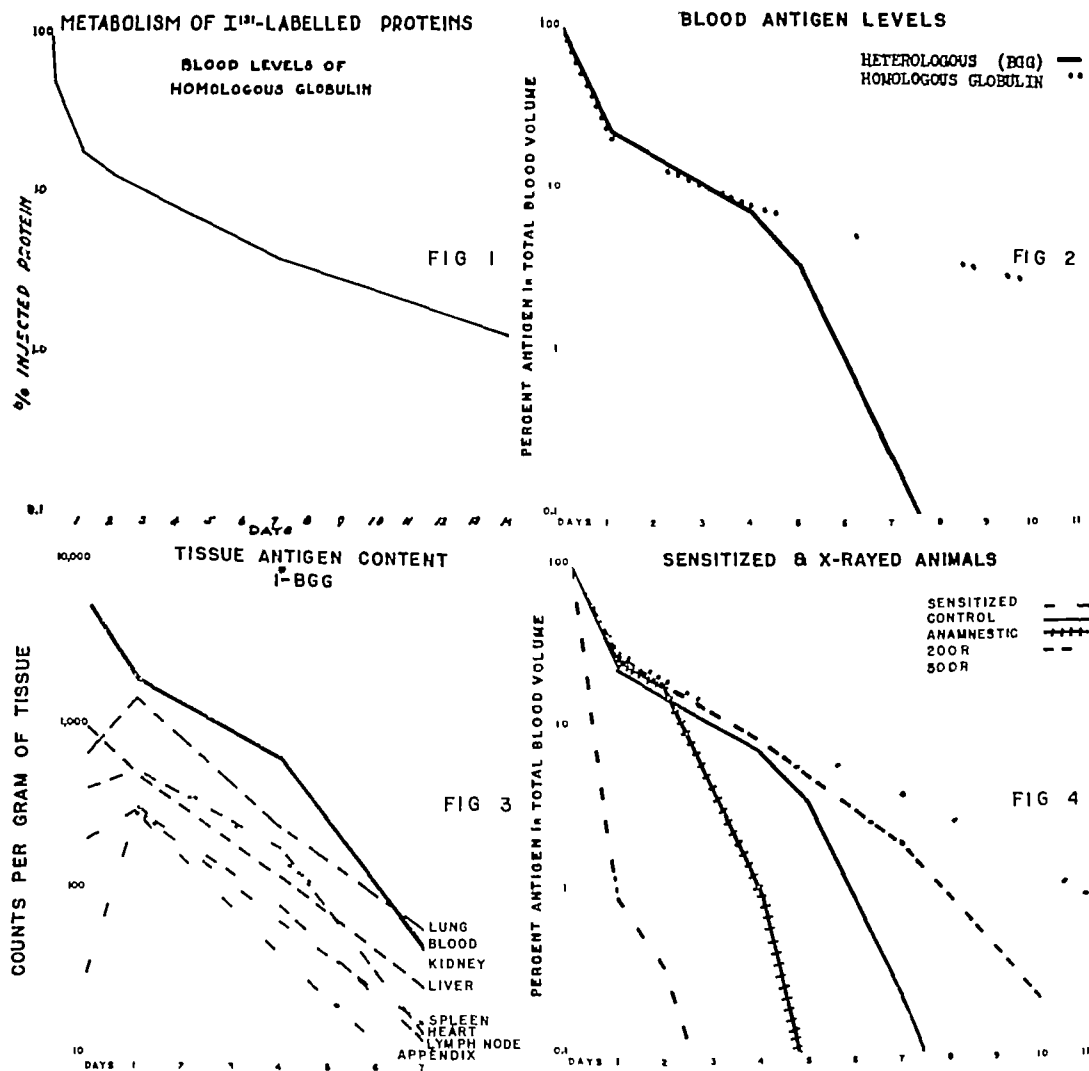
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representing the rate of catabolism of homologous globulin

I*-BGG injected intravenously into rabbits in doses varying from 1 to 500 mg was handled similarly to the I*-RG for the first 4 days (7). The second graph of blood content of labelled protein shows again the disappearance curve for homologous globulin. In addition, the solid line represents the amount of I*-BGG in the blood

globulin at a rate almost identical with that of homologous globulin for 4 days would further suggest that in this initial period the body utilizes the heterologous globulin similarly to its own globulin.

After the fourth day, the disappearance rate of I*-BGG from the blood increased sharply. It is most likely that this increased elimination rate is caused by the appearance of antibody which,



Figs 1 to 4

following a 75-mg injection and shows an equilibration period followed by a 3-day period of catabolism similar to that for the homologous protein. It appears that this antigenic, heterologous protein equilibrates between vascular and extravascular fluids just as does homologous protein. In so doing, the antigen, no doubt, comes into intimate contact with most or all of the body's tissues. The catabolism of heterologous

however, could not be detected by our complement-fixation technique² (8) until all antigen had

² All antibody determinations were done by the complement-fixation technique described by Kent *et al*. This method employs a dilution of antigen (1:500,000) which is optimal for the detection of minimal amounts of antibody with four 50 per cent hemolytic units of complement. The minimal amounts of antibody as detected are of the order of 10 μ g antibody nitrogen per cc of serum.

been eliminated, 7 days following injection. The first antibody to appear probably combines with antigen in extravascular or intravascular fluids and the combination is rapidly eliminated from the circulating fluids and catabolized, as will be discussed later. Since this is accomplished without measurable accumulation of antigen in any organ or tissue, it is evident that the breakdown of antigen is an extremely rapid process and/or one widespread throughout the body.

The elimination of I*-BGG from the various tissues approximately paralleled its elimination from blood, as would be expected with a dynamic equilibrium of the antigen in the body. In figure 3 the amount of radioactivity per gram of tissue or blood following an intravenous injection of 75 mg I*-BGG is plotted against time in days. The activity concentration was higher in blood than in any tissue, and disappearance rates were, in general, parallel with a slight lag in the disappearance of radioactivity from the tissues after the fourth day. All radioactivity levels were insignificant after the seventh day, with no tissue showing measurable retention of antigen. Complement-fixation tests for antigen in the tissues after the seventh day were also negative.

Detectable circulating antibody appeared on the seventh day after virtually all antigen had disappeared. This is in keeping with the findings of Hawn and Janeway, who found disappearance of BGG and appearance of antibody between one and two weeks following injection of 2 gm of antigen (9). Following injection of 75 mg of I*-BGG, the peak antibody level occurred on the tenth day and all detectable circulating antibody disappeared within approximately 20 days after antigen injection. It is apparent that the first antibody is produced in the presence of antigen, but that the major part of antibody production goes on in the complete absence of antigen.

We found considerable support for the view that the increased antigen disappearance rate seen after 4 days in I*-BGG injected control rabbits is caused by appearance of antibody. Figure 4 compares blood antigen levels in 5 experimental groups of rabbits, each with a different immune response. Actively sensitized rabbits with detectable circulating antibody present at the time of injection of antigen showed rapid disappearance of antigen immediately, previously sensitized animals without detectable circulating antibody showed a rapid antigen disappearance beginning 2 days after injection, in keeping with an accelerated, anamnestic response, animals receiving 200 r whole-body γ -radiation showed

delay in and decrease of rapid antigen elimination, corresponding to the inhibition of the immune response resulting from the radiation, animals receiving 500 r γ -radiation showed neither rapid antigen elimination nor any immune response. The appearance of detectable serum antibody in these 5 groups of rabbits always followed immediately upon the rapid disappearance of antigen, as seen in figure 5. Here the 5 antigen disappearance curves are given in temporal relation to the appearance of complement-fixing antibody, indicated by the black bars. In the first 4 instances, rapid antigen disappearance was closely followed by appearance of detectable antibody, while after 500 r neither rapid antigen elimination nor detectable antibody was seen.

Further support for the relationship of antibody to rapid elimination of antigen was found after injection of passive antibody into rabbits

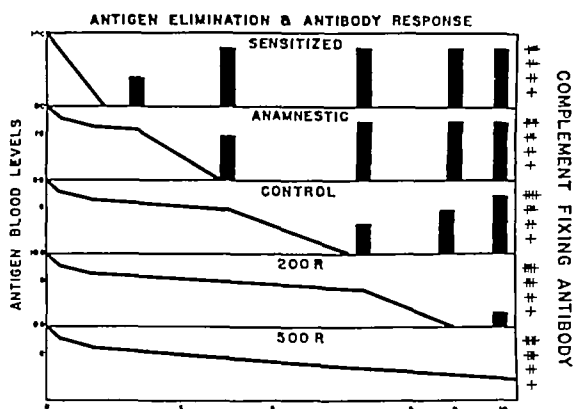


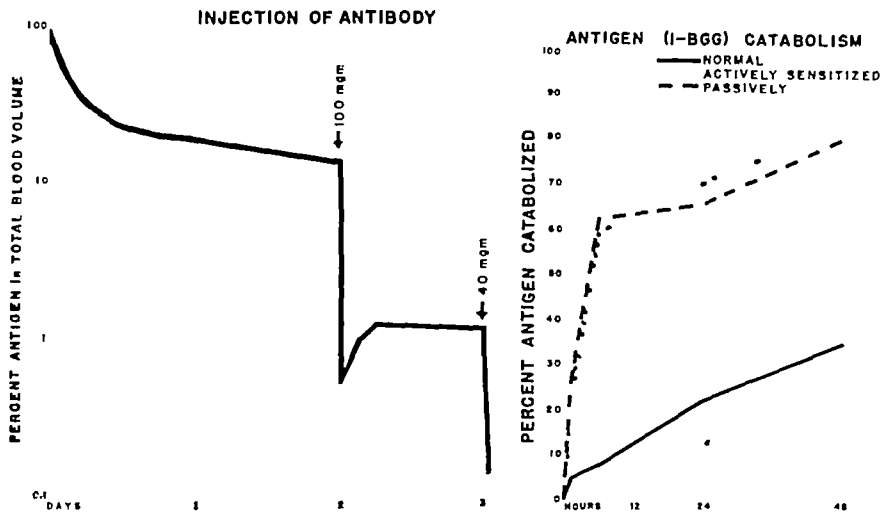
Fig 5

with circulating I*-BGG. In figure 6 the I*-BGG concentration in blood is plotted against an expanded time scale. The injection of passive antibody, 100 mg on second day and 40 mg on third day, caused a prompt drop in blood antigen content. The rise in blood antigen level shortly after the first injection represents the re-establishment of equilibrium of antigen between the depleted vascular fluid and the antigen-containing extravascular fluid.

On the basis of these interrelationships between antigen disappearance rate (ADR) and appearance of antibody, we have used the mathematical expression of ADR as an index of immune response (10). This measure is extremely sensitive and will reveal an immune response which may produce only enough antibody to combine with the antigen present, and not enough to cause a detectable excess in the serum. Such weak antibody responses may occur after moderate

doses of x-ray or following extremely small doses of antigen and result in rapid antigen elimination without detectable antibody. Later demonstration of a specific anamnestic response indicates that such animals have made a previous immune response to the given antigen.

removal of antigen from the blood. In investigating antigen catabolism, we attempted to find whether the increased rate of catabolism in sensitized animals could be accounted for alone by presence of antibody, and therefore, presumably by increased phagocytosis or whether there was,



Figs 6 (left) and 7 (right)

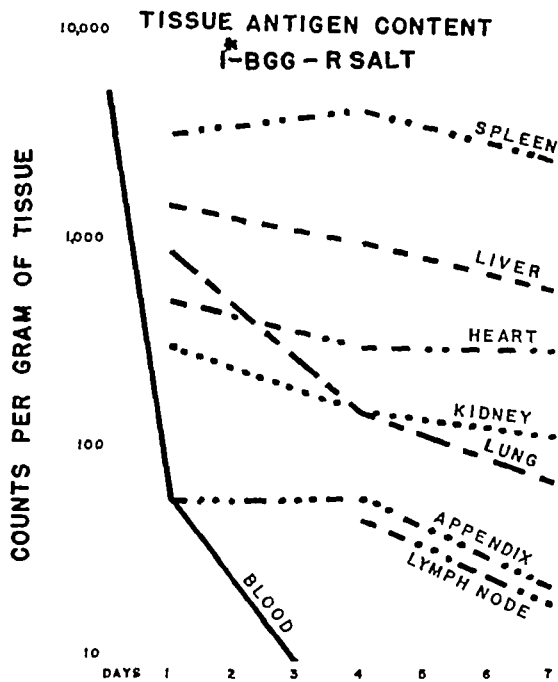


Fig 8

The mechanism of rapid elimination of the antigen-antibody combination from the blood and the associated rapid antigen catabolism are not completely understood. It is most likely that antigen-antibody complexes would be taken up by phagocytic cells, thus accounting for the rapid

also, a cellular enzymatic adaptation associated with the process of sensitization, which facilitated more rapid antigen catabolism (11). To do this, we compared the rates of I*-BGG catabolism in actively sensitized rabbits and in control rabbits given 95 mg of passive antibody, an amount calculated to be approximately equal to the amount in those actively sensitized. Figure 7 shows the rates of I*-BGG catabolism, as measured by non-protein bound I¹³¹ in urine, blood, and tissues in actively sensitized, passively sensitized, and control rabbits for the first 48 hours after intravenous injection. The rates of antigen catabolism for actively and passively sensitized rabbits were almost identical. The antigen disappearance rates in actively and passively sensitized groups were also similar. It appears, therefore, that both the increased rate of disappearance of I*-BGG from the blood and the rapid catabolism found in sensitized animals can be accounted for by the presence of antibody alone. Whether the sensitized animal makes any physiologic or enzymatic adaptation to an antigen such as I*-BGG other than the production of specific antibody cannot be definitely stated at this time. However, there is nothing that happens in the immune catabolism of I*-BGG that cannot be duplicated in a control animal by addition of passive antibody.

These findings agree with the antigen tracer study of human globulin using the fluorescent antibody technique (1). There is, however, a marked discrepancy between our findings and those obtained by workers using BGG or similar proteins labelled with various azo dyes (3). The azo dye-labelled proteins were found concentrated and retained for long periods of time in the reticulo-endothelial system. In an effort to explain these differences, we attached to I*-BGG by azo conjugation one of two compounds, R salt (12, 13) or para-aminobenzoic acid, and traced these doubly labelled antigens by both color and radioactivity. Using these antigens, we found an uptake and retention of antigen by the reticulo-endothelial system, just as described by those using the various azo dye-labelled proteins. Figure 8 indicates azo-I*-BGG concentration in blood and tissues following intravenous injection of 75 mg of the antigen. The blood level fell rapidly while tissue concentrations, especially in those tissues rich in reticulo-endothelial cells, dropped very slowly. The tissue retention of azo-I*-BGG is in marked contrast to the findings

with I*-BGG where no tissue antigen retention occurred (fig. 3). Thus, it appears that azo labelling markedly changes the *in vivo* behavior of protein antigens such as BGG.

SUMMARY

Tracing of the antigen I*-BGG in rabbits indicates that the antigen is handled much like the animal's own globulin until antibodies to it are formed. Before the appearance of antibodies, the antigen exists in equilibrium between vascular and extravascular fluids, has free access to the tissues of the body, and is neither concentrated nor retained by any tissue or organ. With the appearance of antibody, the antigen is rapidly removed from blood and extravascular fluids, probably by phagocytic cells and is rapidly catabolized. There does not appear to be any cellular enzymatic adaptation necessary in the sensitized, rapid catabolism of antigen. Antibody production, although initiated in the presence of antigen, is carried on long after all antigen has disappeared.

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FLUORESCENT ANTIBODIES AS HISTOCHEMICAL TOOLS¹

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THE ANTIBODY molecule can be conjugated with fluorescent compounds without apparent loss of specificity (1-3). This fact makes it possible to employ the precipitin reaction as a method for the histochemical detection of small quantities of material in tissue cells. The only basic requirement for success is that the material sought be sufficiently antigenic so that the requisite anti-serum can be produced.

Purified antisera were chemically conjugated with fluorescein isocyanate to produce a brilliantly fluorescent protein solution in which the antibody molecules as well as the other protein molecules are fluorescent. When such a fluorescent antibody solution is allowed to react with organisms or tissue cells containing homologous antigen, some of the antibody is precipitated over those areas where the antigen is located. The excess of the fluorescent solution can be washed away and the deposited antibody visualized under the fluorescence microscope. This reaction has so far been successfully employed to trace the fate of injected bacterial polysaccharides and foreign animal proteins after injection into mice, and to localize viral and rickettsial antigens in infected cells. In the brief time available, I will summarize the successes which we have so far had in the use of this method, list the failures and point out some of the difficulties.

When foreign substances such as bacterial polysaccharides (4) or animal proteins (5) are injected intravenously into mice, they rapidly begin to leave the blood stream and make their appearance in the connective tissue throughout the animal. They are also found in high concentration in all the phagocytic cells in the body. They can also be found in a low but distinct concentration in lymphocytes in the nodules of the spleen and lymph nodes. In general, the epithelial cells of the animal are well protected against the entrance of these antigenic materials but they all made an appearance in the hepatic cells and in

the endothelium of the renal tubules. There are minor differences in their distribution which there is no time to describe.

The most striking difference among the antigens so far studied is their rate of disappearance. Crystalline egg albumin (dose 10 mg) disappeared within a few hours. Crystalline bovine serum albumin persisted for about 2 days. Human plasma gamma globulin (dose 4 mg) persisted for a week. It is apparent that the rate of disappearance is inversely proportional in a rough way to the molecular size of the antigen. In great contrast to this rather rapid disappearance of protein antigens, the capsular polysaccharides of pneumococcus type II, pneumococcus type III, and Friedländer bacillus type B, injected in comparable doses, persisted for weeks to months. In the case of all six of these antigens, the connective tissue and the phagocytic cells of the reticulo-endothelial system contained antigen after it had disappeared from other locations.

An unexpected finding was that the protein antigens, or fragments of them, could regularly be found in the nuclei of all the cells into which they penetrated. This confirms the data of Crampton and Haurowitz, who found radioactivity in the nuclear fractions of liver homogenates after the injection of proteins labelled with I¹³¹ (6).

The method is not only useful in tracing injected foreign proteins or polysaccharides, its utility has already been demonstrated in the study of cells infected with rickettsiae and with viruses (7). For example, the cells of the monkey parotid infected with mumps virus can be readily visualized by use of labelled antibody against the mumps virus. Similarly, the rickettsiae of epidemic typhus and Rocky Mountain spotted fever have been found in infected cells in the liver and spleen of the cotton rat and in smears of the gastrointestinal tract of individual infected lice. An extensive study is now being carried out by Dr. Barbara Watson of the progress of mumps infection in the embryonated hen's egg. Two other viruses with which preliminary experiments have been successful are

¹ Aided by grants from the Life Insurance Medical Research Fund, the Helen Hay Whitney Foundation and the Eugene Higgins Trust.

influenza A virus in the infected mouse lung and in the infected hen's egg embryo, and the virus of canine hepatitis in the organs of infected dogs. Work with the hepatitis virus is being carried out in collaboration with Dr D L Coffin.

Attempts to perform the same sort of experiments with neurotropic viruses have so far failed. Although it was possible to demonstrate mumps virus in the brain of a monkey with experimental parotitis but without neurological symptoms (8), similar attempts with the Columbia strain of SK virus and Theiler's virus GDVII have been unsuccessful. Attempts to find the Lansing strain of poliomyelitis virus in infected monkey cord and brain and in tissue cultures from human skin and brain have so far failed (9).

The major pitfall in the application of this method is the very regular appearance of what we have hitherto called 'non-specific staining' (3). It is already becoming clear that much of this non-specific staining is the result of so-called natural antibody, and due to the possession of

common antigens by animal tissues of different species and bacteria with which the serum donors are frequently infected. So far in our studies, we have avoided this non-specific staining by absorbing our conjugated antisera with normal tissue from animals whose tissues it was proposed to study. But it is probable that this method is only successful when the normal tissue used for absorption contains appreciable amounts of the antigen belonging to the interfering antigen-antibody system. Much remains still to be worked out with regard to such cross reactions.

Despite this difficulty, the use of fluorescein-labelled antibody as a specific histochemical reagent has already proved to be a useful tool in the localization of antigenic materials in tissue cells. It has the disadvantage inherent in other histochemical methods in that it is difficult to quantitate, particularly so because the amount of light coming from any given cell is very small. Its advantage lies in its apparent high sensitivity, and in its specificity.

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IMMUNOCHEMICAL STUDIES WITH LABELLED ANTIGENS¹

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PREVIOUS experiments with arsanil-azo-proteins had demonstrated that most of the antigen injected into rabbits disappears rapidly from the blood and passes into liver, spleen, bone marrow and lung (1). Similar results are obtained with iodo-ovalbumin tagged with I^{131} (2). Table 1 shows the approximate activity of various organs at different times after the injection of iodinated ovalbumin.

It is apparent from the figures recorded in table 1 that the antigen content of the organs decreases rapidly during the first hours after injection of the antigen, but that the decrease thereafter is very slow. At the end of 29 days the liver still contains 0.018 per cent of the injected activity. If this were present as unchanged iodo-ovalbumin, it would correspond to 15.5 μ g, or 2×10^{14} molecules of iodo-ovalbumin in 62 gm of liver. Since the volume of a liver cell is about 10^{-9} to 10^{-8} ml, each cell would contain approximately 10,000 antigen molecules at the end of the 29th day. Results similar to those recorded in table 1 were obtained when the amount of iodo-ovalbumin injected was reduced to 9 to 11 mg or when the iodo-ovalbumin was replaced by iodinated beef serum globulin.

In a series of experiments we injected in rabbits 2 to 5 doses of iodo-ovalbumin in intervals of 3 days. Only the last of these injections was labelled by I^{131} . The rabbits were killed 24 hours after the last injection. The distribution of the activity in the organs and in the liver fractions was the same as that which followed a single injection of I^{131} -iodo-ovalbumin.

The most interesting result of our experiments is the finding that the injected antigen is rapidly incorporated into the small granules (microsomes) and that it appears shortly thereafter in the large granules (mitochondria) where it remains for long periods of time. The separation of the granules from nuclei and from the supernatant

solution (table 1) was achieved by fractional centrifugation (2, 3). The yields of nuclei, mitochondria and microsomes were about 20 per cent of the total liver homogenate for each of these fractions, while the supernatant solution formed 40 per cent of the homogenate. All four fractions were precipitated by trichloroacetic acid and the precipitated proteins washed with acetone and ethanol. Table 1 shows the relative activities of the washed proteins of the subcellular fractions. When radio-iodinated ovalbumin was added to a normal rabbit liver homogenate under the conditions used in our procedure, only traces were adsorbed to the granules, while the bulk was found in the supernatant solution. This proves that incorporation of the antigen into the granules occurs *in vivo* and not during the fractionation. The finding of the intravenously injected antigen in cellular granules indicates that it passes rapidly through the membranes of the liver cells. Since the molecules of ovalbumin and beef serum globulin are macromolecules which are not able to pass through semipermeable membranes, we have to assume that their incorporation into the liver cells occurs by some process akin to phagocytosis.

The initial high activity of the microsomes and the subsequent appearance of most of the antigen in the mitochondria suggest that the antigen is at first adsorbed to the large surface of the small granules and that it later passes into the larger granules. Another possibility to be considered is the incorporation of the microsomes with their load of antigen into the large granules.

The persistence of the antigen in the large granules is particularly interesting because the mitochondria have been considered as self-reproducing units, i.e. as sites of protein synthesis (4). Possibly they are also the sites of antibody synthesis. This is in accordance with our view that antibody synthesis is essentially protein synthesis modified by the presence of antigen (5).

The distinct activity of the nuclei (table 1) is in agreement with recent findings of Coons and his co-workers (6). It is not clear from our experiments whether the presence of antigen in the nuclei is significant, and whether the nuclei are to be considered as antibody producing sites. The fact that immunity is never transmitted to de-

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² Predoctorate Fellow of the U. S. Public Health Service.

scendants makes a role of the nuclei in antibody synthesis improbable

Labelled antigens were also used in an attempt to elucidate the mechanism of the combination of antigen with antibody. Rabbits were immunized by injections of azoproteins and the immune sera were precipitated by the homologous

to the equation $GB_n \rightarrow GB_{n-1} + B$, where G = antigen and B = antibody. The dissociation constants found were of the order $K = 10^{-6}$ to 10^{-7} . The thermodynamical constants for combination of antigen and antibody, calculated from the values of $1/K$ at two temperatures, are recorded in table 2.

TABLE 1 INTRAVENOUS INJECTION OF IODO-OVALBUMIN INTO RABBITS

AMOUNT OF ANTIGEN INJECTED (MG) TIME (IN HOURS AFTER INJECTION)	38 0 083	80 1 25	70 9 25	43 91	65 212	65 380	86 696
Blood	65 6	13 9	0 947	0 062	0 025	0 014	
Spleen	0 31	0 38	0 050	0 0013	0 0009	0 0011	
Lungs	3 76	0 30	0 042	0 0072	0 0058	0 0049	0 0026
Kidneys	2 07	1 24	0 157	0 024	0 065	0 013	0 0063
Liver	16 1	16 9	2 52	0 151	0 088	0 048	0 018
Liver fractions							
Nuclei	0 81	0 78	0 93	1 49	1 57	1 77	
Mitochondria	1 74	3 58	3 03	2 58	3 31	3 90	
Microsomes	1 95	1 02	0 69	0 29	0 25	0 21	
Supernatant	0 53	0 30	0 34	0 44	0 42	0 39	

The figures pertaining to organs indicate percentage of the injected activity, those pertaining to liver fractions indicate relative activities, when the activity of the liver homogenate in counts per minute per milligram is set equal to 1.0

TABLE 2 THERMODYNAMICAL CONSTANTS FOR THE COMBINATION OF ANTIGEN AND ANTIBODY

Type	ANTIGEN	Amount	IMMUNE SERUM	t_1	ΔF_1	t_2	ΔF_2	ΔH	ΔS
		mg	ml	°C		°C			
Arsanil-beef serum globulin		2 0	6 5	5	-8 0	25	-8 4	-2 0	21
		4 0	6 5	5	-8 0	25	-9 3	-2 1	21
Sulfanilovalbumin		2 0	11 0	4	-8 5	29	-9 0	-2 7	21
		4 0	11 0	4	-8 7	29	-9 2	-3 0	21

ΔF and ΔH in kcal/mole, ΔS in kcal/mole/degree

antigens. The precipitates were washed with small amounts of 0.9 per cent saline solution, then suspended in large volumes (35 ml) of saline solution for the determination of their solubility at different temperatures. After centrifugation it was found that the supernatant solution contained mainly antibody, while only traces of antigen were dissolved. Evidently, the precipitated antigen-antibody complex dissociates according

to the equation $GB_n \rightarrow GB_{n-1} + B$, where G = antigen and B = antibody. The dissociation constants found were of the order $K = 10^{-6}$ to 10^{-7} . The thermodynamical constants for combination of antigen and antibody, calculated from the values of $1/K$ at two temperatures, are recorded in table 2.

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ANTIBODY FORMATION IN SURVIVING TISSUES¹

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AN ATTEMPT has been made to develop a technique for determining the capacity of various tissues for synthesizing antibodies *in vitro*. This technique is designed to avoid the difficulty of determining whether antibody present in a tissue has been synthesized by that tissue or whether it has been transported to the tissue from its origin at another site.

By immunizing animals and sacrificing them at a time when their serum antibody titer is high and, presumably, when antibody is being synthesized actively, it is possible to obtain surviving tissues which are still capable of forming antibody. Incubation of a tissue, which is synthesizing antibody, with an isotopically labeled amino acid permits the utilization of the amino acid for the synthesis of new antibody protein. The capacity of a tissue for forming antibody may be indicated therefore by the presence of isotope in the antibody which is obtained by precipitation with its specific antigen.

METHOD

Young adult rabbits were injected intravenously with a suspension of formalin-killed type VIII pneumococci. Approximately 4 billion organisms were injected 3 or 4 times per week, and the animals were killed by exsanguination after a minimum of 6 weeks of such immunization. Tissues were removed and slices of 0.5-mm thickness were prepared. Approximately 10 to 15 gm of wet tissue (less in the case of spleen control) were placed in 50 cc of medium of the following composition (mm/l): Na, 142.5; K, 10; Ca, 2.5; Cl, 117.5; HCO₃, 40. About 7000 u of penicillin and 10 mg of C¹⁴ carboxyl-labeled glycine (CH₂NH₂C¹⁴OOH) were added to each flask. A stream of 95 per cent O₂ and 5 per cent CO₂ was

passed through the mixtures for 1 to 3 minutes, the flasks were then stoppered, and the preparations were incubated with agitation for 4 hours at 37° C. After incubation, the medium was separated from the tissue slices by centrifugation at high speed. The clear medium was concentrated 5- to 10-fold by negative pressure dialysis.

TABLE 1 C¹⁴ ACTIVITY OF PNEUMOCOCCUS VIII SPECIFIC PRECIPITATE

TISSUE	WET WEIGHT OF TOTAL TISSUE INCUBATED	TOTAL AMOUNT OF CH ₂ NH ₂ C ¹⁴ OOH ¹ ADDED	TOTAL ANTI BODY N	C ¹⁴ ACTIVITY ² OF SPECIFIC PRECIPITATE
	gm	mg	mg	counts/min
<i>Liver</i>				
Control ³	90	90	25.0	2
Experimental	180	120	4.6	93
<i>Kidney</i>				
Control ³	20	20	6.4	8
Experimental	30	30	1.9	7
<i>Spleen</i>				
Control ³	4	10	4.4	0
Experimental	10	10	0.25	630 ⁴

¹ C¹⁴ activity of the glycine is 1.25×10^5 counts per minute per standard dish of glycine of infinite thickness.

² Expressed as C¹⁴ activity per standard dish of specific precipitate of infinite thickness.

³ Tissues from a non-immunized animal with pneumococcus VIII antiserum added to the medium.

⁴ This value is approximate because of the correction of the actual counts (126) to counts at infinite thickness.

against buffered saline. Antibody in the medium was precipitated by the addition of an appropriate amount of pneumococcus type VIII polysaccharide. The specific precipitate was washed with cold saline three or four times and determinations of the radioactivity of the specific precipitate were carried out with a Q-gas counter. All samples were counted for a minimum of 30 min-

¹ This work was supported by a grant from the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council.

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utes, the radioactivity is expressed as counts per minute above background

The possibility that the radioactivity of the specific precipitate might be due to adsorption or co-precipitation of radioactive glycine or of any of its derivatives was considered. To some extent, dialysis reduced the possibility of adsorption, but to test the second possibility the following control experiment was done. Tissues from a normal non-immunized rabbit were removed and prepared in the same manner as in the previous experiment, in addition, however, serum from a rabbit which had been immunized to pneumococcus type VIII was added to the medium before incubation. The preparations were incubated, centrifuged, dialyzed, and the antibody precipitated with type VIII polysaccharide.

RESULTS

Results of these experiments are presented in table 1. Work is now in progress to confirm and to extend these preliminary findings.

The results indicate that the spleen and liver are capable of synthesizing antibody *in vitro*. Synthesis of antibody by splenic tissue has been

described by Parker (1), Fagraeus (2), and Roberts and White (3). Thus far we have been unable to demonstrate such synthesis in kidney tissue. The absence of significant radioactivity in the control indicates that non-specific adsorption or co-precipitation of isotopically labeled products does not account for the isotope activity observed in the specific precipitates of the experimental preparations. However, control preparations differ from the experimental preparations in this respect: in the latter some antibody is presumably intracellular and may be exposed to conditions different from those of the control preparations in which the added antibody may remain extracellular. Whether this consideration is significant cannot be determined at the present time.

DISCUSSION

These findings indicate that this technique affords a means of demonstrating the ability of tissues to form antibodies *in vitro*. By studying the ability of individual tissues and of smaller structural units to synthesize antibody, it may be possible to delineate more clearly the sites of antibody formation.

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BEHAVIOR AND PERSISTENCE OF AZOPROTEIN TRACER ANTIGENS IN MICE¹

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TO DETERMINE the sites from which the initial stimuli to antibody formation arise the fate of certain tracer antigens is under study. A recent paper (1) describes the preparation of some deeply blue, antigenic azoproteins, and the methods for ridding these preparations of unlinked or free dye. Following intravenous injections of these substances into mice some sort of blue material appears in various tissues and remains in them for weeks. However, the visible presence of color in tissues, like the demonstration of radioactivity following injections of radioactive tracer antigens, is no proof that intact antigen is localized in the cells. The dye may be split off rapidly while the dye protein is still circulating in the blood, so that free dye enters the cells, or, the dye may be split off from the protein after it enters the cells, and there be retained. Further, the protein portion of the azoprotein may be broken down sufficiently to lose its antigenicity, either while in the blood or soon after being taken up by the tissues.

To eliminate the first of these possibilities an extremely diffusible dye, echt-saure-blau, has been diazotized and coupled to either bovine γ -globulin (1) or to human albumin.

This dye is not degraded in the bodies of mice injected with it, instead it is so diffusible that it is rapidly eliminated from the body in 2 or 3 hours without being stored intracellularly as granules, or in vacuoles. The dye does not, by itself, form combinations with proteins. Clearly, if such a dye, coupled to a protein and injected into mice, should become split off from its protein while in the blood stream, no blue matter would appear in the cells, if split off after its

entrance into the cells, it would be rapidly eliminated from the body.

By contrast, injections of the two azoproteins, formed from this dye, unlike injections of the dye itself lead to the progressive accumulation and storage of blue granules in many organs, especially in the Kupffer cells of the liver, reticulo-endothelial cells throughout the body, and in the kidney tubules.² Consequently some sort of linked dye protein, not necessarily intact antigen, is taken up and stored. Is this material the original antigen?

STORED BLUE MATERIAL IS RETAINED ANTIGEN

Complement-fixation tests readily answered this question when carried out upon liver emulsions and blood from mice injected intravenously with the azoglobulin. Soon after the injections the deeply colored blood yielded strong reactions for antigen, the lightly colored liver tissue contained some too, but in smaller amounts, a finding that might be attributed to liver blood. However, as time passed, both color and antigen titer decreased in the blood and increased in the liver, until, in two or three days the organ held far more of both than the blood (1). The blue matter accumulating in the liver seemed to be active antigen. After a fortnight only faint traces of antigen remained in the blood, but, by contrast, the blue material, whether antigen or not, was retained much longer in the livers and mesenteric lymph nodes, indeed all the intracellular colored matter did not disappear from these organs for 85 to 120 days.

The finding suggested that antigens may persist in very small amounts, within certain cells, longer than is generally supposed, thereby stimulating prolonged antibody formation. To investigate the matter it seemed wise to study the

¹ The present note summarizes briefly some findings come upon in this laboratory during the course of work carried out with certain azoprotein tracer antigens. Since some of the work has already been published and the remainder is in the process of publication *in extenso* elsewhere, descriptions of the physiological reactions here mentioned and the presentation of data will be omitted.

² Very recent work has shown species differences in the distribution of these azoproteins. In rabbits, for example, the kidney tubules take up far more of the blue material than the livers or mesenteric nodes, although both of the latter contain much. This is the reverse of the state of affairs in the mouse.

persistence of a natural antigen, preferably the protein from which the artificial tracer was derived, because, should the natural antigen be destroyed rapidly in the body, the fate of the artificial tracer would be of little interest. Accordingly an attempt was made to determine the full span of persistence of even the faintest traces of bovine γ -globulin detectable in the blood and certain organs of mice, intravenously injected with it. Since it seemed most unlikely that the minute amounts of antigen to be detected could be successfully extracted from organs for complement-fixation tests, resort was had to a more delicate method for the pursuit of the problem.

EXTREMELY SENSITIVE TEST FOR DETECTION OF TRACES OF ANTIGEN WITHIN TISSUES AND BODY FLUIDS

An earlier paper (2) has described a technique by which extraordinary spasms and other specific vascular reactions in the blood vessels of the ears of anaphylactically sensitive mice are rendered visible under the microscope when the animals are challenged with an intravenous, shocking dose of antigen. These reactions occur even in mice too weakly sensitive to show any of the other signs of anaphylaxis. It now appears that similar ear vascular reactions, hereafter termed EVR, occur in mice injected with various antigens and challenged later with appropriate antisera (reversed passive anaphylaxis). The EVR are so sensitive that one can inject as little as 0.5 to 0.1 μ g of bovine γ -globulin, as antigen, into the peritoneal cavities of normal mice of the Rockefeller Institute strain³ and detect the absorption of some of it by a subsequent injection of very strong rabbit anti-bovine γ -globulin serum. A description of these reactions and their differentiation from certain physiological reactions which occur from time to time in the ears of intravenously injected, normal mice will be presented fully elsewhere (3), in a paper now in press, since the matter is a complicated one, and it cannot be adequately presented in a brief summary.

The extreme sensitivity of the EVR made it seem likely that mice could be used to advantage, as test animals, to detect the presence of truly minute traces of antigen persisting in the tissues

or body fluids of animals previously injected with it.

TESTS FOR PRESENCE OF ANTIGEN PERSISTING IN BLOOD OR TISSUES

Normal mice were injected intravenously with 5 mg of bovine γ -globulin (0.1 ml of a 5% solution) per 30 gm of body weight. At various intervals samples of blood and ground liver, or mesenteric nodes, from these animals (donors) were injected intraperitoneally into normal mice (recipients). Two days later, before antibody formation could take place, the recipients were intravenously injected with strong, rabbit anti-bovine γ -globulin serum. The occurrence of the specific type of EVR indicated the absorption, from the peritoneum, of antigen present in the materials transferred from the donor mice. Suitable controls were employed at all times.

PERSISTENCE OF ANTIGEN

In two series of experiments recipient mice showed specific EVR after transfer of blood from donors injected with the antigen 56 days previously. All reactions after longer periods were negative. The transfer of liver tissue, up to and including 101 days after injecting the donors with antigen, sensitized the recipients sufficiently to yield positive, specific EVR upon the injection of the antiserum. There was therefore a striking similarity in the period of persistence of the bovine γ -globulin, as antigen, in the livers of these donors (101 days) and that of the blue material, whether antigenic or not, in the livers of the other mice injected with the azoglobulin (85-120 days), as mentioned above. The finding called for a direct experiment to find out: Does the transfer of tissues from donor mice, injected with an azoglobulin, to normal recipients lead to the production of positive EVR as long as the transferred tissues show the visible presence of colored material of some sort in them? If so, it is most likely that the colored material is still the antigen.

AN AZOPROTEIN INJECTED INTO MICE SEEMS TO RETAIN ITS ANTIGENICITY AS LONG AS COLOR REMAINS VISIBLE IN TISSUES

A direct test was accordingly made. Since the 101-day period, over which positive EVR had been obtained by the transfer of liver tissue from mice injected with plain bovine γ -globulin, was so much like the 85- to 120-day persistence of color in the livers of other mice injected with

³ Different strains of mice have now been found to vary enormously in their sensitivity to reversed passive anaphylaxis. The strain used for this work is especially sensitive.

the azoglobulin, presumptive evidence seemed to be already at hand to indicate that the colored matter was still antigenic, at least as far as the azoglobulin was concerned. It seemed wise therefore to employ a different protein for the direct test, and, by so doing, to answer not only the question asked above but also two other related questions. Does an azoprotein persist, as an antigen, for the same length of time as the protein from which it is derived, and, do different proteins persist, as antigens, for unequal periods of time?

To answer these questions, a new azoprotein, prepared by coupling to human albumin the same dye that had previously been linked to bovine γ -globulin, was injected into scores of mice, as donors. At weekly intervals thereafter the presence of antigen, persisting in the blood and certain tissues in the donors, was sought by the detection tests mentioned above. As time passed, the blue material, stored in the tissues of these mice injected with the azoalbumin, disappeared far more rapidly than had the colored matter after like injections of the azoglobulin. At the 23rd day the recipients, which got transferred blood from these donors, no longer showed positive, specific EVR. At the 36th day transfer tests made with ground liver tissue, which contained only faint traces of blue material, yielded very weak, positive EVR in the recipients. The transfer of ground mesenteric nodes, which contained more persisting blue material than the livers, gave somewhat stronger detection tests (EVR) for antigen in other recipients.

Highly significant were two series of tests performed 42 and 43 days after injecting the azoalbumin into the donor mice. The livers of these animals showed no remaining blue when examined grossly, or when magnified at low powers (10 or 20 times), under a dissecting microscope. By contrast, the mesenteric lymph nodes held faint traces of blue. The recipients that got transferred liver tissue from these donors yielded negative EVR, whereas transfer of the faintly colored nodal tissue gave positive EVR. Clearly, as long as the blue material remained visible in the tissues, the detection tests for antigen remained positive.

PLAIN ALBUMIN AND DYE-AZOALBUMIN YIELD POSITIVE DETECTION TESTS FOR SIMILAR PERIODS

Scores of other donor mice were intravenously injected with plain human albumin, instead of

the azoalbumin, and transfer tests from these donors were carried out in the same manner. The EVR in the recipient mice became negative at the same time intervals as in the experiments with donor mice injected with the coupled azoalbumin, that is to say, the protein and the azoprotein derived from it, yielded positive detection tests for approximately the same length of time.

It is to be stressed that control tests, too complex to describe here, showed that the appearance of the EVR in all this work could not be attributed to traces of antigen in the antisera reacting with antibody transferred to the recipients from the donors.

WIDELY DIFFERENT PROTEINS YIELD ANTIGEN DETECTION TESTS FOR UNEQUAL PERIODS

The experiment just described answered the last of the questions posed above. Both the plain human albumin and the coupled dye-azoalbumin yielded positive detection tests on the 42nd day, after injecting the donors, but not thereafter, while the bovine γ -globulin yielded positive EVR for 101 days.

BEHAVIOR OF COLORED RESIDUES INDICATES AN UNCHANGED STRUCTURE

It is of much interest that, following the injection of plain globulin into donor mice, the detection tests for that antigen in their tissues remained positive two and one-half times as long as tests for the detection of plain albumin in the tissues of donors similarly injected with that protein. In complete accordance with this, the persistence of colored matter in the livers of mice injected with the azoglobulin and the azoalbumin, respectively, differed in the same manner. Yet the same dye was coupled to both proteins.

Had the presence of color in these tissues, long after injection, been due to dye, split off from the protein to which it had originally been coupled, and either free in the cells or bound to cellular proteins, then its persistence should have been the same in all the tests. But this was not the case. In mice injected with azoglobulin color persisted in the liver for 85 to 120 days, in the animals injected with azoalbumin color could be found in that organ for less than 42 days, and for only a few more days in the mesenteric lymph nodes. These differences are similar to the periods during which positive detection tests can be elicited after injecting the respective azo-

proteins. From all this it would seem to follow that the color behaved as though still coupled to its respective protein.

COMMENT

Since the blue tracer antigens seem to retain their antigenicity as long as blue color can be seen in the tissues they should serve not only to indicate the sites from which the initial stimuli to antibody formation must emanate, when tissues are examined a few days after injecting the animals, but they should show the whereabouts of antigen during prolonged antibody formation.

Little has been said of the distribution of these azoantigens, because a previous paper has fully described the state of affairs during the first few days after injection (1) when the blue material, taken up by the cells, has been definitely shown to be still antigenic. It has seemed best to delay an exhaustive study of the later distribution of

the blue matter seen in the cells, long after injecting mice with the azoproteins, until the work here described could indicate with more certainty whether or not the blue material is still the antigen itself, and until more could be learned about the span of persistence of some natural antigens. Such a study will be undertaken shortly.

In conclusion it is necessary to note only that the presence of intravenously injected azoprotein antigens within certain cells of the reticulo-endothelial system, long after detectible amounts have disappeared from the blood, is highly significant for hypotheses of antibody formation, for among the various reticular cells in lymph nodes and elsewhere, that take up and store these colored antigens, are some of those that may give rise to plasma cells, lymphocytes, and other cells now suspected of carrying antibody. What happens between the storage of antigen by these reticular cells and the appearance of antibody in their daughter cells is yet to be learned.

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RADIOLABELLED ANTITISSUE ANTIBODIES

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THERE have been many studies made in the field of antitissue antibodies especially in connection with certain diseases which appear to have an etiology of autoimmunization in which the mechanism of the disease process is a production by the individual of antibodies against his own tissue and a subsequent reaction of these antibodies with the tissues. Some diseases which appear to be of this category are glomerulonephritis, multiple sclerosis, rheumatic heart disease, certain anemias, periarteritis nodosa, lupus erythematosus etc. Also many attempts have been made since the turn of the century to use antisera prepared against malignant tissue, therapeutically, in the treatment of cancer. It seems reasonable that if an antibody which can produce a physiological effect is present in an antiserum it must first interact with that tissue in order to produce such an effect. Thus, progress would be made if it could be demonstrated that there are antibodies in antitissue sera which actually go to the tissue in question. Further, it is not impossible that, if antibodies can be found which go specifically to a tumor tissue, they can be made to carry physiologically active amounts of radioactivity to the tissue.

We have been able to trace the *in vivo* localization of the injected anti-kidney and anti-lung antibodies by labeling them with radioactive iodine (1, 2) or radioactive sulfur (3) and following the localization of the radioactivity. The radioactive labels can be easily attached to antibody molecules without destroying antibody activity (1, 3, 4) and the antibody can be followed very simply by following the attached radioactivity by a counting or radioautographic technique.

Actually, the fraction of the total protein in a serum which is localizing antitissue antibody is very small. Only a small fraction of the total radioactivity in a labelled serum is attached to localizing antibody since the label is distributed over all of the proteins present. Therefore suitable controls of normal serum or antiserum specific to a heterologous antigen were run. Positive anti-

tissue localizing activity was shown by a significantly greater localization in the tissue from the antitissue serum than from the controls. The background radioactivity was reduced by labeling only the globulin fractions of the antiserum.

It has been possible by the use of such labelled antisera to show that antibodies present in antisera prepared against kidney tissue localize to a high concentration in the kidney with some localization in the liver, and antibodies in sera prepared against lung tissue localize primarily in the lung and kidney. In these experiments the antisera were prepared by injecting rabbits with rat lung or kidney tissue. The globulin fractions were obtained by fractionation with alcohol or ammonium sulfate and were labeled by iodinating with iodine containing I^{131} or by coupling with diazotized *p* sulfanilic acid containing S^{35} .

The duration of the radioactivity which localized in the kidneys indicated that the half life of the localizing antibody in the kidney is of the order of 20 days (5). Thus it appears that the antibody localizing in the kidney is fixed in such a manner that it cannot be metabolized by the cells. Moreover the radioantibody is localized very rapidly, within a single passage through the kidney (6), and the localization takes place primarily in the glomerular tuft (3).

Because of the rapidity of the localization, the localization must take place on some structure on which there is very extensive and complete contact between the blood stream and antigen responsible for the localization. Such contact probably obtains only between the blood and the vascular bed of the organ showing specific localization. Moreover the localization probably takes place on the surface of the cells responsible for this localization. Further evidence supporting this assumption is that the antigen in kidney tissue which causes the localization is insoluble in saline.

We have shown that the antisera prepared against whole kidney tissue contain antibodies which will react with soluble components of kidney tissue and antibodies which can react with insoluble components (7). However it is only

those which react with insoluble components which are responsible for the localization effect. The nonlocalizing antibodies are probably formed against the soluble components of the kidney which are apparently intracellular and the corresponding antibody molecules are nonlocalizing since they apparently do not pass through cell membranes rapidly enough to combine to any large extent with the internal antigens. The parallelism between the properties of the localizing antibody and the nephrotoxic factor is reported by others indicates the identity of these antibodies.

Recently we found that it is possible to elute the antibody which has localized and that this purified antibody can go specifically to the tissue from which it was prepared (8). Thus anti-rat-kidney antibodies eluted from the kidneys of rats receiving radioiodinated anti-rat-kidney serum goes to the kidneys of recipient rats to the extent of 10 per cent whereas only about one-half per cent of the original radioactivity localized in the kidney of the donor rat. However there are also antibodies in anti-rat-kidney serum which localize somewhat in the liver and lung. When

the antibodies are eluted from these organs they have the property of returning to the kidney and also to the organ of the original localization. Thus, radioactivity originally localized in the liver, after elution, goes back to the liver and kidney, while material originally localized in the lung goes back to the lung and kidney. All of these antibodies were originally prepared against the kidney. Similar results were obtained with specific purification of antilung antibodies. The localization in the various tissues reflects the heterogeneity of the antibodies.

That the different organs tested can fractionate the antiserum and preferentially remove certain antibody components indicates differences in the vascular beds of these organs which can be observed with the techniques described here.

In those perivascular diseases which appear to have an etiology of autoimmunization it may well be that the damaging antibody is antibody to the vascular bed of the tissue affected and this damaging antibody localizes as rapidly as it is formed. The specificity of the vascular bed would then determine which organ is affected.

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*Symposium on Hemagglutination by Viruses Which Do
Not Specifically Elute¹*

Chairman GEORGE K HIRST

IN VITRO REACTIONS OF PNEUMONIA VIRUS OF MICE (PVM)

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PNEUMONIA virus of mice (PVM), a latent infectious agent, may produce extensive viral pneumonia and often death in its natural host, the mouse (1). The study of the pneumonia produced by this agent as an experimental model for the study of a viral respiratory disease (2-4) has been materially aided by the demonstration by Mills and Dochez (5) that this pneumotropic virus, under proper conditions, produces hemagglutination of mouse red blood cells. In addition, this phenomenon has permitted detailed investigation of the reactions between PVM and host cells (2, 6-8). This paper will be concerned with these reactions and the essential factors involved.

Tissue fluid expressed from whole (5) or sliced (9) PVM-infected lungs produces agglutination of mouse RBC, whereas suspensions of ground infected lungs do not clump erythrocytes. However, if such lung suspensions are heated at temperatures from 65° to 80°C for proper periods of time (2, 5), hemagglutination of added mouse RBC results. Addition of fresh mouse lung suspension again inhibits the agglutination reaction. This phenomenon can be repeated indefinitely without altering the virus. When heated PVM and mouse RBC are mixed and aliquots removed at intervals, it is readily shown (2) that not only does virus combine with the erythrocytes, but also that spontaneous dissociation does not occur. This is at variance with the influenza-mumps-NDV group of viruses which do release themselves from the bound state (10). These facts suggest that, although virus may be in a free state in the intact lung, disruption of the cells results in combination of PVM with a heat labile cell component. The fact that viral preparations which do not produce agglutination

of mouse RBC are not filterable through Coors no 3 filters, and are readily sedimented by high speed centrifugation, whereas preparations which hemagglutinate mouse RBC are readily filtered but cannot be easily sedimented in high gravitational fields (6, 9) is evidence that PVM actually combines with tissue particles. PVM in the combined state is much larger than the free viral particle, of the order of 140 m μ as compared with 40 m μ (9). That the particles which react with mouse RBC are indeed PVM particles has been established (2).

Dissociation of PVM from tissue component can be accomplished not only by heat or high pH (2, 6), but also, as indicated in table 1, by reducing the electrolyte concentration of the suspension (8). Thus, preparation of PVM-infected mouse lung suspension in distilled water or 0.25 M dextrose or sucrose permits direct hemagglutination of mouse RBC, and the free virus is fully infectious. The addition of NaCl to 0.15 M concentration results in combination of virus and cellular material which indicates that the reduced electrolyte concentration does not alter the capacity of either the virus or lung particles to combine. Furthermore, repeated dissociation and association of the PVM-cell component complex can be accomplished. A similar dependence upon the electrolyte concentration and pH is observed for the PVM-mouse RBC complex (8). The ionic requirements are not specific, either mono- or bivalent cations can be employed without influencing the capacity of lung particles or mouse RBC to bind virus. When free infectious virus is employed to demonstrate PVM-RBC combination, the virus remains infectious when converted again to the free state. Furthermore, the RBC are unaltered by combination with virus, and subsequently show undimin-

¹ May, 2, 1951, Cleveland, Ohio

ished capacity to recombine with virus. Sequential cycles of combination and dissociation of PVM and mouse RBC can be repeated at will by appropriate regulation of the electrolyte concentration and pH of the mixture (8). In contrast, the influenza-mumps-NDV group of viruses irreversibly alter the cell surface of erythrocytes with which they combine, and subsequent combination of virus with the same cells does not occur (10).

Early investigations (2, 7) suggested that PVM combined only with a component in those lung tissues which are susceptible to infection with this agent. Subsequent studies (11), employing more sensitive methods, indicate that, although combination is greatest with particles from cells which are susceptible to infection, PVM can nevertheless combine with a number of mammalian tissues in which this virus does not multiply.

TABLE 1. PREPARATIONS WHICH YIELD PNEUMONIA VIRUS OF MICE IN VARIOUS STATES

STATE	PREPARATION
1 Free infectious	Infected lung—squeeze Infected lung—slice—squeeze Infected lung suspension with <0.01 M electrolyte
2 Free noninfectious	70°C/30 minutes pH 11.0
3 Combined infectious	Infected lung suspension with >0.1 M electrolyte
4 Combined noninfectious	'2' + fresh lung particles

TABLE 2. PROPERTIES OF PNEUMONIA VIRUS OF MICE (PVM) IN RELATION TO ITS STATES

STATE OF VIRUS	PROPERTIES						
	Infectivity mice	Hemagglutination		Combination with lung particles	Specific complement fixation	Specific antigenicity	Size μ
		Unheated	After 70°C				
Free infectious	+	+	+	+	+	+	40
Free noninfectious	0	+	+	+	+	+	40
Combined infectious	+	0	+	0	0	+	140
Combined noninfectious	0	0	+	0	0	+	140

In not a single instance, however, has it been possible to demonstrate the association of PVM with tissue of the chick embryo, an animal completely resistant to infection with this virus (2, 7). Combination between PVM and various adsorbents is likewise demonstrable (11). The combination of PVM with tissue particles or RBC appears to be more dependent upon the ionic composition of the milieu than is the formation of similar complexes by the influenza viruses (cf table 3) (8).

It is now clear that PVM may be obtained in four separate and distinct states: 1) free infectious, 2) free noninfectious, 3) combined infectious, and 4) combined noninfectious (6). The methods by which virus in each state may be obtained are summarized in table 1. Experimental data suggest that for the most part PVM exists in the infected mouse lung in the 'free infectious' state, but whether the other states exist naturally in the infected host has not been determined. Certain data (12) suggest that an infected mouse lung suspension which is able to produce a pro-

gressive infection in the mouse contains both infectious and noninfectious viral particles, both of which produce agglutination of mouse RBC. Table 2 summarizes some of the biologic and physical properties of the viral particles in each state. Although the mechanism by which PVM in the combined infectious state produced infection in the mouse lung was initially puzzling, it has been demonstrated that the lung possesses an enzyme which liberates infectious virus from the combined state (7). The free infectious virus may then combine with susceptible host cells, invade these cells, and multiply to form new particles which then lead to pneumonia (3).

It is evident that the reactions between PVM and tissue particles or mouse RBC are different from those of the influenza-mumps-NDV viruses with comparable cells. Some of these reactions are summarized and compared in table 3. It will be noted that neither inactivated animal nor human sera contains a substance, other than antibody, which reacts with PVM to inhibit combination with mouse RBC, whereas the pres-

ence of such nonspecific inhibitors of hemagglutination by influenza viruses has been widely observed (13) The other reactions likewise clearly

mumps virus interferes with the multiplication of PVM but not with influenza viruses in the mouse lung (15)

TABLE 3 COMPARISON OF CERTAIN REACTIONS OF PNEUMONIA VIRUS OF MICE (PVM) AND INFLUENZA VIRUSES

REACTION	PVM	INFLUENZA
Reaction with RBC	Mouse, hamster	Chick +
Combination RBC and tissue particles	+	+
Spontaneous dissociation	0	+
Virus alteration of 'binder'	0	+
Chemical nature of 'binder'	Protein	Mucoprotein
Electrolyte requirement		
Combination	>0.10 M	>0.0015 M
Dissociation	<0.01 M	
Enzymatic inactivation of 'binder'	Trypsin	RDE (<i>V. cholerae</i>)
Enzymatic inactivation of virus	Trypsin, chymotrypsin	0
'Binder' in serum	0	+

denote the marked differences in biological activities of these viruses. It appears that these viruses have dissimilar host cell requirements for multiplication. 1) the influenza viruses do not interfere with the multiplication of PVM in the mouse lung, nor does PVM preclude the multiplication of the influenza viruses (14), and 2)

DISCUSSION

It has been clearly demonstrated that viruses, animal (8) and bacterial (16), can combine with inorganic materials such as celite and glass, and as such represent artificial complexes. Adsorption of certain viruses to mammalian and fowl RBC may in a sense be equally as artificial a reaction. In certain instances the reaction between virus and erythrocyte has been argued, by analogy, to be similar to or identical with the reaction between virus and the susceptible host cell which supports viral multiplication. That such is not the case with certain neurotropic viruses, GDVII (17), SK-MM group (18), and Japanese B viruses (19), appears probable. By altering the environmental conditions in the test tube, combination of these viruses with the surfaces of certain RBC has been accomplished and agglutination of the erythrocytes occurs. The chemical nature of the surface of the RBC and the chemical constitution of the virus probably dictate the environmental factors required to produce the RBC-virus complex.

Although combination between virus and tissue components or adsorbents may occur in a purely physical sense, it does not follow that certain cell surface constituents are not necessary for combination *in vivo*. Indeed, infection may be limited to those cells which by virtue of their chemical constitution permit cell-virus combination under the environmental conditions which prevail in the host.

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HEMAGGLUTINATION BY VIRUSES AFFECTING THE HUMAN NERVOUS SYSTEM¹

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DURING the past 18 months, Doctors Edward L. Buescher, Robert M. Chanock and I have succeeded in demonstrating and elucidating some of the properties of the hemagglutinins associated with the viruses of Japanese B (Jap B) encephalitis, St. Louis (SLE) encephalitis, West Nile fever and Russian Spring-Summer (RSSE) encephalitis. While each hemagglutinin possessed distinct properties and presented special problems, certain general principles were found to apply to the entire group.

1) Strains of virus which have had a great many passages in mice (over 100) have failed to yield hemagglutinin by methods which proved successful for strains which have had fewer passages.

2) The electrolyte content and pH of solutions used for extraction of infected mouse brains have determined not only the recovery of demonstrable agglutinin, but also the subsequent stability of that which is recovered. Thus, it has been found necessary *not* to use distilled water, hypotonic buffers, or physiologic salt solution buffered just to the acid side of neutrality, since these extract enough inhibitor to obscure the hemagglutinin. It was also necessary to avoid extraction by means of the Waring blender. Centrifugation of the 0.9 per cent NaCl extract at 13,000 rpm for 1 hour (approximately $20,000 \times g$) removed particles, which destroyed the Jap B hemagglutinin within about 3 days at 4°C, but left behind another substance capable of destroying the SLE hemagglutinin within about 2 days. A stable SLE hemagglutinin was obtained only after it was found that the substance which destroyed it was most active on the acid side of pH 7.6 and practically inactive at about pH 8.0.

3) The reactions of this whole group of hemagglutinins are markedly influenced by pH, each

one having its own optimum narrow zone for reaction with the red cells. However, in the case of the SLE hemagglutinin it was found that this pH zone progressively broadened and shifted to the alkaline side as the preparation 'aged' on storage in the refrigerator.

4) The red cells of choice for the Jap B, SLE and West Nile hemagglutinins are derived from newly hatched chicks, while those from older chicks, chickens, and sheep give lower titers, irregular or negative results. The RSSE hemagglutinin thus far has reacted only with sheep cells. Although these viruses are pathogenic for human beings and rhesus monkeys, they do not hemagglutinate their erythrocytes.

5) The hemagglutinins associated with this group of viruses are all highly unstable at 37°C, and to a varying extent, depending on the virus and the pH, also at room temperature and 2°C. The SLE hemagglutinin is much more unstable than that of Jap B. Thus, it was impossible to obtain reproducible or reliable results in hemagglutination inhibition tests with SLE virus, until it was found that at 2°C and at pH 6.9, which was optimum for hemagglutination, the diluted hemagglutinin was destroyed within the brief time required to distribute it in a series of tubes, and appropriate modifications in technique were instituted to overcome this difficulty.

6) All normal sera were found capable of inhibiting these hemagglutinins in dilutions as high as 1:2,000 or more, the titer varying with the species, individuals within the species, and, of course, the amount of hemagglutinin in the test. This may be contrasted with the absence of such normal inhibitor for the hemagglutinin of encephalomyocarditis virus (MM, Columbia Sk). The inhibitor in normal serum behaves like soluble receptor substance, and is present in abundance in animals whose erythrocytes have no receptors for these hemagglutinins. The normal inhibitor for the Jap B, SLE and West Nile hemagglutinins is either lipid or lipoprotein because it proved possible to remove it by extrac-

¹ Aided by a grant from the Commission on Virus and Rickettsial Diseases, Armed Forces Epidemiological Board.

tion of serum with chloroform, benzene, ether, etc., or by precipitation of the protein with acetone. Since specific antibody was unaffected by these procedures, it became possible to measure it without interference from the normal inhibitors in the serum.

7) No receptor-destroying enzyme was found to be associated with the Jap B, SLE and West Nile hemagglutinins. However, hemagglutination could be reversed by repeated shaking at room temperature or 37°C, reversal occurring first in the mixtures containing the smallest amount of hemagglutinin. There is evidence that the reaction between hemagglutinin and the receptors on the red cells is of the equilibrium type. Accordingly, as the uncombined hemagglutinin is destroyed at room temperature or 37°C, particularly on the acid side of neutrality, more hemagglutinin is dissociated from the erythrocyte receptors, and hemagglutination is reversed. That the receptors are unaffected in this reaction is evident from the fact that the erythrocytes are readily reagglutinated by the addition of fresh hemagglutinin.

PROPERTIES AND REACTIONS OF THE JAP B HEMAGGLUTININ (1)

Hemagglutination has been demonstrated with each of 9 different strains of this virus, which have had from 4 to about 70 passages in mice. The Nakayama strain, which in its 70th to 80th passage yielded the optimum titers of hemagglutinin, gave completely negative results with material which had had 183 or more passages in mice. Potent preparations with titers as high as 1:10,000, which remain stable for many months in an ordinary refrigerator at about 5°C, can be prepared as follows. The infected mouse brains, washed free of blood, are ground in a mortar with unbuffered 0.9 per cent solution of NaCl to form a 20 per cent suspension. After extraction in the cold for a period of 4 to 12 hours, the suspension is centrifuged in the cold at 13,000 rpm for one hour (about 20,000 × g), and the supernatant liquid is stored at 5°C. Centrifugation at only 2,000 rpm for 10 to 20 minutes leaves particles in the supernatant liquid which completely destroy the hemagglutinin in about 4 days at 5°C and more rapidly at higher temperatures. Removal of these particles not only yields a preparation that is stable in the cold but one whose titer frequently increases 4- to 16-fold during the first week of storage. Hemagglutina-

tion occurs only in the pH zone of 6.3 to 7.1 and optimally at pH 6.5 to 6.8. Hemagglutination also occurs when the dilutions are made in unbuffered 0.9 per cent NaCl, but the resulting titers vary markedly with different preparations, with period of storage, and other, as yet, undetermined factors. When appropriately buffered, 0.9 per cent NaCl solution is used for preparation of the dilutions, e.g. M/50 or M/100 phosphate at pH 6.5, titers are enhanced as much as 4- to 100 fold, again varying with the type of preparation, period of storage, erythrocytes used etc. This enhancement does not occur when the dilutions are first prepared in unbuffered 0.9 per cent NaCl, and immediately thereafter adjusted to the optimum pH and buffer concentration prior to the addition of erythrocytes. There is no indication that the unbuffered salt solution is deleterious to the hemagglutinin, it appears rather as if the appropriate buffer and pH causes an unfolding or depolymerization of the hemagglutinin. As a matter of fact after storage in the refrigerator for a month or longer as an ordinary saline extract certain preparations undergo a change whereby dilution in unbuffered 0.9 per cent NaCl gives the maximum high titers without any enhancement by buffers at optimum pH. The absence of hemagglutination immediately outside of the required pH zone is not due to rapid destruction of the hemagglutinin, but rather to failure of combination with the erythrocytes. Hemagglutination cannot be reversed by resuspending the agglutinated, sedimented erythrocytes in solutions having a pH outside the required zone.

A 0.25 per cent suspension of chick cells is used (final concentration of 0.125%) because it yields the highest titers. In solutions properly buffered for optimum dispersion of hemagglutinin (H) and combination with receptor (R), the hemagglutinin titer diminishes in a constant ratio as the concentration of erythrocytes increases. Thus, when the dilutions are made in 0.9 per cent NaCl containing M/50 phosphate at pH 6.5, the titer increases 4-fold as the concentration of erythrocytes, in the range of 2 per cent to 0.25 per cent, is decreased 2-fold. These data, as well as those on reversal of hemagglutination without change in the receptors, suggest that the reaction between H and R is of the equilibrium type which may be expressed as $H_x + R_y \rightleftharpoons H_xR_y$, and that the number of R on a single erythrocyte combined with H varies with the concentration of H and the number of erythro-

cytes in the mixture. The lower titers with the more concentrated suspensions of erythrocytes also indicate that a minimum number of R on each cell must be combined with II to result in hemagglutination. It is also worth noting here that, in several simultaneous experiments, the enzymes of *Vibrio cholerae* destroyed the receptors for influenza virus on the chick erythrocytes, but were without effect on the receptors for the Jap B hemagglutinin.

Normal sera from human beings, monkeys, rabbits and mice inhibited hemagglutination, even after the pH was adjusted to the optimum zone required by the hemagglutinin of Jap B virus. The inhibitor in the normal sera reacts with the hemagglutinin in precise multiple proportions giving a straight line relationship with a slope of 1. The inhibitor can reverse the hemagglutination of cells which had combined with small amounts of hemagglutinin (5U or less), and behaves as if it were either the receptor substance of the erythrocytes in soluble form or a substance having an affinity for the same combining groups on the hemagglutinin. The normal inhibitor cannot be removed by dialysis. It is not affected by heating at 65°C, and boiling for 15 minutes does not appreciably alter the inhibitory titer of rabbit and mouse sera but reduces the titer of human serum as much as 75 per cent. The lecithinase of *Clostridium perfringens* toxin, in the presence of CaCl₂, does not destroy the inhibitor but abolishes its resistance to boiling. Treatment with periodate or strong proteinases does not destroy the inhibitor. However, extraction with lipid solvents (ether, benzene, ethylene chloride, chloroform) or precipitation with acetone largely or completely removes the inhibitor from rabbit and human serum and only partially from mouse and monkey serum. Chloroform extraction yields the best results, removing at least 95 per cent of the inhibitor but leaving specific antibody in undiminished titer.

The reaction between specific antibody and the Jap B hemagglutinin also gives a straight line relationship but with a slope of <1, thus differing from the direct proportionality obtaining in the reaction with normal inhibitor. Preliminary observations have indicated that the hemagglutination inhibition produced by specific antibody can be utilized for both diagnostic and epidemiologic survey purposes.

The Jap B hemagglutinin is very heat-labile. At pH 6.5 it becomes inactive at 37°C within

30 minutes, while at pH 7.1 inactivation is only slightly slower. At a room temperature of 24° to 25°C inactivation proceeds at a regular rate somewhat more slowly. At pH 7.0 to 7.1 the activity persists for months at about 4°C. These data apply to 13,000 rpm supernates from which the inactivating substance or enzyme associated with the particles sedimented at this speed has been removed, before removal of this substance inactivation proceeds more rapidly at all temperatures and is complete a few days at 4°C. The effect of freezing and thawing and of lyophilization differs in uncentrifuged and centrifuged preparations as shown in table 1.

The marked instability of the hemagglutinin is also apparent in the fact that mere bubbling of nitrogen gas through 13,000 rpm centrifuged 20 per cent suspension, diluted 1:16 in 0.01 M

TABLE 1. EFFECT OF FREEZING AND THAWING AND LYOPHILIZATION ON JAP B HEMAGGLUTININ

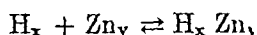
PROCEDURE	20% JAP B MOUSE BRAIN SUSPENSION	
	Uncentrifuged	13,000 rpm supernate
Frozen and thawed once	Active	Inactive
Frozen and thawed 5 ×	Inactive	Inactive
Lyophilization	Inactive	Active

borate-saline buffered at pH 6.8 and kept in an ice bath, was enough to inactivate 75 per cent of it in 5 minutes and 96 per cent in 20 minutes, without significant alteration of pH.

Ultracentrifugation and gradocol membrane filtration experiments indicate that the Jap B hemagglutinin is not smaller than the infectious particle. There is rather a possibility that it may be larger. Centrifugation at 31,440 rpm (average of 58,300 × g) which almost completely sediments the infectious particles, also removes all of the hemagglutinin from the supernatant liquid. Although 75 per cent of the hemagglutinin is removed from the supernatant liquid after centrifugation at 21,740 rpm (average of 27,950 × g), it is difficult to correlate this with the behavior of the infectious particles, because titration of infectivity is not sufficiently accurate within these limits. However, while the average pore diameter (APD) of the membrane, which just allows minimal amounts of infectious virus

to pass, is 50 $m\mu$, and while almost all of the infective particles pass membranes with an APD of 100 $m\mu$ or larger, the hemagglutinin was demonstrable in filtrates from membranes with an APD of 260 $m\mu$ or larger, but not from those with an APD of 200 $m\mu$ or less

Of special interest is the effect of certain simple chemical compounds and of certain bacterial products on the Jap B hemagglutinin. Thus, $ZnSO_4$, which had no effect on hemagglutination by influenza virus, was capable of inhibiting the Jap B hemagglutinin in amounts of 0.03 to 1.92 μg per unit, the more dilute solutions requiring more Zn per unit. It was found that in the concentrations used, the zinc had no effect on the red cells but combined with the hemagglutinin (H) in an equilibrium type of reaction, which may be expressed as



It was possible to reverse this reaction by dilution and by careful removal of Zn from the mixture with H_2S . The experiments with zinc were carried out with collidine or borate buffers. A suitably buffered solution of diethyl, dithio carbamate inhibited the Jap B hemagglutinin in a final concentration of 1:2,000, while 8-hydroxyquinoline and α, α' dipyridyl which also bind heavy metals (although not as well in the presence of proteins) were without effect in 20 times greater concentration. Partially purified preparations derived from cultures of *Clostridium histolyticum* and *Clostridium lentoputrescens*, after boiling for 15 minutes, inhibited the Jap B hemagglutinin in dilutions as high as 1:1,000,000. The effect was on the hemagglutinin and not on the red cells. Further purification of the substances by Dr. A. A. Tytell indicated that the inhibitory activity was associated with certain nucleic acid or protein components which were different from the proteinase in these cultures.

PROPERTIES OF THE ST. LOUIS ENCEPHALITIS VIRUS (SLE) HEMAGGLUTININ

No hemagglutinin could be extracted from mouse brains infected with the Webster no. 3 strain of SLE virus which has had an unknown large number of passages since its isolation in 1933, but was found in several recently recovered strains kindly supplied by Dr. E. H. Lennette. However, the SLE hemagglutinin proved to be much more unstable than the Jap B, and little

work could be done with it until the physico-chemical factors responsible for its rapid degradation were discovered (2). The hemagglutinin is now prepared by extracting the washed, infected mouse brains with enough M/64 borate buffer of pH 9.0 in 0.9 per cent NaCl to yield a 20 per cent suspension. After centrifugation in the cold at 13,000 rpm for one hour, the supernatant liquid has a pH of 8.0 to 8.1 and hemagglutinin titers as high as 1:2,560.

Although hemagglutination occurs with both sheep and chick erythrocytes, 0.25 per cent suspensions of red cells obtained from newly hatched chicks give the best results. The test is performed at room temperature, although 4°C is also suitable. With a fresh preparation, a pH of 6.7 is usually optimum and negative results are the rule beyond pH 7.0. As the preparation is aged on storage at 4°C the pH zone for hemagglutination progressively shifts to the alkaline side and broadens to a point where after a period of about 30 days the reaction occurs best between pH 6.9 and 7.8. At pH 7.0, the SLE hemagglutinin (13,000 rpm supernate + equal volume of phosphate buffer) was destroyed within 5 minutes at 37°C and within 10 to 20 minutes at 24°C, while at 1°C it lost 85 per cent of its activity within 5 to 10 minutes. At pH 7.9, on the other hand, the activity persisted in undiminished titer for at least 160 minutes at 24°C, but was still destroyed within 5 to 10 minutes at 37°C. No receptor destroying enzyme was found, but hemagglutination could be reversed by shaking the tubes, the extent of reversal being influenced by pH, temperature and time. Normal sera contain inhibitor which reacts with the SLE hemagglutinin in the same manner as with that of Jap B. Acetone precipitation proved to be superior to chloroform extraction for elimination of this inhibitor, and the precipitated protein contained the specific antibody in undiminished titer. Tests on acute and convalescent sera from 9 patients with St. Louis encephalitis (kindly supplied by Dr. E. H. Lennette), as well as on 50 normal sera, showed that the hemagglutination inhibition test can be used for diagnostic purposes.

HEMAGGLUTININ OF WEST NILE FEVER VIRUS

Although only exploratory work has been done thus far with this virus, a hemagglutinin was obtained in 0.9 per cent NaCl extracts of in-

fect mouse brains which reacted with chick and sheep erythrocytes at a pH of 7.0 to 7.5 (optimum about 7.3) at room temperature. It was thus apparent that the three antigenically related viruses could be differentiated by the pH zones in which they produced hemagglutination (see table 2). Since the pH zone changes markedly for 'aged' SLE hemagglutinin and possibly also for that of West Nile, the data shown in table 2 apply only to relatively fresh preparations.

Little work has been done thus far on the physico-chemical factors which influence the stability and behavior of the West Nile hemagglutinin, but a peculiar phenomenon was observed with reference to the temperature requirements

allowed to sediment at 4° C. Chloroform extraction removed the normal inhibitor and anti-West Nile sera produced specific inhibition. A common antigen between the Jap B, SLE and West Nile hemagglutinins was also demonstrated by hemagglutination inhibition tests with chloroform extracted sera.

HEMAGGLUTININ OF RUSSIAN SPRING-SUMMER ENCEPHALITIS (RSSE) VIRUS

Infected mouse brains extracted for several hours in unbuffered 0.9 per cent NaCl, yielded preparations which hemagglutinated sheep erythrocytes (but not chick, human 'O' or rhesus) at pH 7.5 (but not pH 5.5, 6.5 or 8.5) and at room temperature (26° C) but not at 4° C. These properties differentiate it not only from the hemagglutinins of Jap B, SLE and West Nile viruses but also from that of the encephalomyocarditis-MM-Columbia Sk virus. Preliminary observations suggested the possibility that the RSSE hemagglutinin, unlike those of Jap B, SLE and West Nile, may destroy the receptors on the sheep cells. Normal sera had inhibitory activity which could *not* be removed by extraction with chloroform. While normal guinea pig serum had an inhibitory titer of 1:20, anti-RSSE guinea pig serum had an inhibitory titer of 1:160. The RSSE hemagglutinin is very unstable at pH 7.0 to 7.1, even at 4° C, and much more work remains to be done with it.

TABLE 2 DIFFERENTIATION OF ANTIGENICALLY RELATED VIRUSES BY pH ZONE OF HEMAGGLUTINATION

VIRUS	HEMAGGLUTINATION AT INDICATED pH		
	6.5	6.7	7.4
Japanese B	++++	+++	0
St. Louis	0 to ±	+++	0
West Nile	0	0	+++

TABLE 3 INFLUENCE OF TEMPERATURE ON HEMAGGLUTINATION OF CHICK ERYTHROCYTES BY STORED OR FROZEN AND THAWED WEST NILE HEMAGGLUTININ

TEMPERATURE OF REAGENTS AT TIME OF MIXING	TEMPERATURE FOR SUBSEQUENT SEDIMENTATION OF ERYTHROCYTES	RESULT
25° C	25° C	Negative
25° C	4° C	Positive (1:2560)
2°-4° C	4° C	Negative

for the reaction between stored hemagglutinin and chick cells (see table 3).

Other tests also indicated that while 25° C was required for initial combination, 4° C was required for appearance of hemagglutination. It should be noted, however, that this phenomenon obtained only for *stored or frozen and thawed* hemagglutinin and chick cells, since fresh preparations hemagglutinated both chick and sheep cells at 25° C and stored or frozen and thawed preparations still agglutinated sheep cells at 25° C. However, higher titers were obtained when mixtures prepared at room temperature were

HEMAGGLUTININS FOR WESTERN (WEE) AND EASTERN EQUINE ENCEPHALITIS (EEE) AND POLIOMYELITIS VIRUSES

Extraction with unbuffered, 0.9 per cent NaCl has thus far yielded no hemagglutinin for multiple-passaged and recently recovered strains of EEE, WEE, and poliomyelitis viruses in surveys utilizing chick, sheep, rhesus and human type 'O' erythrocytes and diluents buffered at pH 5.5, 6.5, 7.5 and 8.5. One recently recovered WEE strain gave suggestively positive results with chick and sheep cells at pH 5.5 at room temperature, but this observation has not yet been followed up. Extracts of several recently recovered strains of poliomyelitis virus, centrifuged at 2000 rpm, regularly produced hemagglutination of human type 'A' and 'O' cells (but not of chick or sheep cells) at pH 5.4 to 6.2 and at 37° C, but not at room temperature or 4° C. While extracts of normal monkey cords were negative under the

same conditions, the hemagglutination could not be inhibited by specific antiserum and appeared to be due to a substance in the infected tissue with an isoelectric point in this particular pH zone. At the moment it is difficult to indicate the procedures or conditions which may lead to the

demonstration of hemagglutinin for the polio-myelitis group of viruses. From what has been presented thus far, however, it is clear that the viruses affecting the human nervous system provide an interesting field for future studies on hemagglutination.

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HEMAGGLUTINATION BY GDVII AND THE COL SK, COL MM, MENGO ENCEPHALOMYELITIS AND ENCEPHALOMYOCARDITIS VIRUSES

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THE AGGLUTINATION of sheep erythrocytes by Col SK and Col MM viruses first was reported in 1947 by Hallauer (1) Olitsky and Yager (2) extended this work by not only confirming the findings of other investigations but by adding the viruses of encephalomyocarditis and Mengo encephalomyelitis. At the same time, Lahelle and Horsfall (3) reported that the GDVII strain of mouse encephalomyelitis virus would agglutinate human O cells in the cold. Later Verlinde and de Baan (4) and Bremer (5) also showed positive sheep cell hemagglutination by Col SK and Col MM viruses. This series of papers (1-5) brought to light for the first time the fact that several neurotropic viruses would cause the agglutination of certain erythrocytes under very exacting requirements of incubation temperatures and that such hemagglutinations were specific in that hemagglutination inhibition (HI) could be demonstrated when immune serum was added in the usual HI test. Interest was thus stimulated to discover other neurotropic viruses capable of agglutinating erythrocytes. Results of the work of Verlinde and de Baan (4) and of Sabin and Buescher (6) have now extended this list of viruses to include Japanese encephalitis, St. Louis encephalitis and others, each of which has its own peculiarities of temperature of incubation, preparation of the antigen, type of cells agglutinated and the pH range. It is believed that the future may reveal other neurotropic viruses to agglutinate erythrocytes provided the exacting conditions for each are discovered.

Lahelle and Horsfall (3) demonstrated that the GDVII strain of mouse encephalomyelitis virus would agglutinate human O red cells at 4° C but not at 23° C or 37° C, that the hemagglutination was inhibited by homologous immune sera and by FA antiserum but not by antiserum against other neurotropic viruses. They failed to demonstrate that FA virus would agglutinate

any of the erythrocytes tested under their experimental conditions.

Lahelle and Horsfall (3) observed that where GDVII virus is readily adsorbed by human O erythrocytes at 4° C, it was quickly eluted from them at 37° C and that this adsorption and elution could be carried on an indefinite number of times. By taking advantage of this property, it was possible to effect about a 10-fold concentration.

Fastier (7) investigated the mechanism of GDVII hemagglutination by heating the antigen to 56° C for 30 minutes. This reduced the titer considerably but did not influence the adsorption and elution process with human O red cells. Unlike PR8 influenza virus, treatment of human O cells with periodate had very little effect on the elution of GDVII virus. In addition, extracts of human O cells inhibited influenza hemagglutination to a much greater degree than was apparent for the GDVII strain. He concluded that the mechanism of hemagglutination of GDVII and PR8 were not closely related. Fastier (8) later investigated the inhibition of GDVII virus hemagglutination by normal tissue extracts. He concluded that saline extracts of normal mouse organs were, in some varying degrees, capable of inhibiting GDVII virus hemagglutination. Sera deriving from the mouse, rat, guinea pig and rabbit exhibited this property to the largest extent. The inhibitor present in mouse brain and serum reacted similarly to the effects of heat and other agents tested. Studies on the distribution of the inhibitor revealed that fetal mouse brains and adult tissue contained comparable amounts. Fastier showed that the inhibitor could be extracted in saline solution, further, that this material could be concentrated by 50 per cent saturation with ammonium sulfate at pH 7.0. This extract appeared to be incapable of inactivating GDVII virus when used in place of immune serum in neutralization tests.

Before beginning a discussion of the Col MM, Col SK, encephalomyocarditis and Mengo en-

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cephalomyelitis viruses, it should be pointed out that Warren and Smadel (9) and Dick (10) have published conclusive evidence that these viruses, probably indigenous in rodents, are identical, such evidence being based on the results of neutralization, complement-fixation and cross-immunity tests. Therefore, in this presentation, these viruses will be referred to as the EMC group as suggested by Warren and Smadel (9).

Each of the viruses of the EMC group will agglutinate sheep cells at 4° C and will elute if the temperature of incubation is raised above 20° C. Like GDVII hemagglutination such adsorption and elution may be carried on an indefinite number of times and this method may be employed to concentrate the virus. Homologous immune sera will inhibit hemagglutination as well as heterologous immune sera prepared against any of the members of the group. Thus the cross-hemagglutination inhibition (HI) test provides further serologic evidence of their identities.

Gard and Heller (11) working with the Col MM strain of the EMC group have attempted to refine the method employed and standardize a technique of determining HI titers in human sera, a tool to be used to determine the importance of the disease in human beings. A very dilute erythrocyte suspension, 0.05 per cent, was employed to increase sensitivity and the resulting loss of stability was overcome by the addition of an extract of sheep brain in the place of bovine plasma albumen employed by Olitsky and Yager (2). A 10 per cent sheep brain extract clarified by centrifugation at 12,000 *g* was employed as the diluent for the virus propagated in mouse brain. These authors failed to find inhibitors similar to those reported by Sabin and Buescher (6), effective against Col MM hemagglutinin in either infected or normal brain tissue. Hemagglutination was not inhibited by pseudomucinous ovarian cyst fluids which evidenced high inhibitory activity against influenza virus hemagglutinin. In addition, and unlike Japanese encephalitis virus (6), Col MM was shown to be independent of pH range (6.0-9.0). However, ionic composition of the suspending medium was a factor since hypertonic salt solutions suppressed hemagglutination. The presence of positive or negative ions in solution, exerted much greater influence. It was shown that mono- and divalent cations had no obvious effect. On the other hand, multivalent anions caused considerable inhibition about proportional to their charge. Four units of virus would be completely inhibited in the standard HI test by

0.0002 M hexametaphosphate. Evidence was brought forth to show that this action was on the red cell rather than upon the viral particles. For example, if the number of units of virus were increased in the HI test, a corresponding decrease in the HI titer of the serum was observed. On the other hand, the HI titer of hexametaphosphate was independent of the number of units of virus employed to the HI test. In addition sodium hexametaphosphate failed to neutralize the Col MM virus in the standard neutralization test. Gard and Heller (11) propose the use of a standardized inactivated immune rabbit serum which like the test sera is adsorbed in the cold with sheep erythrocytes. The hyperimmune rabbit serum is, in addition, adsorbed with normal mouse brain. An HI test with adequate controls is conducted employing 0.2 ml of serial dilutions of serum, 4 agglutinating units contained in 0.1 ml. After incubation for half an hour at room temperature, 0.1 ml of a 0.2 per cent RBC suspension is added and the test is incubated at 4° C. Results are expressed as inhibiting activity in comparison to a standard serum and expressed as the HI index, since the HI titer of a serum is dependent upon the amount and state of the virus employed in the titration. An index of below 1 was classified as negative, 1 to 4 doubtful or weak and values above 8 were considered positive. Results obtained on 384 sera deriving from patients who exhibited CNS symptoms were examined and of this group 73.2 per cent were negative, 14.3 per cent weakly positive and 12.5 per cent positive. Of 39 sera deriving from patients with various diseases other than the involvement of the CNS, no positive reactions were obtained. The 146 sera obtained from blood donors and pregnant women were examined, and 88.4 per cent were negative, 10.9 per cent exhibited weakly positive results and 0.7 per cent were positive. Gard and Heller, however, point out that the positive sera failed to neutralize Col MM virus in the neutralization test and believe that the inhibiting capacity of the sera is not due to the presence of specific antibodies and is therefore, in its present state, not a usable tool for determination of past infection with a virus of the EMC group. On the other hand, since Warren, Smadel and Russ (9) have found antibody to the different members of this group in patients having a mild febrile illness, and since Seligmann and Jungeblut (12) have demonstrated positive neutralizing antibody against the Col SK virus

in normal sera, the question of whether positive reactions obtained by Gard and Heller may not have been due to inapparent or non-recognizable infection. Further work is needed to clear up this point.

Olitsky and Yager (2) attempted to discover other neurotropic viruses that would cause hemagglutination of erythrocytes. Eastern equine encephalitis, Western equine encephalitis, Venezuelan equine encephalitis, Japanese B encephalitis, St. Louis encephalitis, Russian Far East encephalitis, vesicular stomatitis (2 strains), Theiler (FA strain), Theiler (TO strain), poliomyelitis (Lansing strain) poliomyelitis (MEF1 strain), West Nile, rabies, lymphocytic choriomeningitis, herpes simplex and louping ill viruses were all tested for hemagglutinins using sheep, human O, chick, horse, hamster, dog, cat and guinea pig erythrocytes. In no instance was a specific hemagglutination observed that could be inhibited by homologous immune serum. Many nonspecific false or spontaneous hemagglutination reactions did occur, particularly when a mouse brain virus or normal mouse brain suspensions were employed with hamster cells (4). Normal and immune sera was shown to contain an inhibitor (HI) against this hamster hemagglutinin. In addition an agglutinin for dog, cat and guinea pig erythrocytes is contained in normal or virus-infected mouse brain suspensions.

Yager *et al.* (13) employing substantially the same technique used by Olitsky and Yager (2) attempted to extend the hypothesis that hemagglutination might depend upon selection of eryth-

rocytes deriving from a particular species of animal or bird since GDVII will agglutinate only human O cells and the EMC group only sheep erythrocytes. In the course of this investigation, erythrocytes deriving from 25 species were employed using eight viruses as antigens. In no case could a specific hemagglutination be demonstrated.

Bremer (5) demonstrated with reference to hemagglutination of sheep cells by Col SK and Col MM viruses that potassium (C_1) enhances agglutination while calcium (C_2) elutes the virus from the cells thus inhibiting agglutination.

Sabin and Buescher (6) have pointed out that in the case of Japanese encephalitis virus hemagglutination not only is the selection of an erythrocyte important as is the ionic state of the medium and also the pH range. In addition, continued passage in mice will cause the loss of the ability of the Japanese encephalitis virus to agglutinate red cells, so that a positive test will result only with viruses in the earliest mouse passages. This finding we have been able to confirm (14).

In conclusion, the EMC group and GDVII strain of Theiler virus will agglutinate erythrocytes, hemagglutination being inhibited by homologous immune serum, but in each of these instances, many variables are encountered and different conditions underlie the reactions. The demonstration of hemagglutination by additional neurotropic viruses is a possibility but the road to this achievement has many barriers as illustrated by the experience of the workers in this field.

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AMERICAN ASSOCIATION OF IMMUNOLOGISTS

Symposium on Growth of Bacterial Virus in the Host Cell¹

Chairman MARK ADAMS

MECHANISM OF BACTERIOPHAGE REPRODUCTION²

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THE STUDY of phage reproduction centers around the question of how the specific material substrate of the virus is replicated in many copies inside the host cell. A number of approaches have been developed, some of which have been well illustrated by the reports presented by the other participants in this symposium.

The biochemical approach (1-3) analyzes the changes in amounts of gross chemical fractions of the virus-infected system (proteins, RNA, DNA), it studies the origin of virus components by isotopic and by nutritional methods, it follows the fate of the components of the infecting virus and of certain host cell components, particularly enzymes. The results from this approach to date indicate that the new virus is made from building blocks synthesized, partly before but mostly after infection, by the host enzymes, and that in all probability the only *specific* material to be formed in phage-infected bacteria (in infections normally leading to lysis rather than to lysogenicity, that is, to symbiosis between virus and bacteria) is virus material. Phage infection apparently allows the pre-existent enzymes of the host to pile up the building blocks, but redirects the specific organization of these towards its own ends.

We have recently obtained cytochemical data (4) that suggest a possible interpretation of the reorientation of specific syntheses by phage infection. Chromatinic staining of bacteria infected with the same phages used in the biochemical studies reveals an almost immediate disruption of the 'nuclear bodies' of bacteria after infection.

This is followed by a filling up of the cell with a finely granular type of Feulgen positive material (chromatin), which we tentatively identify with phage material because of its absence whenever infected bacteria fail to produce active phage, either because of genetic peculiarities of the host or because of pre-treatment of the phage, for example, with proper doses of radiation. Nuclear disruption itself, however, is always present whenever the bacterium is killed by the phage, whether active phage production follows or not. It seems reasonable to suppose that the peculiar 'killing' of bacteria by phage, which suppresses cell reproduction but not cell metabolism, is a direct consequence of the disruption of the nuclear, presumably genetic, apparatus of the bacteria. Furthermore, I wish to suggest that the redirection of the syntheses results from the replacement of the genetic apparatus of the host with that of the virus, under the influence of the new set of genetic determinants brought in by the virus, virus is produced instead of bacterial protoplasm. In particular, the failure to synthesize new bacterial enzymes, as illustrated by the lack of enzymatic adaptation (5), would result from alteration of the corresponding genes. For production of an adaptive enzyme, there is supposedly (6, 7) a need for energy, for building materials (N sources, internal or external), for the specific substrate, and for the corresponding gene, in phage-infected bacteria, the gene would be the missing (or incapacitated) ingredient. According to this hypothesis of 'genetic parasitism', we should consider cases of lysogenicity as examples of 'genetic symbiosis' between phage and bacterium.

It is important to remember that neither these experiments nor any chemical results tell us anything as yet as to *how* the infecting phage acts in impressing specificity on the newly formed

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material. Actually, we have as yet no idea as to what chemical fraction of the phage (or of any other biological material) is responsible for specificity, nor as to what role, if any, the nucleic acid or protein fractions of a so-called nucleoprotein may play in replication of specificity.

The 'activity cycle' approach attacks the problem of reproduction at the biological level, by tracing the specific virus material through its ability to reproduce. Reproduction takes place in the latent period between infection and lysis, at the end of which several hundred new active particles may be produced. Premature disruption of infected bacteria (8, 9) reveals, first, an 'eclipse' period, during which no active virus is recoverable, followed by the appearance and gradual increase of virus activity in the second half of the latent period. The early eclipse (an apparently general occurrence in virus infections) suggests a reorganization of the virus material into a non-infectious form.⁴ What goes on during the eclipse period? Genetic studies throw some light here. As has been described in this symposium, mixed infection with phage particles of two related types differing by at least two characteristics (such differences can originate by mutation) gives rise to recombinant types in rather precise proportions (11, 12). The fact that recombinant types are present with similar frequency in the final yield and among the very first active particles that appear inside the bacterium around the middle of the latent period (9) indicates that, whatever the reorganization that makes recombination possible, it must take place as early as the formation of the first active virus or earlier. The recombinants of reciprocal types formed within individual bacteria are not present in equal or even correlated numbers (9, 13), this suggests that they do not result from reciprocal exchanges of parts among pairs of mature particles. Moreover, the particles of each recombinant type in individual bacteria are distributed at random, without any sign of 'clonal' arrangement. This suggests that recombination occurs

at a stage in production of virus after which that virus does not act any more as a model for further virus production.

I have recently obtained very different results by studying the distribution of spontaneous phage 'mutants' (rather than recombinants) produced in individual bacteria. This distribution, based on 95 bacteria-liberating mutants of phage T2 out of over 5000 examined, is definitely clonal and quite similar to the one expected if each new mutant gave rise by successive reduplications to a 'clone' of mutant elements. We know that in phage spontaneous mutations occur only during reproduction (14), probably during actual replication of genetic material. The above result suggests then that there is a phase of logarithmic reduplication of phage material, during which mutations occur but recombinants are not yet formed. Recombinants, then, are probably formed after reproduction of phage material but not after the formation of active particles. A possible interpretation is that individual specific units or groups of units composing the phage reproduce severally and that the new materials are then taken up by an assembly mechanism (working after reproduction or more likely concurrently with it) that organizes them into mature particles (15), recombination would simply express the chances for the alternative forms of a given genetic unit to be brought together with those of another. This picture requires, of course, confirmation by other evidence before it can be considered as anything more than a working hypothesis. One of its features, which I find particularly appealing, is that it considers the virus, as we know it in its morphologically complex extracellular state, as a sort of resting stage, which appears to be the terminal form of a complex process of reproduction. In studying phage production, we would, then, be dealing at some point directly with events taking place beyond the geometrical and chemical limitations placed by such a highly organized structure as an active phage particle. This strengthens my confidence that phage workers—with different tools but with shared enthusiasm—are all prying into the level itself at which the mystery of biological replication is enacted.

⁴ Possibly, inside lysogenic bacteria the phage is always in such form but for the occasional outbursts of phage liberation (10).

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BIOCHEMICAL STUDIES ON MULTIPLICATION OF BACTERIAL VIRUSES¹

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THE PURPOSE of this paper is to summarize those chemical studies on virus-infected bacteria which suggest the mode of multiplication of the bacterial viruses.² In this survey we shall not consider the important subjects of adsorption or lysis but primarily those major synthetic steps culminating in virus formation. These steps have been studied in a system whose technical attributes permit controllable virus infection of essentially all the bacterial host cells. Various aspects of the biology and manipulation of the system have been summarized in numerous reviews.

We shall deal in the main with some even-numbered T phages, T2, T4, and T6, which parasitize *E. coli*. These tadpole shaped viruses are about 120 m μ long and 80 m μ across and contain large amounts of nucleoprotein organized within the head, which is bounded by a distinct membrane. T2 and T6, for instance, contain about 40 per cent of desoxyribose nucleic acid (DNA), and at least 55 per cent protein (1-4). There is some discussion going on as to whether these 2 components might not represent the entire phage. The absolute purity of various types of purified preparations creates a rather difficult problem.

We have recently shown (5) that when ultracentrifugally purified preparations of these viruses are found to contain phosphorus (P) other than DNA-P, these preparations still contain bac-

terial debris. This debris then has been shown to contain most, if not all, of the non-DNA-P. In any case well over 95 per cent of the total P is DNA-P, in contrast to the host which may contain three to five times as much ribose nucleic acid (RNA) as DNA.

When the host cells of a growing culture are infected, the cells stop multiplying. In Warburg respirometers, the increase in the rate of O₂ consumption observed in the growing culture is stopped by virus infection but respiration continues unchanged (2). This is true also on treatment of cells with virus inactivated by ultraviolet light. This simple experiment indicates that the production of respiratory enzymes has been halted, however, energy production appears to be maintained by the host's enzymes, a view which is strengthened by the findings that the RQ of the infected cell is unchanged as is the ratio of carbon burned to that assimilated. Of course maintenance of respiration alone is no measure of the maintenance and development of utilizable energy, evidence on the quantity of assimilation is essential to this conclusion.

In addition it is found that N and P are assimilated, and that nucleoprotein is being made in considerable amounts (3, 6). However, although the P entering normal cells passes through intermediates and is deposited in large measure in RNA and DNA, the P entering infected cells ends essentially in DNA, the nucleic acid characteristic of virus. This P appears there in such amounts and at a rate which suggests that it is the same P which in the uninfected cell would have appeared in the two nucleic acids. That is, there appears to be a basic shunt at some stage of P utilization which throws this P into the path of DNA synthesis. Much of this P may be isolated later in virus.

This shunt in P utilization has seemed to us to be crucial to the economy of the infected cell in establishing the parasitic relations which are being described. In line with our interest in the

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² Some recent essays on virus multiplication have implied a rigorous separation of the levels of organization being examined by chemical and biological investigations as well as of the deductions possible from the two lines of work. The inacceptability of this viewpoint in general is especially manifest in the field of virology where it is the task of the biochemist to study genetic duplication at molecular and intermolecular levels.

precise site of the shunt, we have recently studied and demonstrated an enzymatic pathway for ribose phosphate formation from 6-phosphogluconic acid in cell-free systems (7)

It has been shown by means of radioactive P in several laboratories (3, 8, 9) that about 75 to 80 per cent of the P appearing in virus comes from the medium after infection. It has not yet been resolved whether the extra 20 to 25 per cent comes from low molecular phosphorus-containing intermediates or from host DNA.

In the course of these studies we have shown (3, 8) that the P appearing in DNA does not pass

shown later, is increasing, we can say that above initial virus cell ratios of 4, the rate of virus DNA synthesis is independent of the numbers of intracellular virus particles.

It has also been shown that the maximal rate of virus DNA synthesis in infected cells was the same whether the infecting virus was T2r, T4r, or T6r (10). The amount of the DNA produced approaches the amount of DNA found in the virus liberated from these cells (10).

At this point, some conclusions may be formulated. The host bacterium weighs about 10^{-12} gm, the virus 10^{-16} gm. In an infected cell the respira-

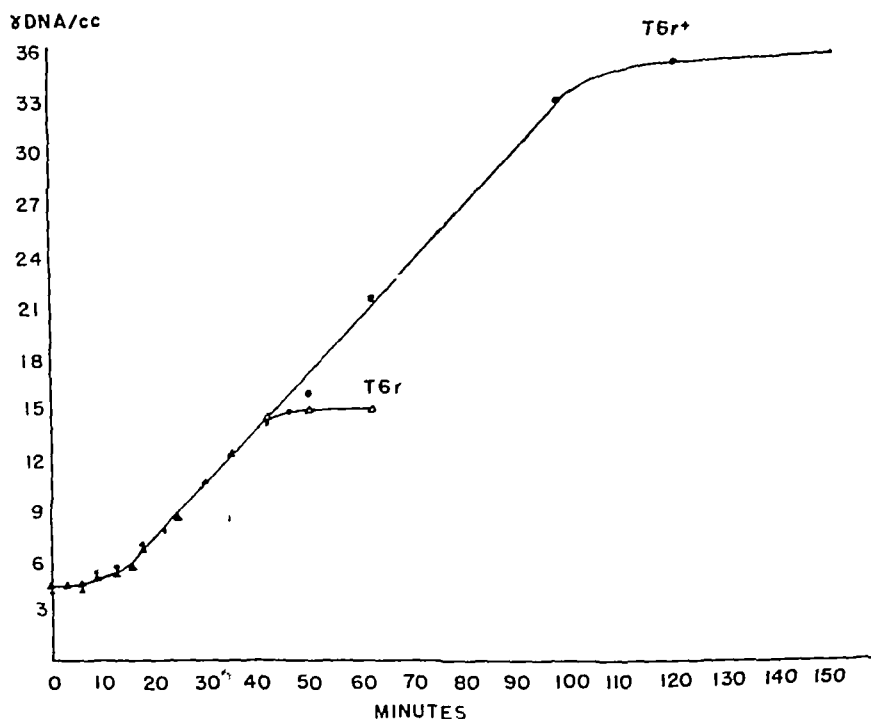


Fig. 1. A comparison of synthesis of DNA in cells multiply infected with T6r+ or T6r virus (10)

through an appreciable fraction of RNA, i.e. RNA is not a precursor of DNA in this system. Indeed the bulk of the original nucleic acid of the host, the RNA, appears to be quite inert under conditions of infection. Recent unpublished experiments of Hershey *et al* indicate that once the virus DNA is formed, its P also does not have an appreciable turnover.

The course of DNA synthesis in *E. coli* infected with each of 2 related virus strains, e.g. T6r+ and T6r, is presented in figure 1 (10). It can be noted that there is a lag of about 10 minutes before DNA synthesis begins, that synthesis in each case falls on the same curve, and that this synthesis occurs at a constant rate for most of the course of synthesis. Since intracellular virus, as will be

tion and quantitative assimilation pattern of P and C resembles the capabilities of the enzymes of the host. Furthermore the amounts of material synthesized imply the continued activity of these enzymes in the infected cell. Nevertheless the compounds produced are built in the main into virus, and at rates independent of the numbers of virus particles and, to a certain extent at least, independent of the kind of virus. We conclude from these considerations that the complex virus polymers are being synthesized by host enzymes and, in addition we note that these rates of synthesis of virus substances are constant, unlike most instances of bacterial growth and division which are exponential processes.

Since this suggested that intracellular virus

formation was also a nonexponential process, it was important to study the correlation of DNA synthesis and the formation of complete virus. After Dr Doermann worked out the method to be described in his paper, we compared the formation of DNA and intracellular T4 virus in the same infected cells in joint experiments (11). In figure 2, it can be seen that the rates of synthesis were essentially similar, i. e. that intact virus formation is also an additive and not an exponential process. However, it should be noted that DNA synthesis does not finish off the virus—there is an additional step involving several minutes to be explored.

Protein is synthesized from the very beginning of infection (fig. 3). There is considerable synthesis, again at a constant rate, and independent of the genetic character of the virus (10). There are many important difficult problems to be solved about this protein, one of which is the question of whether it is all virus protein. It is suggestive but not conclusive that bacterial respiratory systems are not being synthesized and Monod and Wollman (12) have shown that certain adaptive enzymes cannot be synthesized in infected cells. Fowler and Cohen (13, 14) have shown the dependence of virus synthesis on amino acids and other substances in the medium by many methods and these data as well as the serological specificity of the phages imply *de novo* synthesis of phage protein. These questions are also being explored by Kozloff *et al.* (15) who have shown with isotopic N that 92 per cent of virus protein N comes from the medium after infection and not from the host N existing prior to infection.

In line with the demonstration of nutritional requirements for virus synthesis, we have shown that specific amino-acid antimetabolites such as 5-methyl tryptophan among others can reversibly block virus formation (16, 17, 13). Thus a knowledge of the synthetic steps in virus formation leads to a rational chemotherapy of virus infections (11). It is not intended to minimize the technical problems of obtaining such data and developing a chemotherapy for virus diseases of man and other forms. However, it must be emphasized that a methodology for a rational chemotherapy of virus infections does exist.

From the ultraviolet absorption spectra of infected cells, it is possible to get a picture of purine and pyrimidine formation (18). The spectra for DNA, the virus, and the bacterial host, all have marked peaks at 2600 Å due to the purine

and pyrimidine absorption. If we follow the increment at this wave length in suspensions of infected cells and multiply by the density, con-

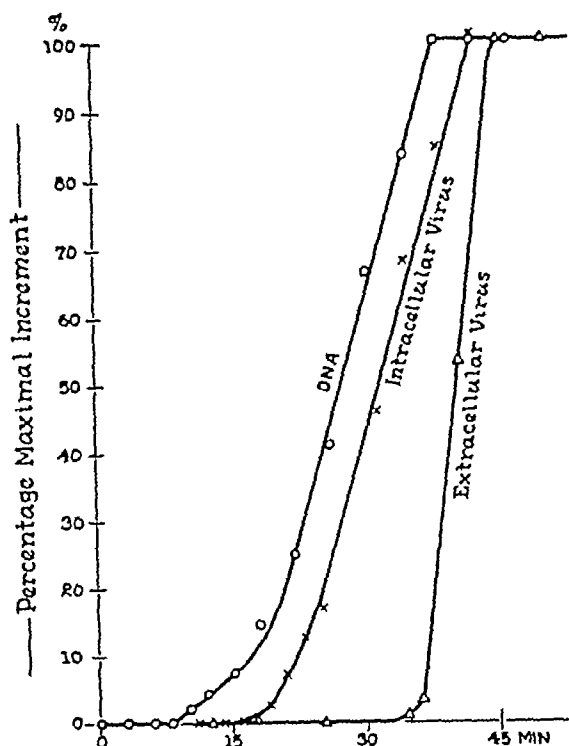


Fig. 2 THE SYNTHESIS OF DNA and intracellular T4r during the latent period of virus multiplication (11)

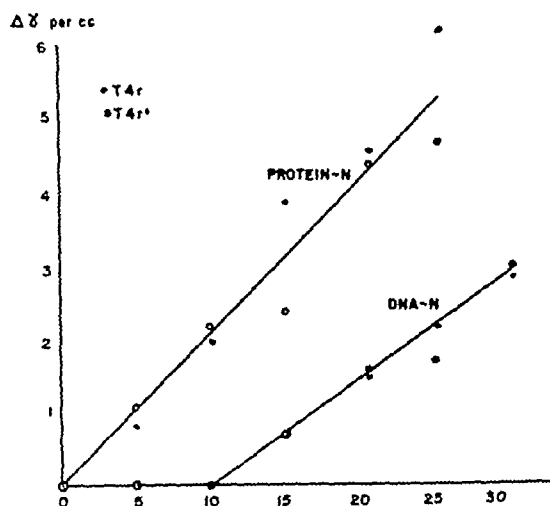


Fig. 3 A COMPARISON OF PROTEIN AND DNA synthesis in cells multiply infected with T4r⁺ or T4r⁻ virus (10)

centration factor established on DNA, we can convert this increment to DNA. If we simultaneously estimate protein-bound DNA chemically, the curves are found to be quite parallel but not coincident. These data would seem to imply that purines and pyrimidines are synthe-

slightly greater than the density increment in normally infected cells. The spectrum of the increment maintained a peak at 2600 Å and this absorption was presumably due to synthesis of purines and pyrimidines. Thus there was a total inhibition for a definite period at the level of DNA polymer synthesis, but not apparently of purine and pyrimidine. We have measured the appearance of intracellular virus by Doeimann's method in this system and it was found that the appearance of intracellular virus was displaced as was the inception of DNA synthesis, i.e. the appearance of one particle per cell coincides closely with the beginning of DNA synthesis in this system. Thus a total inhibition of DNA synthesis and complete virus formation occurred for a limited reshuffling period despite the presence within the infected cell of a full complement of active genetic units. Therefore we have concluded that if DNA synthesis is essential to genetic duplication, the reproduction of genetic units requires the interdependent activity of all the undamaged genetic units. The alternative is that an increase in DNA has nothing to do with the reproduction of genetic units (18). Clearly this system may provide a critical test of the bare chemical requisites of genetic continuity.

In a study of P uptake in this mutual reactivation system we have found that there is no binding of P to protein before DNA is formed, i.e. there is no synthesis of protein-bound nucleic

acid at all. As can be observed in figure 5, inorganic P was converted to organic P and these organic phosphates, possibly some early stage of DNA precursors, accumulated within the cell in an acid soluble form. Obviously the precise nature of the small amounts of these phosphates which accumulate in this system is an important problem. Although there was no net increase in protein-bound P or nucleic acid, protein synthesis proceeded nevertheless, i.e. there was an increase in trichloroacetic-acid precipitable nitrogen almost as fast as in the normally infected cell. In another approach to the same problem, Raff and Cohen have shown that the removal of tryptophan from the medium in this system prior to DNA synthesis was even faster than in normally infected cells (23) (fig. 6). We do not yet know whether this system is synthesizing virus or bacterial protein under these conditions.

Thus while normally infected cells permit a study of DNA synthesis uncomplicated by RNA synthesis, cells infected with irradiated virus may under special conditions permit the study of protein synthesis uncomplicated by any nucleic acid synthesis as well as the study of interrupted stages of DNA synthesis. Indeed the bacterial virus systems are important biochemical tools in the study of protein and nucleic acid synthesis no less than their importance in the study of the basic problem of how a virus multiplies.

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INTRACELLULAR PHAGE GROWTH AS STUDIED BY PREMATURE LYSIS¹

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EVER since the beginnings of research on the reproduction of bacteriophage, one of the most imposing difficulties has been that the infected host cell presents a closed door to the investigator. At the beginning of the growth cycle, one or more virus particles become attached to the bacterium and after a certain length of time the cell bursts, yielding anywhere from a few to a thousand new phage particles. The purpose of this symposium is to summarize what is known concerning the events transpiring behind that closed door. From Dr. Cohen's discussion it is seen that considerable information is available concerning the chemical changes which occur within the infected cell, and Latarjet's (1) experiments with x-ray survival of phage inside infected bacteria have given some quantitative information about the intracellular picture. Yet in the absence of a method for titrating intracellular bacteriophage, it has been difficult to translate these results into a biological framework. My purpose in this discussion will be twofold: first, it will be to describe briefly the methods which have now been developed for estimating intracellular virus and to summarize the results which have been obtained; second it will be to present genetic data, coupled in part with intracellular assays. Similarly to the results presented by Dr. Cohen, these results will force us to the conclusion that the infecting particle must undergo some alteration prior to reproduction, and that its multiplication is not accomplished by serial fissions of the whole infecting virus particle.

With reference to the first objective, we have devised two methods of titrating the intracellular

bacteriophage population. Either of these methods gives satisfactory results under the proper conditions. Due to lack of time it is impossible to describe the methods in all particulars, nor is it possible to give all the details of evidence for accepting the results which have been obtained with them. They will, however, be briefly described and compared. Both methods depend on disrupting the infected bacterium, and since we know of no methods that will do this instantaneously without inactivating the virus, they both depend also on arresting phage reproduction during a period in which the cells are broken down. The first technique, which will be referred to as the cyanide-lysis method, relies for stopping phage growth on the introduction of 0.01 M cyanide. In the presence of this inhibiting agent, the bacteria are opened up by adding a large excess of a second phage type. In our experiments T6 has been used exclusively for this purpose, and the adsorption of large numbers of this second phage causes lysis of the cells (2). In order to assay the phage whose growth we are studying in the presence of a large excess of the lysing agent T6, it is necessary to plate against the bacterial strain B/6 which is specifically and totally resistant to T6, while all the other phages which we have used form plaques when plated against B/6.

The second method, which will be called the sonic method, was devised in cooperation with Dr. T. F. Anderson in his laboratory at the Johnson Foundation of the University of Pennsylvania. This method, by contrast with the other, does not depend on cyanide to stop virus multiplication, but depends on reducing the temperature to 5° or lower during a 5-minute interval in which the infected cells are exposed to intense sonic vibrations. This treatment disrupts some 90 to 95 per cent of the infected bacteria. This method is unfortunately applicable only to those phages which show moderate resistance to sonic energy. Among the members of the T series these are T3, T7, and possibly T1 (3).

The first results which I would like to discuss

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²The author is indebted to Dr. M. Demerec and the staff of the Department of Genetics, Carnegie Institution of Washington, for providing facilities and a stimulating environment for these experiments, and to the staff of the Biology Division, Oak Ridge National Laboratory, for material and intellectual aid in preparation of the manuscript.

are seen in figure 1, and are from an experiment done with Dr Anderson. Its purpose was to compare the results obtained with the two methods described above. Separate aliquots of a T3-infected bacterial suspension were treated simultaneously with the two methods. From a third untreated aliquot control lysis was determined. Attention should be directed to several aspects of the results shown in the figure. It is seen that, in

to 95 per cent of the cells are disrupted. From the figure it can be seen that the titer of plaque-forming particles per ml in the early stages with sonic treatment is between 5 and 10 per cent of the infected bacterial titer. As the yield of phage per cell approaches one, the small fraction becomes negligible.

The agreement in the ascending portions of the curves can be taken to indicate that both meth-

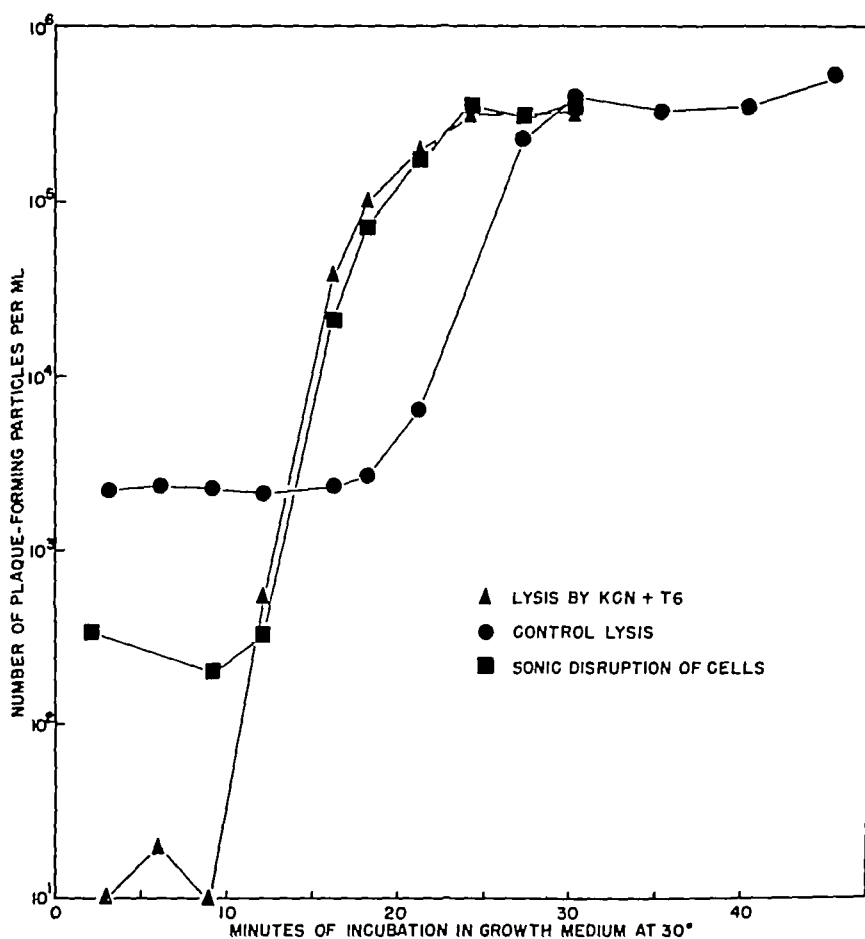


Fig 1 COMPARISON of cyanide-lysis procedure with sonic method for estimation of intracellular bacteriophage

the ascending portions of the curves, there is close agreement in the results from the sonic disruption treatment and the cyanide-lysis treatment. The only discrepancy is found in the earliest points, where both curves fall far below the control lysis curve. This difference is due to a difficulty in technique, as is seen from the following considerations. Any infected, unlysed cell will make one plaque. In the case of the cyanide and T6-treated aliquots very nearly 100 per cent of the cells are broken down by the treatment. With the sonic treatment, however, only some 90

ods give about the same intracellular phage estimate. It is furthermore evident that the two procedures are independent of each other since separate techniques of disruption have been employed and since different mechanisms were used to stop phage growth. The agreement between the results, therefore, constitutes convincing evidence for accepting these methods as adequate for titrating the intracellular bacteriophage population.

Results very similar to those obtained with T3 were found when the intracellular development

of T4 was followed by the cyanide lysis technique. The curves were similar in shape, but spread out over the longer latent period of T1. Again none of the original infecting particles were recovered, even when the multiplicity of infection was in excess of 5 phage particles per cell.

The question which naturally follows here is how the results of intracellular titrations fit with the intracellular chemistry studies which were presented by Dr. Cohen. He has already answered this question, and I wish only to recall to your attention the striking parallel in the accumulation of newly synthesized deoxyribose nucleic acid in the cell and the subsequent increase in mature phage particles.

A little more detail may be added to the results of the intracellular titrations by investigating the

whole cultures are plated out. When this was done with bacteria infected with 5 to 10 T2H particles per cell, the results shown in figure 2 were obtained. The average yield of phage per cell in the 5 experiments ranged from 30 to 60, and the individual bursts were plotted as percentage of the average in a given experiment. The average yields per cell from those allowed to lyse normally was around 300. It is seen that there is a wide range, percentage-wise, in the cells which were disrupted ahead of their normal lysis time, indicating that the cells are not uniform in the time at which the first phage particle appears in them. The spread found is similar to that obtained from normally lysing T1-infected cells by Delbrück (4) and T2H-infected cells by Hershey and Rotman (5).

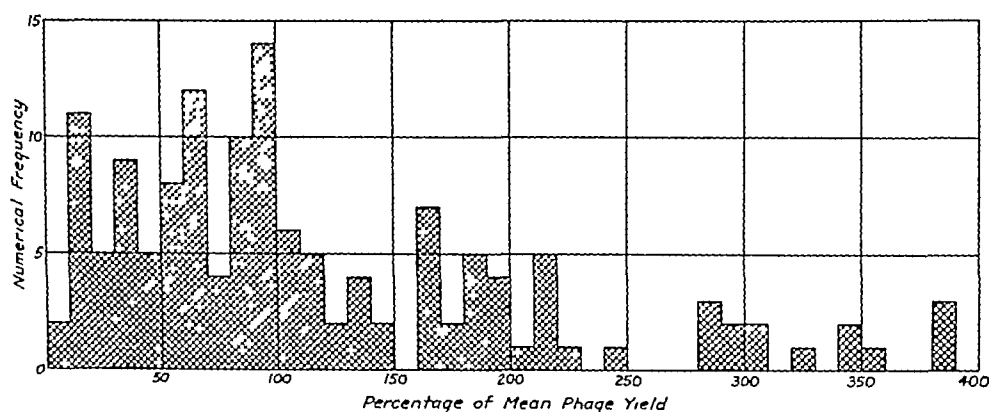


Fig. 2. DISTRIBUTION of intracellular bacteriophage particles among individual bacteria as found by cyanide-lysis procedure.

phage present in individual bacterial cells. Since the experiments of Delbrück (4) published five years ago it has been known that there is a wide range in the yield from individual cells which lyse normally. It seemed worthwhile, therefore, to adapt the cyanide-lysis procedure to titrating the phage within single bacterial cells. The technique depends on diluting the infected bacteria to a point where single drops will have a small probability of containing an infected bacterium. From this highly diluted culture single drops are dispensed into many individual tubes at some stage before one wants to induce lysis. The majority of the cultures which contain an infected bacterium under these conditions will contain only one. The cultures are incubated at the desired growth temperature until the time of lysis induction. They are then placed in an ice bath, and cyanide and T6 are added quickly. After a suitable period for lysis induction, the

TABLE 1. OCCURRENCE OF GENETIC RECOMBINANTS IN THE CROSS T2H_{r1} x T2H_h DURING THE LATENT PERIOD

EXPER. NO.	TIME OF CYANIDE TREATMENT	NO. PHAGE PER CELL	TOTAL RECOMBINANTS OBSERVED		PERCENT RECOMBINANTS
			<i>rh</i>	<i>r⁺h⁺</i>	
1	20	23	6	4	2.0
2	20	60	49	48	2.2
1	25	36	2	6	1.6
3	27	67	45	36	2.4
2	29	102	18	16	2.3
1	30	113	11	8	3.0
1	Control burst	288	31	16	3.0
2	Control burst	516	72	59	3.5
3	Control burst	365	15	12	2.3

The number of bursts obtained in these experiments is slightly lower than would be expected on the basis of the number of infected

bacteria known to be present. This is to be expected, however, if a few of the infected cells do not yet contain any mature phage particles at the time of induced lysis. The fair agreement between the number of bursts and the expected number indicates clearly that the treatment used for inducing lysis is obtaining phage from all of the cells and not merely from a few which might be sensitive to the lytic procedure.

From the results so far presented, the major conclusion appears to be that the infecting particles, whether they be one or many per cell, are not recoverable as such, and that they have undergone some change that prevents them from forming a plaque when the cell is disrupted. This is in agreement with the much earlier experiments of Delbrück and Luria (6) in which they infected cells simultaneously with T1 and T2 (α and γ). Under these conditions, T2 completely suppresses the multiplication of T1. Furthermore, when the yields from these cells were examined, not even the infecting particles of T1 were found.

I would like now to turn to some of the genetic experiments which shed a little more light on the problem of intracellular reproduction of bacterial viruses. From the data so far presented one might still believe that the intracellular multiplication is accomplished through binary fission of the infecting virus particle. The non-exponential nature of the curves of intracellular virus increase could be explained by assuming some limiting synthetic reaction, and the early disappearance of the infecting particles might be due to a temporarily irreversible attachment to the bacterial components. The genetic experiments, however, furnish convincing evidence against the hypothesis of binary fission.

The basic experiments in this regard have been done by Hershey and Rotman (5). They infected bacteria simultaneously with two types of T2H which differed from each other in two differentiable hereditary loci. The yield from such bacteria consisted of the two parental genotypes, but in addition, two new types which were identified as the other two combinations of the genetic markers. When the yields from individual bacteria

were analyzed, the two genetic recombinant types were found in equal proportion and furthermore the recombinants occurred in statistically the same proportion in each of the individual cells. In other words, there were no yields containing unusually large clones of the recombinant types. Even the mere fact that recombination of genetic material does occur would be difficult to explain on a binary fission hypothesis. However, assuming such a hypothesis, and admitting the possibility that whole phage particles could transfer genetic properties, one must still account for the non-clonal distribution of the recombinants. The amendment would, therefore, have to be added to the hypothesis that recombination occurs only in the later stages of multiplication.

We have attempted to answer the question whether any of the first completed phage particles may be of the recombinant types. A cross was made after the technique of Hershey and Rotman (5), but instead of allowing the latent period to go to completion, it was interrupted at several points with the cyanide-lysis technique. The cross used was one of those described by Hershey and Rotman, namely $T2H_{r13} \times T2H_h$. When the cells are allowed to lyse normally, this cross gives 2 to 3 per cent recombinants. This particular cross with low recombination was selected in preference to high recombination crosses for the following reason: on the binary fission hypothesis the absence of clones would more definitely preclude the early occurrence of recombinants than in cases where a high percentage of recombination occurs.

The results of the experiment (table 1) show that even when there is a small number of phage particles per cell, the percentage of recombinants is about as high as is found when cells are allowed to lyse normally. On the hypothesis of binary fission, these early recombinants would necessarily grow into clones. Hershey and Rotman's evidence is unequivocal that no clones are found. One must therefore conclude that the phages of the T-even series do not multiply by binary fission, and further that the first completed phage particles are not themselves the parents of those viruses which appear later within the same cell.

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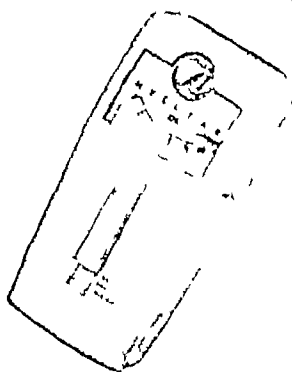
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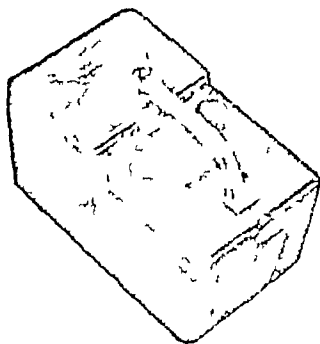
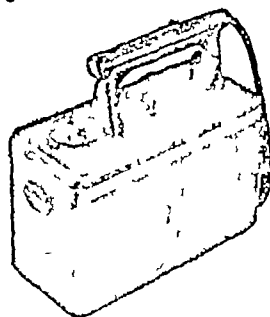


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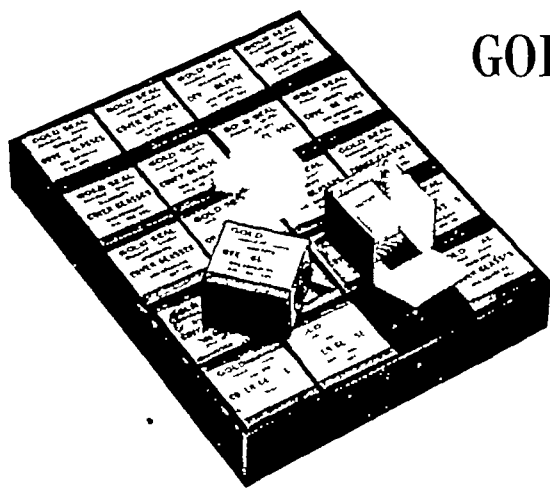
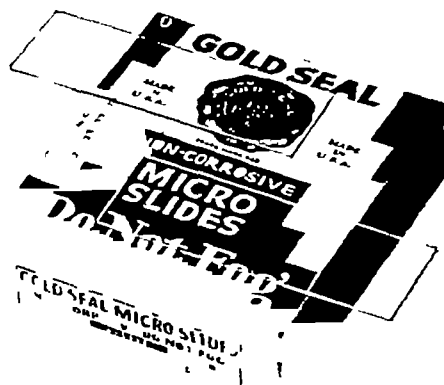
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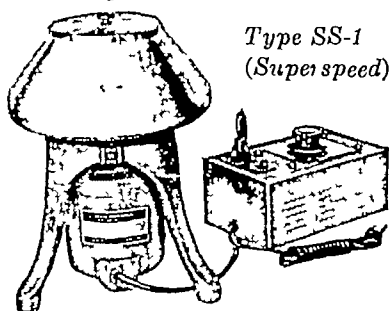
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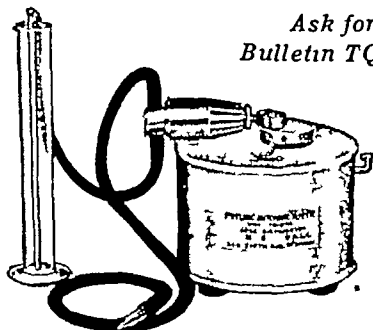
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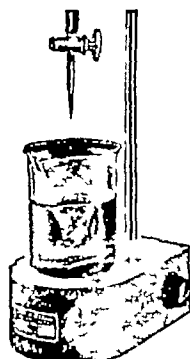


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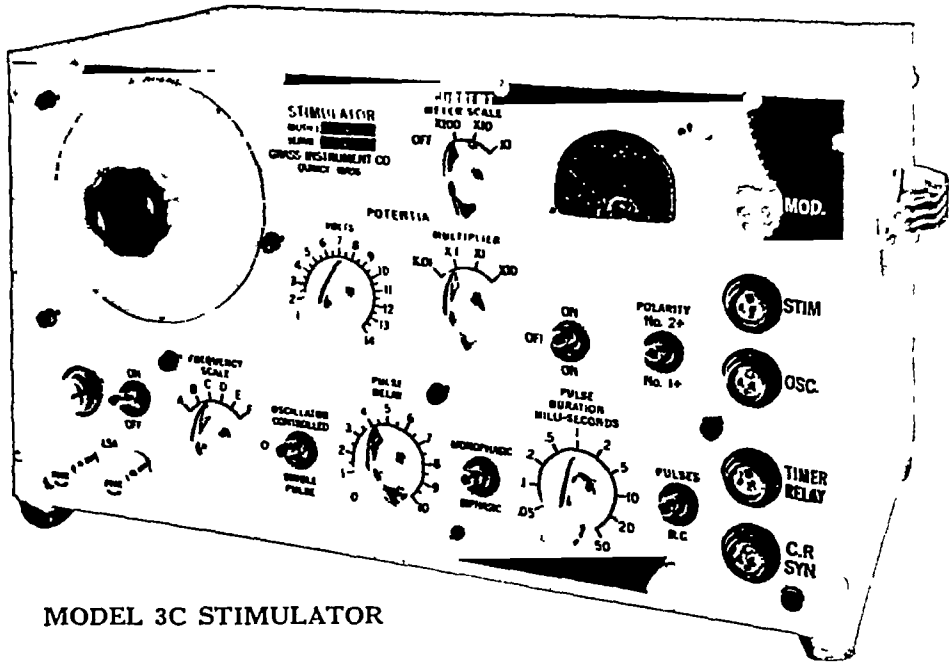
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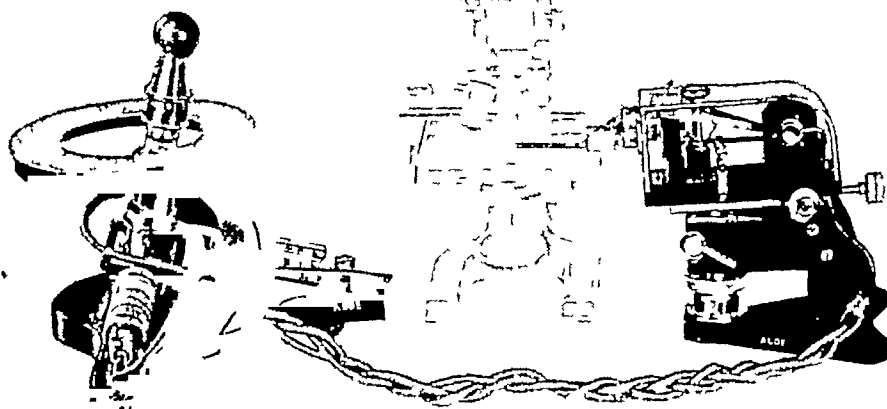
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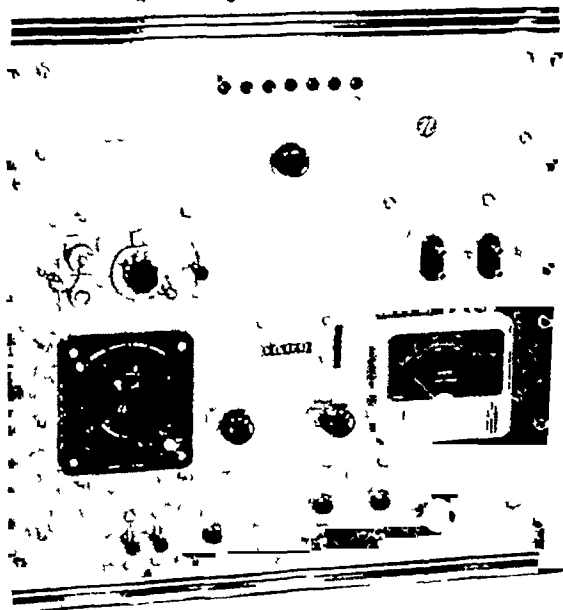
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ATLANTIC CITY, NEW JERSEY, APRIL 17-21, 1950

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(An asterisk * following an author's name indicates "by invitation")

Optimum frequencies for sonic disintegration of paramecia EUGENE ACKERMAN (introduced by J A E EISTER) *Johnson Fndn, Univ of Pennsylvania, Philadelphia*

The disintegration of *Paramecium caudatum* by intense sound waves of audible frequencies was studied. Sonic fields were generated by a magnetically driven, steel diaphragm which formed the bottom of a cup which contained the aqueous suspension being treated. Frequency of vibration, time of exposure, and concentration of cells before and after treatment were measured. Sonic velocities were measured with a calibrated phonograph cartridge in contact with the driving magnet. The ratio of the concentration of the survivors, N , at a time t , to the original concentration N_0 , obeyed the relationship $\ln(N/N_0) = Kvt$ where V represents the peak velocity of the water. The rate factor, K , is independent of the frequency except in the range 1,100 to 1,300 cycles/sec, where K rises to a maximum at 1200 cycles/sec of 6 times its value outside this range. Different maxima were found for 3 other *Paramecium* species, the optimum frequencies increasing as the size decreased. These results were checked with mixtures of 2 species. Vibrational modes of cells are readily excited by stresses in the water due to cavitation. Spherical cells in this size range would have appropriate resonant frequencies if they had interfacial tensions of about 3 dynes/cm or a cortex with the rigidity of a fibrin gel. *Trichomonas foetus* and mammalian erythrocytes, which are much smaller, gave constant K values throughout this range.

Adrenalectomy and proteinuria in the rat T

ADDIS,¹ J MARMORSTON,* H GOODMAN* AND A SELLERS * *Inst for Med Research, Cedars of Lebanon Hospital, Los Angeles, Calif*

The normal rat responds to the intraperitoneal injection of 4 units of hog renin diluted in 4 cc of normal saline solution by an intense proteinuria, averaging 31.7 mg of protein/hour. Protein excretion drops to 1 mg/hour in response to the same dose of renin in the bilaterally adrenalectomized, salt-maintained animal. Desoxy corticosterone acetate (0.5 mg daily, subcutaneously), aqueous

adrenal cortex extract (2 cc daily, intraperitoneally), and 11-dehydro-17-hydroxycorticosterone acetate (compound E) (10 mg daily, subcutaneously) administered to the salt-maintained, adrenalectomized rat restores its ability to respond to intraperitoneal renin injections by proteinuria as massive as that found in the intact normal animal. The intraperitoneal injection of 2 or 3 grams of human serum albumin (6% solution in normal saline) produces an intense transient proteinuria in the rat averaging 510 mg for the 18 hours following the injection. Following bilateral adrenalectomy, the protein excretion after human serum albumin injection is reduced to 75 mg for the 18-hour period after the albumin injection. The spontaneous proteinuria found in the normal male rat is significantly reduced in the adrenalectomized, salt-maintained animals.

Sex difference in proteinuria of normal rats T

ADDIS,¹ J MARMORSTON,* H GOODMAN* AND A SELLERS * *Inst for Med Research, Cedars of Lebanon Hospital, Los Angeles, Calif*

The rate of spontaneous protein excretion in normal male and female rats is identical until 50-60 days of age (approximately the age of puberty), at which time, the rate of protein excretion in the male rises sharply several fold above that found in the female. This increased rate of protein excretion in the normal male rat persists until at least 200 days of age. Observations were not made after this age. The rate of spontaneous protein excretion in castrate male rats is not essentially different from that found in the female at all ages from 30-200 days. In order to determine whether the increased protein found in the urine of male rats had its origin in the kidney or in the accessory sex glands, normal male and female rats of 120-130 days of age were injected with 5 cc of normal saline solution subcutaneously. At the end of one hour, the base of the distended bladder was clamped and the urine withdrawn by puncture through an avascular portion. The protein content of the bladder and voided urines are essentially the same. It is concluded that the protein excreted by the normal male and female rat comes directly from the kidney, il-

¹ Deceased.

¹ Deceased.

though the bladder is not entirely eliminated as a minor source of some excreted protein

Labile factor of prothrombin conversion its consumption in normal and abnormal blood coagulation BENJAMIN ALEXANDER, GRETA LANDWEHR AND ROBERT GOLDSTEIN (introduced by HERRMAN L BLUMGART) *Med Research Labs, Beth Israel Hospital, and the Dept of Medicine, Harvard Med School, Boston, Mass*

Rapid prothrombin conversion to thrombin requires a plasma component called "Labile Factor" (L F). Whereas its concentration in plasma is known its level in serum is obscure. Such information is necessary to elucidate its role in coagulation and to permit precise determination of serum prothrombin by measuring its prothrombin time. Prothrombin was determined by a modified one-stage procedure in which the material was diluted with fresh plasma rendered prothrombin-free by BaSO₄. Prothrombin times were interpolated on standardization curves obtained on normal plasma similarly diluted. L F was measured by its ability to rectify the retarded prothrombin conversion of aged plasma devoid of the factor. Plasma and sera were first adsorbed by BaSO₄ to remove prothrombin and spea. They were then added (whole or diluted) to stored pooled plasma (1-4) and the prothrombin times of the mixtures were determined. Normal serum, separated one hour after coagulation, contains much less L F than plasma. Accelerating coagulation by thromboplastin supplements reduces serum L F even further. Conversely, hemophilic and thrombocytopenic sera contain large amounts of L F. Similarly, dicumarol hypoprothrombinemic blood, with or without thromboplastin supplements, yields sera rich in L F. Thus Labile Factor is consumed in relation to the amount and velocity of prothrombin conversion, suggesting its role as reagent rather than catalyst. The significance of these observations in determining serum prothrombin and prothrombin consumption are discussed. Also, the relation between L F and prothrombin-converting-accessory factors reported by others are reviewed.

Influence of diaphragm on portal blood flow and venous return ROBERT S ALEXANDER *Dept of Physiology, Western Reserve Univ School of Medicine, Cleveland, Ohio*

Changes in the dynamics of portal blood flow associated with contraction of the diaphragm have been studied in anesthetized dogs by means of optical manometers and direct measurements of blood flow. Contraction of the diaphragm produced by phrenic nerve stimulation results in a rise in effective portal pressure in spite of a reduction in portal inflow from the mesenteric bed.

This is dependent upon an increase in resistance to blood flow in the mesenteric circuit and a proportionately greater increase in resistance to hepatic blood flow. These resistance changes are interpreted as being the result of mechanical compression of abdominal structures by diaphragmatic contraction. In addition, phasic changes in blood flow associated with diaphragmatic contraction suggest a 'milking' action of the diaphragm upon the portal system. In confirmation of this, intermittent stimulation of the phrenic nerve at short intervals markedly increases portal blood flow in spite of the changes in resistance to arterial inflow. This increased blood flow is not dependent upon changes in intrathoracic pressure. Comparisons have been made between these observations and comparable observations made during the normal respiratory cycle. It is concluded that the action of the diaphragm in milking blood from the portal system is an important factor contributing to venous return to the right heart, especially with rapid rates of respiration.

Effect of spinal cord lesions on positive and negative conditioned reflexes in dogs WILLIAM F ALLEN *Dept of Anatomy, Univ of Oregon Med School, Portland*

This study represents the results of transections of the mid-thoracic pyramidal or extrapyramidal fibers on acquired auditory-hindleg conditioned reflexes. Transections of the lateral pyramidal tract produced but little effect on the ipsilateral positive conditioned reflex and none on the negative reflex. Hemisection of the cord eliminated the ipsilateral hindleg conditioned reflex for 1000 trials, but the positive conditioned stimulus finally elicited a walking reflex of the hindlegs that started with the normal leg. The positive conditioned reflex was abolished for both hindlegs by a lesion that hemisected the left side of the cord, cut the right lateral pyramidal tract and a median strip of the right ventral pyramidal tract, but left uninjured many of the right ventral and lateral reticulospinal fibers. In this dog the positive conditioned stimulus evoked movements of the forelegs which dragged the hindlegs on the floor. The negative conditioned stimulus prevented this response, but did not stop a slow clonic movement of the right hindleg that began and ended with a daily session of tests. Transection of both ventral reticulospinal tracts produced no effect on the positive conditioned reflex for either hindleg and slight effect on the negative conditioned reflex. Transection of the ventral reticulospinal tracts and many of the right lateral reticulospinal fibers did not effect the positive conditioned reflex for the right hindleg, but eliminated the right negative conditioned reflex in over 1000 trials. A like transection which included

a large proportion of the left lateral reticulospinal fibers together with the ventral reticulospinal tracts did not affect the left hindleg positive conditioned reflex, but the negative conditioned reflex required over 2000 tests before re-establishment occurred. It would therefore appear that the reticulospinal tracts are as essential for this negative conditioned reflex as the pyramidal tracts for this positive conditioned reflex.

Effect of daily exposures to 18,000 feet simulated altitude upon life cycle of the rat PAUL D. ALLTAND AND BENJAMIN HIGHMAN (introduced by HEINZ SPECHT) *Natl Insts of Health, Bethesda, Md*

Sprague Dawley rats were exposed to 18,000 feet 4 hours daily beginning at 14 days of age and terminating at 540 days or prior death. Seventy-two rats were killed for study at intervals between 37 and 540 days, while 20 males and 30 females were observed for longevity. In contrast to the findings observed in rats similarly exposed to 25,000 feet, they showed no significant reduction in longevity compared with controls. Nine of 20 male rats died during exposure period, but 7 lived 700-798 days, 7 of 30 females died during exposure, but 14 lived from 700-928 days and 1 survived (1026 days). Males gained less weight than controls during exposure, but females grew normally. Hematocrit values (78% in males, 70% in females) remained at high levels from 100-350 days of age and then they declined to approximately 67% at 540 days. The polycythemia rapidly disappeared after cessation of exposures. Pathologic changes attributable to altitude were rarely severe. Some exposed animals showed fatty degeneration and hemosiderosis of the liver and kidney. Such changes tended to be more severe in animals with pneumonia and were seen only occasionally in controls. A few exposed rats showed slight thickening of the valvular leaflets, but there were no cardiac vegetations, renal infarcts, or massive intestinal hemorrhages as previously reported (1949) in rats exposed to 25,000 feet. These findings indicate that the life cycle of rats is not significantly altered by discontinuous exposures to 18,000 feet.

Cortical representation and spinal pathways of visceral afferents V. E. AMASSIAN (introduced by T. C. RUCH) *Dept of Physiology and Biophysics, Univ of Washington School of Medicine, Seattle*

Afferents distributed with sympathetic nerves to the viscera are represented in the trunk region of the cortical sensory areas of the cat and monkey. Under pentobarbital, latencies of the initial surface positive wave from single shock stimulation of the splanchnic nerve in the cat were 9.5-12 and

8-10.5 msec for contralateral areas I and II. Ipsilateral responses of 12-14 msec latency were found in area II, where the responses were best seen. The spinal pathway for the early splanchnic response is in the posterior columns. The evidence is that section of the posterior column at C₁ abolishes the early response, while section of anterior and lateral columns leaves it unimpaired. A series of action potentials appears in the posterior columns on splanchnic stimulation, and antidromic stimulation of the posterior columns gives a series of similar latency in the splanchnic nerve. From the latency of the initial wave, the conduction velocity of the fastest splanchnic afferents, expressed as an average for the pathway below the medulla, is estimated at 45 metres/sec, corresponding to fibers of 7.5 μ or over. Many of these are derived from pacinian corpuscles. No evidence could be found that the rapidly-conducting, visceral afferents initiate the early cortical response through an interaction with somatic lumbar or intercostal afferents below the medulla. This may have implications in theories of the reference of visceral sensation. Recordings from the anterolateral regions of the spinal cord have confirmed the presence of another ascending pathway with properties differing from those of the posterior columns.

Proteins obtained by long-continued mild extraction of whole mammalian muscles WILLIAM R. AMBERSON, SYLVIA HIMMELFARB* AND CAROLYN M. STOUT *Dept of Physiology, Univ of Maryland School of Medicine, Baltimore*

In extension of work previously reported (*Federation Proc* 7 2, 1948, *J Biol Chem* 181 405, 1949), whole rabbit muscles have been repeatedly extracted for as long as 4 months by the use of 5% and 10% potassium pyrophosphate solutions, at 0 °C. In the present work the muscles have not been frozen before or during extraction. Individual extraction periods are usually 2 or 4 weeks. The various series of resulting protein solutions have been subjected to electrophoretic analysis, and the time course of extraction of the several components followed. The major components, A, C, and D, reappear in many successive extraction periods, with the C component (myosin) gradually forming a larger percentage of the total extracted protein. Mobilities are not markedly altered for many weeks. Total protein extracted may finally reach as much as 10% of the original wet weight. After the first week a new component, which we designate as C', appear in the electrophoretic patterns. Its mobility is intermediate between C and D. It becomes well developed on the descending limb, but is very small on the ascending limb. The sum of the areas of C and C' on the descending limb is nearly the same as that of the same com-

ponents on the ascending limb. In the presence of C' the form of the D elevation is also modified, on the descending limb. It appears that C' is in some way related to both C and D components, but its identity has not been established.

Factors affecting quantitative comparison of direct and indirect effects of x-rays on enzymes

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It is not at present possible to state definitely the relative importance of direct and indirect ('activated water' reactions) X-ray effects in irradiated living tissue. However, since both must occur to some extent, it is desirable to learn more of their characteristics in systems which are simpler than living cells. A number of comparisons have appeared in the literature, particularly on viruses, but less data is available on proteins having the molecular sizes of enzymes. Pepsin has therefore been irradiated, both in solution and after drying, and the loss of enzymatic activity determined. The loss in solution can, as was reported earlier, be increased several-fold merely by warming the solution of the enzyme after the irradiation. Also, in the case of the dry enzyme, a previously unrecognized factor is of importance in the quantitative result. When the number of molecules of pepsin inactivated per ion pair formed is calculated, the value is somewhat larger for the direct effect than for the indirect effect. The exact ratio depends upon conditions. However, there is nothing approaching the difference of 4000 to 1 reported by Lea for tobacco mosaic virus. In a 20-25% pepsin solution a substantial fraction of the inactivation would be calculated as due to the indirect reaction. Additional data is needed, since this suggests that the indirect reaction cannot yet be eliminated as insignificant within the cell, especially if the sensitive entities are small.

Diencephalic mechanisms for facilitation and inhibition

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Recent studies have shown facilitatory mechanisms existing in the diencephalon to be associated with important changes in the electrical activity of the cerebral cortex. Inhibitory mechanisms have also been reported to have been activated from structures in the diencephalon and rostral mesencephalon which cause changes in electrocortical activity. Relationships between motor facilitation or inhibition and the changes in electrocortical activity have remained obscure.

In the present experiments stimulation points from the septum to the red nucleus of the cat have been mapped for their effects upon a) cortical induced movements, b) electrically recorded spinal reflexes, and c) electrocorticograms. Results of these experiments to date have shown the most marked facilitation of cortically induced movements and spinal reflexes from stimulation of the habenulopeduncular complex. Facilitation has also been obtained from the red nucleus, tegmental fasciculus, posterior hypothalamus, subthalamus and from portions of the thalamic reticular system, antero-medial nucleus and ventralis lateralis. Stimulation points producing inhibition have been found in the septum, dorsal hypothalamic area, and caudate nucleus. We have been unable to demonstrate a constant relationship between cortical electrical responses to these stimuli and the effect upon cortically induced movements, most of the motor effects being apparently downstream rather than via the cortex, as controlled by cortical ablation.

Potentialiation of diuretic action of salyrgan by ammonium chloride

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It is well recognized that the prior administration of ammonium chloride potentiates the action of mercurial diuretics, and that this potentiation is synergistic rather than merely additive. It is possible that the increased efficacy of mercury under these conditions is due a) to increased acidity of the body fluids, i.e. to a reduction in plasma pH, b) to alteration of anion pattern of the body fluids, i.e. to an increase in the plasma concentration of chloride at the expense of bicarbonate, c) to an increase in the acidity of the urine, or d) to other unrecognized mechanisms. An analysis of the possible relationship of these factors to the potentiation of salyrgan-theophylline in the dog is being attempted in the following way. An acidosis produced by the administration of 10 gm. of ammonium chloride on the day preceding the experiment lowers plasma pH to about 7.30 and increases plasma chloride and depresses plasma bicarbonate by about 10 mEq/l. respectively. A synergistic increase in sodium and chloride excretion is demonstrable on administering 1 or 2 cc. of salyrgan-theophylline (40-80 mg. Hg). An acidosis induced by the inhalation of 7 per cent CO_2 in air accompanied by an equivalent or greater drop in plasma pH but with a slight increase rather than a decrease in plasma bicarbonate does not enhance the activity of the mercurial diuretic. It would appear from experiments to date that plasma acidity is not a significant factor. Further studies are in progress.

Ascorbic acid and ketosteroid cytochemistry in the rat's adrenal cortex following prolonged injections of epinephrine HABEEB BACCHUS (introduced by C E LEESE) *Dept of Physiology, George Washington Univ School of Medicine, Washington, D C*

In an attempt to elucidate the relationship of ascorbic acid to secretory activity in the adrenal cortex, adrenals of rats receiving two injections of epinephrine daily for 8 days and for 15 days were examined using histochemical techniques for the detection of ketosteroids and of ascorbic acid. The resistance phase of the General Adaptation Syndrome (observed after 16 injections of epinephrine) is characterized by considerable enlargement, and increased ketosteroid content of the fascicular zone. The glomerular zone is shown to be normal in size and in ketosteroid content. The exhaustion phase (seen after 26 injections of epinephrine) is characterized by maximum hypertrophy of the fascicular zone, with considerable depletion of ketosteroids from the fascicular and reticular zones. The alarm reaction has been shown in previous work to be characterized by depletion of ketosteroids from the fascicular and reticular zones of normal sizes. Ascorbic acid cytochemistry indicated that two 'cell types' exist in the normal cortex, one 'type' with ascorbic acid diffusely distributed in the cytoplasm, and the other 'type', with ascorbic acid aggregated peripherally in the cytoplasm. The alarm reaction is characterized by the preponderance of the latter 'type' of cells. The resistance phase is characterized by the normal distribution of the cell 'types', the cells being larger than normal. The exhaustion phase is characterized by the occurrence of considerably enlarged cells which exhibit decrease in ascorbic acid content. These shifts may have some significance in the secretory activity of the cells of the adrenal cortex.

Ketosteroid and ascorbic acid cytochemistry observed in the adrenal cortices of rats flooded with either sodium or potassium chloride, or given prolonged injections of DCA HABEEB BACCHUS (introduced by E C ALBRITTON) *Dept of Physiology, George Washington Univ School of Medicine, Washington, D C*

The relation of the glomerular zone of the rat's adrenal cortex to increased intake of sodium chloride and of potassium chloride was tested by ketosteroid and ascorbic acid cytochemical techniques. A progressive structural and functional atrophy of the glomerular zone was observed in rats supplied with 2.5% NaCl as the sole of fluid intake (sodium chloride 'flooding'), for 9, 15, and 30 days. Adrenals of control animals exhibited normal ketosteroid and ascorbic acid distributions. The appearance of the zone of NaCl flooded ani-

mals was similar to that of animals receiving daily injections of desoxycorticosterone acetate for 30 days. Progressive structural and functional hypertrophy of the glomerular zone was observed in animals flooded with 2.5% KCl for 9, 15 and 30 days. One hour after intraperitoneal injection of either 0.85% NaCl or 2.5% NaCl, no changes were observed in the glomerular zone, but the alarm reaction was observed in the inner zones, of the cortex. These findings indicate that a) possibly the glomerular zone and not the cortex as a whole, is involved in controlling the sodium potassium ratio in the body, and b) the glomerular zone is not a 'stress' zone.

Effects of varying dilutions of Ringer's solution on the blood capillary bed of the rat mesoappendix THEODORE BALOURDAS* AND ROBERT CHAMBERS *Dept of Biology, New York Univ, Washington Square College of Arts and Science, New York City*

An account of the method and the topography of the vessels has been published (CHAMBERS AND ZWEIFACH *Am J Anat* 75 173, 1944). The caecum is extruded through an incision in the abdomen of the rat and the mesoappendix gently spread over a glass ring and mounted for microscopic observation. The mesoappendix and the exposed viscus is kept moist by a constant drip of Ringer's solution containing 1% ash-free gelatin and kept at 38° C. The present experiments were performed as above, except that the constitution of the drip is varied for the purpose of noting the effect of changing the environment of the capillary bed. The exposed mesoappendix was first subject to a drip of normal Ringer-gelatin solution for 10-15 minutes. For the experimental study only those preparations were used which were normal under previously determined standards and which exhibited the usual threshold sensitivity of one part of a standard solution of epinephrine (1:1000) in 1 to 4 million Ringer's solution. The drip was then changed to $\frac{1}{2}$, $\frac{1}{4}$ or $\frac{1}{8}$ of normal and, after 10-15 minutes, the epinephrine sensitivity again tested. The sensitivity to epinephrine rose appreciably with time and with increasing dilution of the drip solution. Threshold sensitivity rose progressively from 1:20, 1:240 and 1:600 million. The most pronounced increase occurred when the dilution of the drip was $\frac{1}{8}$ of the Ringer's solution in which the epinephrine sensitivity rose in one case to the extreme value of 1:1200 million. At the end of the experiments of 1 to 2 hours, the drip solution was changed to normal Ringer's solution. The sensitivity fell but, in the experiments so far, the original value did not return to that noted at the beginning of the experiment.

Auricular pressure during exercise in dogs with decreased cardiac competence and cardiac fail-

ure A C BARGER, B B ROE AND G S RICHARDSON (introduced by E M LANDIS) *Dept of Physiology, Harvard Med School, Boston, Mass*

To investigate the relation between cardiac competence, exercise and venous pressure (LANDIS *et al*, 1946) we have studied work tolerance and auricular pressure in dogs during exercise on the treadmill before and after the production of the following cardiac lesions a) pulmonary insufficiency, b) pulmonary stenosis, c) combined pulmonary stenosis and insufficiency, d) tricuspid insufficiency, and e) combined tricuspid insufficiency and pulmonary stenosis. Auricular pressure at rest in most normal dogs was close to zero, and did not change markedly during exercise. Pulmonary insufficiency had no measurable effect on auricular pressure at rest or during exercise, or on work tolerance. Pulmonary stenosis produced a slight elevation of resting auricular pressure with little further change during exercise (one exception). Work performance was normal, and the subsequent avulsion of the pulmonary valves had remarkably little effect. With tricuspid insufficiency resting auricular pressure was elevated 70-100 mm saline. Exercise produced a further rise of 50-100 mm saline, but no detectable decrease in ability to do work was found. Three dogs with tricuspid insufficiency were then subjected to pulmonary stenosis. Resting auricular pressure was 165-250 mm saline, and rose to as high as 425 mm saline during exercise of much lower intensity. All 3 dogs so treated developed a syndrome of cardiac failure, with marked reduction in work tolerance. Heart-lung preparations indicated clearly that cardiac insufficiency was present.

Effect of albumin on renal oxygen consumption in man HAROLD G BARKER, JOHN K CLARK, ARCHER P CROSLY AND ALVIN J CUMMINS (introduced by I S RAYDIN) *Harrison Dept of Surgical Research, School of Medicine and Hospital, Univ of Pennsylvania, Philadelphia*

Renal oxygen consumption has been generally thought to vary directly with changes in renal blood flow in contradistinction to other organs and the body as a whole where oxygen uptake remains constant when blood flow changes. This relationship has been reported by others with reduced renal blood flows but has not been previously tested at increased flow rates. The rapid iv administration of 75 gm of albumin was used in 5 normal human subjects as a means of increasing renal blood flow. Renal hemodynamic effects were essentially those observed by others, namely a large increase in renal blood flow (direct PAH Fick), variable or unchanged glomerular filtration rate (mannitol clearance), depressed PAH excretion, and a fall in hematocrit. In all 5 subjects

the renal A-V oxygen difference decreased approximately in proportion to the increase of renal blood flow so that renal oxygen consumption changed but little. The elevated renal blood flow following albumin is probably partly a result of renal vasodilatation secondary to the increased blood volume and partly due to decreased viscosity. The decreased filtration fraction probably indicates that the vasodilatation is in the efferent arteriole although in the absence of a direct method for measuring glomerular filtration rate it might be argued that albumin leads to reabsorption of the test substance. In one other subject we found no change in PAH Tm in the face of an increased renal blood flow following albumin. This makes a Trueta shunt (diversion from cortex to medulla) explanation of our findings unlikely.

Anti-thyroxine effects of thyroxine analogues

S B BARKER AND H B DIRKS, JR*, H M KLITGAARD,* S WAWZONEK* AND S C WANG* *Depts of Physiology and Chemistry, State Univ of Iowa, Iowa City*

Work has been continuing on the evaluation of potential inhibitors of thyroxine in a mammalian organism, the rat, using the following criteria: 1) lowering the BMR's of normal animals, 2) lowering the thyroxine elevated BMR's of thyroidectomized animals, 3) reversal of thyroxine depression of estrogen sensitivity of thyroidectomized animals. Among the compounds showing ability to overcome thyroxine effects were iodinated phenoxyacetic acids, 4-hydroxy- and 4-benzoyloxy-3,5-diodobenzoic acids, N-(4'-hydroxy-3', 5'-diodobenzoyl)-3, 5 diiodotyrosine, N-(2, 5-dihydroxyphenyl)-pyridinium acetate and 2 dimethyl-aminomethyl-dibenzofuran hydrochloride. The third test appeared to yield the most sensitive changes, with molar ratios of inhibitor thyroxine required to produce approximately 50% decrease in thyroxine effect being about 50:1 to 100:1. Considerably larger amounts were necessary in the other two procedures.

Electrolytes in the muscle of young chicks J S BARLOW* AND J F MANERY *Dept of Biochemistry, Univ of Toronto, Toronto, Canada*

The breast muscle of chicks, 3 days old, showed an entirely different electrolyte pattern from that of adult hen muscle. It contained 83% water, 10% more than the adult muscle. The chloride concentration (68.2 mEq/kg) was 8 times that of adult muscle, the sodium (96.6 mEq) was 3.5 times, while the potassium (45.5 mEq/kg) was about one-half that of adult muscle. In the chick serum, these constituents are not significantly different in concentration from that in adult serum. Good indirect evidence of the extracellular position of the muscle chloride was obtained in two ways. Firstly, the chloride diffused freely

from the muscle into Ringer's solutions in which the chloride had been replaced by nitrate. Secondly, the proportion of extracellular space measured histologically agreed well with the estimates of the extracellular fluid volume calculated from chloride analyses, i.e. about 50% of muscle volume. When this estimate of the extracellular volume was adopted, the intracellular potassium concentration was about the same as that of the adult muscle but a much larger intracellular sodium concentration was found. This sodium was found to diffuse freely into Ringer's solution in which sodium was replaced by potassium or by lithium and to exchange rapidly with radioactive sodium. The data suggest that the sodium not accommodated in the extracellular water is situated outside the cells, perhaps bound to some organic solid.

A transitory carbohydrate substance occurring in anaerobic assimilation of yeast cells J. PERCY BAUMBERGER AND FRANK W. FALES * *Physiology Dept., Stanford Univ. Med. School, Stanford, Calif.*

The authors previously showed (*J. Biol. Chem.* 173 1, 1948) that a large fraction of the sugar added to a yeast cell suspension goes temporarily into a nonreducing, nonhydrolyzable form. Further study has added the following information as to the characteristics of this 'hidden' sugar: 1) It is still in carbohydrate form as it is demonstrable by Dreywood's Anthrone Reagent which is specific for the hydroxymethylfurfural group, but it is insoluble and occurs inside the cell. It is not trehalose as it is not extractable by alcohol. 2) The 'hidden' sugar is a product of cell synthesis since its formation is inhibited by sodium azide at a concentration greater than that necessary to stop glycogen formation. 3) The 'hidden' sugar finally appears in the carbon balance sheet as the substrate is gradually depleted. It has therefore a transitory existence and its accumulation and disappearance follow a regular course. 4) Metabolically the 'hidden' sugar is characterized by being very rapidly fermented anaerobically. This is demonstrated by washing the yeast cells free of substrate at suitable stages in the course of the formation and breakdown of the intracellular 'hidden' sugar. The rate of fermentation of the washed cells is found to vary directly with their 'hidden' sugar content. 5) Similarly the 'hidden' sugar is very rapidly oxidized. This is demonstrated as above with washed cells. The cells are suspended in air saturated buffer and the oxygen consumption rate is followed polarographically. This rate is found to also vary directly with the 'hidden' sugar content.

Dual effect of positive intrapulmonic pressure on muscular response JOHN W. BEAN AND LEONARD

E. ELWELL * *Dept. of Physiology, Univ. of Michigan, Ann Arbor*

Application of positive intrapulmonic pressure causes an augmentation of tibialis anticus contractions elicited by slow (1-2/sec.) single shock motor nerve stimulation in anesthetized dogs. This has been tentatively ascribed to an influence on the neuromyal junction induced by the drop in blood flow (recorded by thermopile) which results from PIP application. The finding that augmentation occurred in direct stimulation of the normal muscle, but was missing in such stimulation of the chronically denervated muscle supports this interpretation. Furthermore, augmentation is also missing in curarized muscle. It was found, however, that curarization does not provide an entirely fair means of differentiation of effects, since it was demonstrated that curarization not only paralyzes the nerve endings, but also markedly depresses irritability of the muscle itself, both in the normal and chronically denervated preparation. In contrast with its augmentatory influence on contractions of low frequency, PIP invariably causes a profound diminution of contractions elicited by higher frequency of stimulation (10-20/sec. or above). PIP therefore exerts an incrementory influence on contractions of low frequency and a decrementory influence on contractions of higher frequency. The incrementory influence may be operative during higher frequency stimulation, but if so, it is entirely masked and superseded by the decrementory influence. This decrementory influence is attributed to the diminished blood flow and O₂ supply, but unlike the incrementory influence it is readily demonstrable in direct stimulation of the chronically denervated muscle.

Intestinal blood flow JOHN W. BEAN AND M. SIDKY MOHAMED * *Dept. of Physiology, Univ. of Michigan, Ann Arbor*

In an extension of work, reported earlier, the relation between blood flow into an isolated loop of dog intestine, perfused by heparinized dog blood, was studied by thermopile and drop methods. Findings that individual rhythmic contractions provide important propulsive force to blood flow, and that increasing rhythmic activity increases flow are confirmed and further demonstrated by the fact that the rhythmic contractile phase increases outflow but diminishes inflow, while the reverse is true of the relaxation phase. Flow is markedly affected by change in tonus; decreased tonus greatly augments flow, even in the absence of rhythmic contractions; increased tonus decreases, or even temporarily occludes the flow. Intermittent tonic contractions serve also in propulsion of blood. Tonic occlusion of flow is followed by a temporary supra normal flow, pro-

sumably due to temporary accumulation of vasodilator substances CO_2 (6-15%) increases flow, followed occasionally by a gradual diminution to below the pre- CO_2 level. These changes are attributed to an initial increase of gut activity, the subsequent pronounced quiescence and decreased tonus and to vasodilation. Low O_2 (7-10%) increases flow, decreases gut activity with slow recovery. Acetylcholine increases flow even in the absence of gut stimulation, with larger doses the increased flow is temporarily occluded by strong tonic contractions. Increased intraluminary pressure (12-20) mm Hg causes an initial short increase of outflow and a subsequent pronounced decrease of both inflow and outflow in spite of the augmentation of gut activity by the increased pressure.

Ketosis in diabetic rats CLARISSA H. BEATTY
Depts of Biochemistry and Physiology, Univ of Oregon Med School, Portland

Little ketosis occurs in fasting rats (BUTTS AND DEUEL *J Biol Chem* 100 415, 1933), therefore, ketosis in rats has usually been studied after administering ketotic substances such as butyric acid. While investigating the suitability of diabetic rats for studying the effect of various compounds on ketosis (West and Beatty, to be published), a high level of ketonuria was noticed in many alloxan diabetic animals. The first week following alloxan, ketonuria as high as 33-42 mg/100 gm/24 hr was found without comparably high urinary glucose or non-protein nitrogen excretion. Moreover, as ketonuria fell glucosuria and urinary non-protein nitrogen excretion frequently rose. During the following weeks ketonuria usually decreased to a lower and relatively constant level, then rose terminally. For weeks some rats maintained ketonuria above 4 mg/100 gm/24 hr, a value at least 5 times that of the non-diabetic animal. Depancreatized rats did not show this severe early ketonuria. In animals with sufficient pancreas removed to produce diabetes in 1-2 weeks, ketonuria commenced at low levels and, within a matter of days, reached plateau values between 3-5 mg/100 gm/24 hr. These animals were not followed to the terminal stages. When diabetic animals were fasted 24 hours, ketonuria fell to non-diabetic levels, and glucosuria decreased sharply. Restriction of food intake may result in a decrease in glucosuria without a comparable drop in ketonuria. These data indicate that some diabetic rats show sufficient ketosis to render them suitable for investigation of this metabolic upset.

Comparison of arterial O_2 saturation with tissue O_2 levels VIVIAN G. BEHRMANN, WALTER D. GRIEST*, GEORGE H. MANGUN* AND FRANK W. HARTMAN* *Dept of Laboratories, Henry Ford Hospital Detroit, Mich*

Continuous recordings of arterial O_2 saturation, tissue O_2 , respiration and blood pressure were obtained on anesthetized dogs. The blood O_2 saturation was recorded photoelectrically by the oxyhemograph while tissue O_2 concentration was determined voltametrically. Relative changes in available tissue O_2 were measured by inserting a tungsten wire electrode for a known distance into the tissue, and applying an Ag-AgCl electrode to a nearby convenient area. Across the terminals of this circuit an E M F of -0.66 v was applied, the resultant current, varying with available O_2 was amplified by a contact modulated D C amplifier and recorded by a D C milliammeter. O_2 concentration was measured in muscle, spleen, kidney, or liver during each procedure for the purpose of comparison. Hyperoxic as well as mild and severe hypoxic states were established by altering the O_2 content of the inspired air. The contour of the oxyhemograph and the tissue O_2 tracings show a striking similarity. Development of and recovery from hypoxemia were reflected by parallel changes in tissue O_2 except for a measurable time lag. Using the arterial O_2 saturation level as a control, one may compare the time lag and the extent of O_2 change in the tissues studied. Since it is obvious that the tissue O_2 consumption as well as its circulatory supply are prime factors in affecting the available O_2 concentration in the tissues, experiments were performed in which the tissue O_2 consumption was reduced by a histotoxic agent, (cyanide), and circulatory changes were created by vasomotor drugs.

Simultaneous high- and low-speed electromyography A. L. BENNETT AND A. L. DUNN*
Dept of Physiology and Pharmacology, Univ of Nebraska College of Medicine, Omaha

A method of recording action potentials with simultaneous, parallel, high- and low-speed traces upon the same photographic record has been developed and used in electromyography. A continuous trace from one C-R tube is recorded on paper (6 cm Electrocardiograph no 1115) moving at speeds from 5-20 cm/second. Simultaneously a second beam is repeatedly swept across the face of another 5 inch C-R tube so that high speed traces are made parallel to the continuous trace. The rapid traces are automatically repeated at regular intervals so that they form an almost continuous line. The linear speed of the sweep may be adjusted to provide a time-base of 1-10 millsec/cm. The necessity of replacing the conventional slit at the photographic surface with an opening large enough to accommodate each sweep introduces the problem of fogging due to background illumination. To prevent this a blanking circuit releases the beam only during the useful portion of the sweep on the one tube. The other tube is

masked with a slit aperture at the tube face. This method of recording has facilitated the analysis of single complexes from units which can be identified in terms of rhythm and rate of repetition in the parallel, continuous tracing. Such analyses have revealed wide variations in phase relationships and duration of complexes both from innervated and denervated muscle units. Often it is essential to have the combined recording in order to identify the type of unit which is spontaneously active.

New methods of direct calorimetry and dermal radiometry T. BENZINGER AND C. KITZINGER (introduced by EUGENE F. DuBOIS) *Naval Med. Research Inst., Bethesda, Md.*

Total caloric output can be continuously recorded as a single thermoelectric potential by means of the gradient calorimeter, with a sensitivity of 35 μ /watt, and a half response time of 4.2 sec. for the gradient layer (Benzinger, T. and C. Kitzinger *Rev. Scient. Inst.* In press, 1949). Radiated heat loss from the entire body surface can be continuously recorded as a single thermoelectric potential by means of the 4 π radiometer, with a sensitivity of 18 μ v/watt, and a half response time of 0.25 sec. The 4 π radiometer can be applied to the internal surface of a gradient calorimeter. The rapid response of each allows the study of reflex changes in peripheral circulation. (The index of peripheral circulation is obtained from total heat loss, average skin temperature, and rectal temperature, according to DuBois' and Hardy's formula, while average skin temperature follows from radiated heat output according to the Stefan-Boltzmann equation.)

Use of N-acetyl 4-aminoantipyrine in measurement of total body water in dog and man EUGENE Y. BERGER,* BERNARD B. BRODIE,* JULIUS AXELROD,* MARCELLE F. DUNNING,* YETTA POROSOWSKA* AND J. MURRAY STEELE *Research Service, Third (New York Univ.) Med. Division, Goldwater Memorial Hospital, New York City*

Antipyrine, because of its uniform distribution throughout the water of the body and its slow rate of excretion and transformation, has been used in the measurement of total body water in man. It is impractical to use for this purpose in dogs, however, because the rate of transformation is too rapid in this species. A derivative of antipyrine, N-acetyl 4-aminoantipyrine, has also been found to distribute in tissues in proportion to the water content. In addition, its metabolic alteration is slow enough in the dog to permit its use in this animal in the measurement of the total body water. In contrast to antipyrine, which is bound to a small extent on plasma proteins, N-acetyl 4-aminoantipyrine is only negligibly bound. Preliminary comparisons of the volumes of distribution of antipyrine and N-acetyl 4-aminoanti-

pyrine were carried out in man. The agreement was good enough to suggest that N-acetyl 4-aminoantipyrine may be useful in both man and dog as a measure of body water. N-acetyl 4-aminoantipyrine has certain advantages over antipyrine for use in man. Its estimation involves a colorimetric procedure which, though somewhat less accurate than that of antipyrine, does not require an ultraviolet spectrophotometer. The compound is almost quantitatively excreted in the urine so that if its urinary excretion is measured, the volume of distribution may be calculated on the basis of a single plasma level.

Effect of reduced cardiac output on renal circulation ROBERT M. BERNE AND MATTHEW N. LEVY (introduced by CARL J. WIGGERS) *Dept. of Physiology, Western Reserve Univ. Med. School, Cleveland, Ohio*

Cardiac output was acutely reduced in open-chest dogs anesthetized with pentobarbital by means of graded constriction of the pulmonary artery. Glomerular filtration rate and renal plasma flow were measured by creatinine and PAH clearances, respectively. The Fick principle was used to determine cardiac output. Experiments were divided into periods of preoperative and postoperative controls, of pulmonary artery constriction, and recovery following release of constriction. Compression of the pulmonary artery sufficient to elevate central venous pressure without lowering mean arterial pressure below 80 mm. Hg resulted in a fall in cardiac output and renal blood flow. The latter fell proportionately less than the former, yielding an elevated renal fraction. Values returned toward control level upon release of the constriction. Glomerular filtration rate either remained constant or fell slightly, while the filtration fraction rose uniformly. With reduction of cardiac output, total peripheral resistance increased to a much greater degree than did renal resistance, and tended to return toward control level with release of the pulmonary artery. Renal resistance remained elevated and showed no statistically significant change between periods of constriction and release. Application of Lammport's formulae to our data reveals a decrease in afferent and an increase in efferent arteriolar resistances and a slight increase in post-arteriolar resistance. These studies suggest that the reduced renal fraction of congestive heart failure is not directly due to a reduction in cardiac output per se.

Metabolism of leukemic cells *in vitro* ROBERT M. BIRD, JOHN A. CLEMENTS AND LILLIAN M. BECKER (introduced by F. GLADENATSCHE) *Dept. of Physiology, Cornell Univ. Med. College, New York City*

The metabolic characteristics of leukocytes obtained from peripheral blood of leukemic patients

in relapse have been studied *in vitro*. Data derived using the Summerson constant volume differential manometer were compared with chemical analyses for glucose utilization and lactic acid production. Leukocytes from both myelogenous and lymphatic leukemia exhibited an oxygen consumption averaging 1.1 c mm/mg dry wt/hr with a Respiratory Quotient of 0.85. Those from myelogenous leukemia were characterized by a moderate glycolysis, total aerobic acid production averaging 5.6 c mm/mg/hr, lactic acid formation, 16 μ g/mg/hr, and glucose utilization, 28 μ g/mg/hr. Glycolysis in leukocytes from lymphatic leukemia was very low, the rate being about one-fifth of that of myelogenous leukemic cells. Anaerobic glycolysis was also greater in myelogenous leukemia averaging 9.2 c mm/mg/hr as contrasted to 3.1 c mm/mg/hr in lymphatic leukemia. Factors influencing the metabolic rates were studied. When the total number of leukocytes in the experimental vessel was below 25×10^6 per ml, all rates tended to be elevated. When the proportion of myeloblasts exceeded 60%, oxygen consumption was significantly increased and glycolysis depressed. Specimens obtained from patients with myelogenous leukemia under treatment with urethane or radiation showed average rates lower than the untreated group, but the difference of the means was not statistically significant. By altering the pH of the medium from 7.5 to 7.1, anaerobic glycolysis was reduced. Leukocytes from non-leukemic patients exhibited metabolic rates slightly higher than those of untreated leukemia.

Inactivation of posterior pituitary antidiuretic hormone by liver extracts JAMES H. BIRNIE (introduced by ROBERT GAUNT) *Dept of Zoology, Syracuse Univ, Syracuse, N Y*

The *in vitro* studies of Heller and Urban and the *in vivo* observations of Eversole, Birnie and Gaunt indicate that the liver plays an important role in the inactivation of posterior pituitary antidiuretic hormone. We have attempted to determine some of the properties of the system (enzyme) responsible for this inactivation. Systems capable of destroying both the antidiuretic and chloruretic activities of pitressin during *in vitro* incubation have been found in cell-free extracts of liver tissue. Preparations from kidney, spleen, whole blood and skeletal muscle contained some but progressively less activity. The inactivating system from liver tissue appears to have the properties of an enzyme. It is heat-labile, being destroyed by exposure to 80°C for 10 minutes. Optimum activity during incubation is between pH 6.2 and 7.5. At pH 6.5 the rate of inactivation of pitressin increases appreciably with a rise in temperature from 0° to 37°C. Further increases in temperature are associated with a sharp decline

in activity. One ml of a 1-10 aqueous extract adjusted to pH 6.5 is capable of inactivating 100 millunits of pitressin in 30 minutes at 37°C. The activity of liver extracts is almost quantitatively removed through adjustment of the reaction to pH 5.2 or by half-saturation with ammonium sulphate at pH 6.5. Activity of the enzyme is destroyed by exposure to 0.0005 M CuSO_4 or ZnSO_4 . Liver extracts also destroy the pitressin-like antidiuretic activity of fresh rat blood serum. There is a decrease in the pitressin-inactivating ability of extracts prepared from livers of rats following adrenalectomy. This may help explain the rise of serum antidiuretic substance after adrenalectomy and contribute to an understanding of the altered water metabolism in adrenal insufficiency.

Effect of exercise on renal function in dogs WILLIAM D. BLAKE (introduced by JOHN R. BROBECK) *Lab of Physiology, Yale Univ School of Medicine, New Haven, Conn*

Kattus *et al* (*Bull Johns Hopkins Hosp* 84:344, 1949) have demonstrated that in man the assumption of the upright position and the act of walking may reduce the percentage of filtered sodium excreted without any associated decrease in glomerular filtration rate. In order to evaluate further this response in terms of mechanisms, similar studies are being conducted on dogs. The usual clearance techniques are being used to estimate renal function. The intravenous infusion of creatinine, para-aminohippurate and NaCl in solution is maintained throughout the course of the experiment. After the collection of 2 or 3 urine samples at rest the dog is exercised on a treadmill especially constructed for the purpose. The collection of urine samples is continued immediately after the cessation of exercise and for several periods thereafter. In some experiments it has been possible to demonstrate a reduction in sodium excretion coincident with and immediately following exercise without change in filtration rate but this has not been an invariable finding. The possible mechanisms responsible for this change in sodium excretion, when it occurs, will be discussed.

Pigment layer factor in visual purple regeneration

ALFRED F. BLISS *Dept of Physiology, Tufts College Med School, Boston, Mass*

Hosoya and Sasaki (*Tôhoku J E Med*, '38) reported that partly bleached visual purple regenerated almost completely in the dark if extracted from retinal tissue containing an excess of the black pigment layer of the eye. We have investigated some properties of the pigment layer factor with the aid of extracts of visual purple prepared as follows. The rods from dark adapted frog retinas were centrifuged from 2.3 sucrose-water solution (Saito, *ibid*). Their visual purple

was extracted with aqueous digitonin. The extract at pH 6.5 was divided into 2 parts which were simultaneously bleached 90% in 1 minute by white light. One of the pair was immediately treated with alkaline sodium desoxycholate to prevent regeneration (CHASE AND HAIG, *J G P*, '38). After 2 hours in the dark at 25° the other was similarly treated, and both were then completely bleached to determine the regenerated visual purple as the difference between the falls in optical density of the 0- and 2-hour samples. This method was necessary because much of the density was due to photostable material. Such unfortified rod extracts gave no significant regeneration. Regeneration was best induced by adding 0.5 M NaCl extracts of fresh or lyophilized frog pigment layer homogenates to the visual purple. Water extracts were inferior. Salt extracts of beef pigment layer were about 0.1 as active as frog. Frog brain, liver and ATP were inactive. The amount of regenerated visual purple seemed more dependent on the amount of pigment layer than on the degree of bleaching. The factor was destroyed in 1 minute at 100°, but was not inactivated by dialysis against salt, or by alcohol or ammonium sulfate precipitation in the cold.

Cardiac output in the rat and its relationship to air embolism F. R. BLOOD* AND F. E. D'AMOUR
Biologic Research Laboratories, Univ. of Denver, Denver, Colo.

A method has been developed for determination of cardiac output in the rat at normal and simulated high altitudes using the Fick principle. The rat is anatomically suitable for this technic since it is possible to introduce a small metal tube directly into the heart through the jugular vein through which samples of mixed venous blood can be obtained. The cardiac output in the rat at Denver's altitude was found to be 46.5 cc/min. When the altitude was increased it was found that the cardiac output decreased and that the occurrence of air embolism was in some way related to the relative decrease in cardiac output. There was a significant difference in the cardiac output at high altitude in rats developing air embolism as compared with those in which no embolism developed. Animals with a cardiac output greater than 20 cc/min. did not develop air embolism while, in most cases, those with a cardiac output less than this value did show air embolism at autopsy.

Free energy efficiency of assimilation of acetate and ethanol by *Chilomonas paramecium* J. J. BLUM* AND JOHN O. HUTCHENS
Dept. of Physiology, Univ. of Chicago, Chicago, Ill.

Excess heat produced when measured amounts of acetate or ethanol were added to cultures of *Chilomonas* was measured using a differential microcalorimeter. Measurements were made with

and without a nitrogen source present. Fraction of substrate assimilated, and free energy efficiency of assimilation were calculated. With no N-source (only starch and fat synthesized) 59.8% of added acetate and 81.9% of ethanol were assimilated. The free energy efficiencies of assimilation are 26.4% and 19.8% respectively. Assuming only starch to be synthesized yields slightly higher values for assimilation, 63.4% for acetate and 87.2% for ethanol, with corresponding efficiencies of 31.0% and 23.4% respectively. To calculate free energy efficiency of assimilation of ethanol, the cost of synthesis of starch and fat from acetate was employed. Available energy was taken as the sum of that necessary for conversion of all the ethanol to acetic acid plus that available from oxidation of 18.1% of the acetate. The data indicate that the energy from the ethanol to acetic conversion must be used in part. With a nitrogen source (ammonia) present ca. 43% of acetate appeared to be assimilated (Compare 55% estimated from carbon balance sheets by HUTCHENS *et al.*, *J. Cell Comp. Physiol.* 32, 117, 1948). With protein involved, precise calculations of percentage assimilation based on thermal data are impossible at present as are calculations of the free energy cost of protein synthesis. Dependence of percentage assimilation on the nature of end products may depend on 1) cost of protein synthesis, 2) efficiency of protein synthesizing mechanisms or 3) complex kinetic phenomena.

Action of M.B., 2,4-DNP, NaN₃, and ethyl carbamate on oxygen uptake of mitotically active and blocked embryonic cells JOSEPH HALL BODINE
Zoological Lab., State Univ. of Iowa, Iowa City

An extensive quantitative study has been made on the effects of these compounds upon the oxygen uptake of mitotically active and blocked grasshopper embryos. Pure solutions as well as combinations of reagents have been employed. The cellular respiratory mechanism is approximately 60% sensitive and 40% insensitive to ethyl carbamate and sodium azide in reversible doses. Methylene blue exerts its marked stimulating effects upon the 60% sensitive fraction while 2,4-dinitrophenol exerts its stimulating action upon the 40% insensitive fraction. Apparent antagonisms between such compounds as methylene blue, ethyl carbamate and sodium azide are thus readily explained. Sodium azide has little if any effect upon the oxygen uptake of mitotically blocked embryonic cells.

Influence of phlorhizin on intestinal absorption of hexoses E. M. BODANOV* AND S. B. BARKER
Dept. of Physiology, State Univ. of Iowa, Iowa City

Hexose absorption experiments on anesthetized rats gave the following coefficients of absorption (mg. absorbed per 100 gm./hr.) in glucose 116.6

D-galactose 101.2, D-fructose 49.3, D-mannose 19.9, L-sorbose 7.1 Phlorhizin (subcutaneously in oil) inhibited significantly the absorption of glucose (38%) and galactose (44%). Fructose absorption in phlorhizinized rats was 28% greater than in controls. The effect of phlorhizin on mannose (-26%) and sorbose (-90%) was not statistically significant. The normal coefficients were lower than those obtained by Cori and others in unanesthetized rats, indicating a general depression of the absorption processes by anesthesia (Nembutal). The lower coefficients were, however, in the same relative order, except for galactose. The difference between glucose and galactose coefficients in these experiments is not statistically significant. The lack of phlorhizin inhibition for fructose absorption is in contrast to previous reports and may indicate the presence of fructose phosphorylating and dephosphorylating enzymes resistant to phlorhizin action.

Adrenal stress and metabolism of ascorbic acid

WALTER M. BOOKER, RAYMOND L. HAYES* AND FRANCES M. DENT * *Depts of Pharmacology and Oral Medicine, Howard Univ College of Medicine and Dentistry, Washington, D C*

Animals under stress, given ascorbic acid, excrete more ascorbic acid in the urine than is given, indicating not only an inability to control the administered ascorbic acid, but a loss of ability to hold the stored ascorbic acid. When groups of animals under stress are given the same amount of ascorbic acid as the above group and are injected with adreno-cortical extract, the urinary excretion is significantly less and the plasma and cell levels are higher. In the ACTH treated groups, the urinary ascorbic acid rises to reach a peak by the 3rd day and falls on the 4th day. When ACTH animals are given ascorbic acid, the urinary output of ascorbic acid is higher by the third day than that of the ACTH treated above. The drop is similar to the above group, although not as great. The ACTH group, antagonized by ACE, shows a much less urinary output of ascorbic acid, and when given ascorbic acid, the urinary excretion is much less than the ACTH-ascorbic acid group. The evidence seems to show that day-to-day injections of ACTH stresses the adrenals to such a degree that the content of the ascorbic acid decreases. ACE antagonizes this stress, although at the present dosages have not been worked out between the two substances where the effects of ACTH have been off-set. ACTH animals show a decrease in adrenal ascorbic acid, with an increase in adrenal cholesterol. ACE treated animals tend to reverse this picture. ACTH treated animals show a low plasma ascorbic acid level as compared with those treated with ACE and ACTH,

but the ascorbic acid tends to hold fairly well in all ACTH treated groups.

Influence of adreno-cortical hormone on the day-to-day blood levels and excretion of ascorbic acid in dogs

WALTER M. BOOKER, RAYMOND L. HAYES* AND FRANCES M. DENT * *Depts of Pharmacology and Oral Medicine, Howard Univ College of Medicine and Dentistry, Washington, D C*

To study the possible rôle of the adreno-cortical hormone in the maintenance of blood levels from day to day, dogs were arranged in 4 groups. *Group I* dog chow plus 30 gm of Delcos granules per day, *Group II* 200 mg ascorbic acid plus 30 gm of Delcos granules per day plus dog chow, *Group III* 200 mg ascorbic acid plus 30 gm of Delcos granules per day plus 0.1 cc adreno-cortical extract plus dog chow, *Group IV* vitamin C free dog chow plus 30 gm of Delcos granules. Plasma and cell ascorbic acid, as well as urinary ascorbic acid, were determined daily. Cholesterol, chlorides, sodium and potassium were determined on each animal daily. The experiments ran 2 weeks. The plasma levels of the animals receiving ascorbic acid plus adreno-cortical hormone were consistently higher than any other group during the first week. During the second week, the plasma levels of the adreno-cortical treated animals (*Group III*) were not markedly higher than the plasma levels of animals receiving only ascorbic acid (*Group II*), but the blood cell levels were much higher than this group. The lowest plasma levels, as expected, were in the vitamin C free group (*Group IV*), but the cell levels of this group did not fall until after the plasma level had fallen significantly during the beginning of the second week. Blood cell conservation of vitamin C is suggested. When the regimen of the groups was rearranged after a 2-week run giving cortical extract to *Group II*, no extract to *Group III* and ascorbic acid to *Group IV*, the group receiving cortical extract continued to show the highest blood levels of vitamin C.

Further studies on the vomiting center

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Chronic lesions in the *ala cinerea* of the medulla oblongata (vomiting center of Hatcher and Weiss, 1923) in 5 dogs increased the intravenous apomorphine threshold for emesis to an indeterminate level (more than 50 times control), whereas the threshold to orally administered CuSO_4 was increased only slightly. Thus, the vomiting center is not located in the *ala cinerea*, rather, the latter constitutes an afferent station for the emetic response. Lesions in the floor of the IVth ventricle

extending into the dorsolateral portion of the reticular formation (reactive area for emesis by electrical stimulation, Borison and Wang, 1949) have resulted in significant increases in CuSO_4 threshold as well as marked refractoriness to apomorphine. It follows therefrom that the center for emesis resides in this deep structure of the medulla. Attempts are now being made with the use of small radon implants to destroy a circumscribed portion of the lateral reticular formation without damaging the floor of the IVth ventricle. Dogs in which all afferent pathways from the gastrointestinal tract have been severed still vomited to orally administered CuSO_4 but only to large doses and with latencies prolonged to a minimum of 90 minutes. The long delay suggests that the response is due to the action of absorbed copper. This contention is supported by the fact that CuSO_4 injected intravenously elicits emesis within 3 minutes. The emetic response to intravenous copper, like apomorphine, was not elicitable after destruction of the *ala cinerea*, thus indicating that this structure serves as a chemoreceptor for emesis.

On the nature of the elastic mechanism in actomyosin threads JEAN BOTTS AND MANUEL MORALES (introduced by KENNETH S. COLE) *Naval Med Research Inst, Bethesda, Md, and the Dept of Physiology, Univ of Chicago, Chicago, Ill*

Thermodynamic analysis of tension as a function of temperature for various extensions permits resolution of tension into 'entropy and enthalpy forces'. For small extensions of a thread maintained wet ('native') the positive tension was entirely entropic, the enthalpic contribution was negative. The reverse resulted in threads allowed to dry under stretch ('vulcanized') and then re-wet. Electron microscope and thread solubility studies showed that substances capable of hydrogen bonding inhibited gelation of actomyosin to an extent roughly paralleling their bonding ability, suggesting that hydrogen bonds, as well as purely electrostatic attractions, were responsible for maintaining the actomyosin network, especially as regards infra-filamentary linking. Absorption studies in the near infra red tended to confirm the foregoing observations. If the enthalpy be taken to approximate the internal energy, the native thread corresponds exactly to the state of resting muscle postulated by Bull, and more recently by Riseman-Kirkwood and Katchalsky, for its 'rest length' seems a compromise between an entropic contractile force and an electrostatic repulsion between charges fixed along the linear elements. Presumably on neutralizing the charges the system would contract under a purely entropic force. An earlier thermodynamic study by Woods

on myosin films probably employed a system 'vulcanized' in the extended state. Whether the actomyosin within the muscle fibril is more nearly in the 'native' or the 'vulcanized' state now becomes an important issue to settle.

Experimental differential inflow obstruction in dogs' hearts ROBERT J. BOUCEK,* JOHN H. GRINDLAY *Mayo Clinic, Rochester, Minn*

Cellophane bands placed about the venae cavae for periods of 5 days resulted in a reproducible picture of circulatory failure related to obstruction of right inflow which was progressive for 2 weeks and was followed by complete recovery after 4 weeks. The failure was characterized by a subnormal arterial pressure (av, 80 mm Hg), parallel reduction in glomerular filtration, renal plasma flow and urinary output (av, $\frac{2}{3}$ of normal). Increases in venous pressure (av, 100 mm H_2O) became established after the appearance of ascites and edema. The blood volume was normal, as was the value for serum protein, the protein content of the ascitic fluid was high (av, 3.4 gm %). Obstruction of left inflow, successfully produced in one animal, by the placing of cellophane bands about the pulmonary veins, resulted in severe hyperpnea with markedly increased roentgenologic density in the lung fields. This was followed by generalized edema. The volume of circulating blood was increased. Glomerular filtration, output of urine and arterial pressure remained normal. A slight increase in venous pressure occurred late after failure became evident. Acute pericarditis produced by Dakin's solution resulted in either of the two types of failure or a combination thereof. One type resembled the late phase of right-inflow stasis with a high venous pressure and an abrupt occurrence of ascites. The second type of failure resembled the picture of left-inflow stasis, with minimal change in renal function, a large increase in blood volume, and the occurrence of pulmonary edema, followed later by a slight increase in venous pressure and by edema.

Factors in hyperpnea of bronchial obstruction and atelectasis HARBEN J. BOUTOURLINE-YOUNG* AND JAMES L. WHITTENBERGER *Dept of Physiology, Harvard School of Public Health, Boston, Mass*

This investigation was prompted by the observation that in 3 newborn infants with atelectasis who were stimulated with electrophrenic respiration there was a reduction of 20-25% in minute volume during subsequent spontaneous respiration. This appeared to accompany expansion of the lungs, presumably a decrease in atelectasis. In dogs under various types of anesthesia, there was a consistent increase in respiratory minute volume following obstruction of one main

bronchus Thus the unobstructed lung was being ventilated at a rate generally more than twice its previous level The results were not affected by the use of local anesthesia at the site of obstruction Evidence was adduced for the following factors in hyperpnea a) *Mechanical* Because of the decreased expansion of the obstructed lung with inspiration, the intrapleural negative pressure was raised and the other lung accordingly expanded more b) *Dead space* The upper airway composes a given fraction of the total lung volume, in bronchial obstruction the alveolar space is approximately halved, without proportionate reduction in upper airway dead space The ratio of alveolar space to dead space is therefore decreased, resulting in increased ventilation requirements to maintain normal $p\text{CO}_2$ c) *Chemical* Although arterial oxygen saturation was reduced during obstruction, arterial CO_2 values remained normal, indicating that in this type of insult to lung function, the perfusion-ventilation ratio compensates for impaired CO_2 elimination, but not O_2 uptake d) *Reflex* Ventilatory reflexes due to local chemoreceptor or pressoreceptor stimulation have not been fully evaluated

Superprecipitation of actomyosin by inosine triphosphate WILLIAM J BOWEN AND S S SPICER * *Experimental Biology and Medicine Inst , Natl Insts of Health, Bethesda, Md*

Superprecipitation of actomyosin by adenosine triphosphate (ATP) is described by Spicer and Gergely in another abstract The addition of inosine triphosphate (ITP) instead of ATP is without effect, but if 0.3 ml of aqueous muscle extract (prepared by extracting muscle with 2 volumes of water) is added with ITP, superprecipitation does occur Muscle extract deproteinized with 2% perchloric acid is more effective than the original extract Ash from deproteinized muscle extract retains about 25% of the activity of the extract ITP with 0.001M MgCl_2 causes complete superprecipitation Superprecipitation does not occur when muscle extract or any of the above preparations from it are added without ITP The superprecipitations by ITP and ATP, respectively, differ in that approximately twice as much ITP (1.0 mg) as ATP is required and that superprecipitation by ITP requires 5 times longer (approx. 5 min) than that by ATP These results indicate that an organic factor, in addition to Mg, must be supplied for ITP to cause superprecipitation The possibility exists that ITP acts by being converted to ATP If this is true, then it should be possible to demonstrate spectrophotometrically the formation of adenine in this reaction Also, myokinase in the presence of myosin (since myokinase removes ADP by dismutation but not IDP) would cause larger quantities of inorganic phosphorus to be liberated

if ATP is formed than if not Both of these approaches were tested and neither gave evidence of formation of ATP Experiments are now in progress to determine whether this effect of ITP in the presence of muscle extract can be explained on other bases

Role of the vagus in control of respiration EMIL BOZLER AND B H BURCH * *Dept of Physiology, Ohio State Univ , Columbus*

To determine the role of the pulmonary afferent fibers the following experiment was carried out One end of a thin rubber tube was attached to the left vagus of an anesthetized dog just above the hilum The tube was passed through the back to the outside The right vagus was cut in the neck After the chest was closed and normal respiration was resumed, 0.2 cc of 2% cocaine was introduced through the tube to block the vagus The block abolished the Hering-Breuer reflexes and produced the following changes in respiration 1) The amplitude of respiration was diminished, 2) respiratory rate was increased, 3) The speed of the inspiratory act was markedly diminished The differences between these results and those of cervical vagotomy probably are due to the presence of extrapulmonary vagal fibers influencing the rate of respiration and to the discharge of impulses from the cut end of the nerve It may be concluded that vagal afferent fibers have a strong augmenting effect on inspiration as suggested by Gesell This effect is not caused by mechanical receptors because vagal block slows inspiration also while the chest is open and while respiratory movements and lung inflation are asynchronous and because this effect does not depend on the volume of the lungs Afferent impulses of pulmonary origin are predominantly excitatory and exert a continuous drive on respiration

Water and electrolyte excretion by the harbor seal (*Phoca vitulina*, L) S E BRADLEY, G H MUDGE* AND W D BLAKE * *Dept of Medicine, Columbia Univ College of Physicians and Surgeons, New York City, and Mt Desert Island Biological Lab , Bar Harbor, Maine*

Measurements of glomerular filtration rate by the creatinine clearance and of urinary sodium, potassium and water output have been made in 24 studies of 6 weanling Harbor Seals under varying conditions of hydration and food intake, and during diving apnoea The post-prandial renal hyperemia and apneic ischemia with corresponding changes in glomerular filtration, detected in earlier studies and confirmed in these, provided an opportunity to examine the relationship between filtration and water or electrolyte excretion In general, water and electrolyte output changed in the same direction as the filtration rate However, sodium,

potassium or water loading resulted in increased excretion, in large part as a result of decreased tubular reabsorption independent of changes in filtration. Water diuresis was associated with diminished sodium and potassium output. Pitressin and mercupurin had opposite effects on water excretion, but both augmented the elimination of electrolytes. It is interesting that a marked fall in output and urinary concentrations of sodium and potassium occurred in association with reduced filtration during apnea despite preliminary loading or administration of a mercurial diuretic. These results suggest that glomerulotubular imbalance may develop during renal ischemia in the seal and give rise to a relatively excessive reabsorption of electrolyte from the glomerular filtrate. The creatinine U/P ratio tended to fall during apnea indicating that water reabsorption was relatively decreased. This phenomenon could not be explained by reduced pituitary activity since it was observed in one animal during apnea following pitressin. However, the creatinine U/P ratio rose during apnea following sodium load, suggesting the possibility that effective water reabsorption may depend in part upon a critical concentration of sodium in the tubular urine.

Influence of respiration upon the dynamics of experimental atrial septal defects G A BRECHER AND D F OPDYKE *Dept of Physiology, Western Reserve Univ Med School, Cleveland, Ohio*

Since it is established that left atrial pressure exceeds right atrial pressure during most of the cardiac cycle in open-chest dogs it is important to know what effect changes of intrathoracic pressure have on the interatrial pressure gradient. Interatrial septal defects were produced, the chest closed, and the effect of changing intrathoracic pressure studied in acute experiments. When the left to right inter-atrial pressure difference was small during the expiratory pause the pressure gradient became reversed for a longer duration of the cardiac cycle during inspiratory movements. Rapid and extensive intrathoracic pressure decreases caused a greater and longer lasting gradient reversal. During dyspneic inspiratory movements the atrial pressure reversal lasted in some cases for several consecutive cardiac cycles. Neither alternating positive pressure inspirations with an artificial respirator nor maintained increased positive intrathoracic pressure during Valsalva-type experiments caused a reversal of the left to right inter-atrial pressure gradient. The importance of the respiration for a reversal of the blood flow through an interatrial shunt can be evaluated by an analysis of the phasic changes of atrial pressure curves.

Carbohydrates in human cervical mucus MARY ALICE B BRECKENRIDGE* AND W T POMMER-

ENKE *Dept of Obstetrics and Gynecology, Univ of Rochester Med School, Rochester, N Y*

That human cervical mucus is penetrable by sperm *in vitro* only at the ovulatory phase of the menstrual cycle has been demonstrated. *In vitro* studies also indicate that human sperm are dependent on anaerobic glycolysis for motility. Carbohydrates are present in the female genital tract, and the possibility that these are utilized in sperm metabolism presents itself. This work on cervical mucus has been continued to identify, by a chromatographic method, the carbohydrates and to quantitatively estimate individual simple sugars and polysaccharides throughout the menstrual cycle. In 6 normal subjects, followed through a total of 14 cycles, glucose was the only simple sugar consistently found, and the 0-36 mg % glucose showed no consistent cyclic variation. Whether or not the free glucose is utilized by sperm in passage through the cervical canal, it would appear that the glucose content of the mucus is not a factor in the cyclic penetrability.

Adrenal function in gravitational and acceleratory shock S W BRITTON AND C R FRENCH* *Physiological Lab, Univ of Virginia, Charlottesville, Va*

Normal rats are very resistant to the head-up position on the tilt-table, and survive for 12-48 hours, although considerable torpidity is shown throughout the period. Respiratory and various reflex activities may be used as criteria of an animal's condition. Hyperglycemia occurs within the first few hours, followed by prolonged hypoglycemia. In 30 tests made 1-7 days after bilateral adrenalectomy, marked sensitivity to the vertical posture was shown, some animals died within one hour, and most of the others became comatose or died within 2 hours. Operated rats treated with cortico-adrenal preparations showed resistance to gravity almost equal to normal animals. Adrenaline administration only slightly improved tolerance. On exposure to accelerations on the centrifuge, adrenalectomized rats showed extreme sensitivity, in 26 cases, 11 died and all others were comatose within 12 min at 2 g. This was a relatively mild exposure for normal animals.

An inhibitor of insulin-inactivating system (insulinase) of liver ROBERT H BROTH-KAHN, BEN SIMKIN* AND I ARTHUR MIRSKY *May Inst for Med Research, Jewish Hospital, Cincinnati, Ohio*

Previous studies have demonstrated the existence in liver extracts of a system (insulinase) capable of inactivating insulin during *in vitro* incubation. During the process of purification of this system by acetone precipitation it was noted that the redissolved precipitates containing the insulinase activity, when reconstituted to their original

volume, were more active than the crude extracts. This observation indicated the presence of an insulinase-inhibiting substance in the crude extract and in the supernatant from the acetone precipitate. The existence of this inhibitor has been confirmed and it has been partially purified by adsorption on charcoal, and elution with glacial acetic acid followed by precipitation with absolute alcohol-petroleum ether mixtures. Concentrates of this substance are quite active and completely suppress the *in vitro* destruction of insulin by insulinase but do not inhibit the action of the more common proteolytic enzymes. Intravenous injection of large amounts have no effect on the blood sugar of the fasting rabbit. The distribution and properties of this inhibitor will be described.

Origin of the cerebellar waves JOHN M. BROOKHART, G. MORUZZI* AND R. S. SNIDER *Depts of Physiology and Anatomy, Northwestern Univ Med School, Chicago, Ill*

Spike potentials of single cerebellar neurons superimposed upon a baseline of typical cerebellar waves have been recorded from the cerebellar cortex in unanesthetized decerebrate cats using fine, penetrating wire electrodes and conventional oscilloscopic recording equipment. Localization of the tips of electrode wires left *in situ* was accomplished by microscopic examination of thick, fixed, stained and cleared blocks of cerebellar tissue. Low voltage wave activity and freedom from spike potentials characterized recordings from the molecular layer and white matter. Higher voltage waves, occasional spike potentials, and massive injury discharges upon movement of the electrode characterized recordings from the Purkinje cell and granule cell layers. These findings indicate the great localizing ability of the wire electrode and the origin of the cerebellar waves from the Purkinje and/or granule cell layers. Temporary cerebellar ischemia or administration of ether produced abolition of spike potentials before wave activity was affected. This change was reversible, wave activity recovering before spike potentials. Summation of spikes of the type recorded from these electrodes cannot therefore be considered as a source of cerebellar waves. The waves may originate from spike potentials of small, unmyelinated axons having a selectively high resistance to anoxia, or from variations in the state of polarization neural membranes, or from some unknown source.

Excitability of mammalian heart during the cardiac cycle the auricle CHANDLER! McC BROOKS, OSCAR ORIAS,* JEROME L. GILBERT,* ARTHUR A. SIEBENS,* BRIAN HOFFMAN* AND E. E. SUCKLING* *Dept of Physiology and Pharmacology, Long Island College of Medicine, the State Univ of New York, Brooklyn*

Excitability of the auricle throughout the cardiac cycle is determined by observation of stimuli needed to produce extrasystoles. Stimuli are applied to the auricle through attached non-polarizable electrodes from a stimulator delivering rectangular waves of variable intensity (from 0 to 30 ma) and duration (from 0.1 to 15 msec). The heart is driven by a pulsing-oscillator at any desired rate. Single testing stimuli can be applied at any position in the cardiac cycle within an error of ± 2 msec. Strength-duration curves are obtained and reveal that transition from an absolutely refractory state to a normal resting state of excitability is not smoothly progressive. Within the relative refractory period there are intervals of greater followed by intervals of lesser excitability as recovery proceeds. No supernormal-period is found. The latency between testing stimulus and the response increases progressively as the stimuli are placed earlier in the refractory period. A local excitatory state created by a single strong testing stimulus may outlast the refractoriness which follows normal driven beats. Progressive increases of testing-shock intensity at any instant within the 'vulnerable-period' produce first extrasystoles, then fibrillation and finally no response at all (a 'disappearance-phenomenon'). The 'disappearance-phenomenon' is thought to be due to large field effects of very strong stimuli. Like fibrillation, it can be obtained only during a 'vulnerable' portion of the relative refractory period.

Some biological and chemical properties of the citrovorum factor HARRY P. BROQUIST,* E. L. R. STOKSTAD AND T. H. JUKES *Lederle Labs Division, American Cyanamid Co., Pearl River, N. Y.*

Recent studies have indicated that concentrates of natural materials active for growth of *Leuconostoc citrovorum* reverse the toxicity of folic acid antagonists for some microorganisms under conditions in which pteroylglutamic acid (PGA) is ineffective. In the present study further evidence for the ability of citrovorum factor (CF) concentrates to reverse 4-amino PGA for *L. citrovorum* was found. In studies with mice it was found that CF concentrates given either in the diet or by injection could protect mice against the toxic effects of 4-amino PGA. PGA was without effect in alleviating this toxicity. In counter current distribution studies by the method of Craig, it was found that the distribution curves for CF activity or folic acid activity were indistinguishable. In other experiments it was found that CF was readily converted by exposure to HCl to a compound with properties similar to folic acid. These findings coupled with the ability of CF concentrates to reverse the toxicity of 4-amino PGA in microorganisms and mice support the view that CF is

a hitherto unrecognized biologically active form of PGA

Secretion of the gluconeogenic factor by the adrenal KATHARINE A BROWNELL *Ohio State Univ, Columbus*

The gluconeogenic factor secreted by the dog adrenal was assayed by virtue of its ability to cause deposition of liver glycogen. Blood was collected from the left lumbo-adrenal vein, tied at its entrance to the vena cava in a heparized nembu-talized dog. Transfusion of blood from a donor dog replaced that removed from the subject. The hormone was separated from the adrenal blood plasma by extraction with ethylene chloride. A 5% ethyl alcohol solution of the hormone was injected in 4 equal doses over an 8-hour period into adrenalectomized, 24-hour starved mice. The amount of liver glycogen deposited was an indicator of the gluconeogenic hormone present. Under the conditions of the experiment an appreciable amount of gluconeogenic hormone was always being secreted. Injection of ACTH increased the amount secreted per unit of time, but large amounts of epinephrine appeared to have little influence under experimental conditions.

Axon as model of synapse: new evidence of similarity of conduction and transmission THEODORE HOLMES BULLOCK *Dept of Zoology, Univ of California, Los Angeles*

Death of the all or none impulse at a predisposed locus and rebirth of a new impulse beyond (BLAIR AND ERLANGER, 1939, in frog fibers) was restudied in invertebrate fibers with 4-gun cathode ray tube. The necessary and sufficient condition is a lower curve of local response decrement with distance than of return of excitability. Two conditions that seemed necessary in the frog experiments are shown not to be essential: current eddies through nodes of Ranvier and a locus of artificially heightened excitability (cathode) within the critical distance (ca. 1 mm). Present results emphasize the importance of actively (though decrementally) propagated local response and the short distance over which crucial changes in excitability may occur. One-way conduction was directly shown in single fibers (cf BISHOP AND ERLANGER, 1926, ROSENBLUTH *et al*, 1949) with multichannel recording. Its locus is very restricted. It can occur without polarization and as a result of either static conditions or fluctuating conditions associated with immediately preceding impulses. After-discharge in fibers frequently takes the form of one-way initiation. The synapse-like character is emphasized by long and variable delays. One-way inhibition is a consequence. Excitability may locally change in discontinuous manner through several steps in rapid, erratic succession. It is apparently critical that immediately preceding impulses fol-

low a certain temporal and spatial pattern. The explanation seems to lie in local response properties. The result amounts to genuine integration and introduces long, variable local delays. Finally, a labile facilitation of conduction rate has been found in fresh preparations of earthworm giant fibers.

Influence of tetraethylammonium chloride on the circulatory responses to the Valsalva maneuver IVAN L BUNNELL, DAVID G GREENE AND WILLIAM W KUNZ (introduced by FRED R GRIFFITH, JR) *Depts of Physiology and Medicine, Univ of Buffalo School of Medicine and the Buffalo General Hospital, Buffalo, N Y*

The response of the arterial blood pressure to the Valsalva maneuver has been noted previously to be a useful indication of the completeness of autonomic blockade by various blocking agents. Normally, after intrathoracic tension is released, there is an immediate drop in arterial pressure, followed by a rise to a level higher than that which existed before the maneuver. This 'overshoot', a manifestation of increased vasoconstrictor activity, is abolished by autonomic blockade. The adaptive reflexes normally resulting from the maneuver produce not only changes in vasoconstrictor activity, but also changes in heart rate. The presence of the latter provides another indication of an actively functioning autonomic nervous system. In the present study, arterial blood pressure has been measured in unanesthetized human subjects with a Hathaway blood pressure recording system attached to an indwelling arterial needle. The influence of intravenous infusions of tetraethylammonium chloride for varying periods of time in abolishing both the 'overshoot' and the changes in heart rate will be described, and discussed with regard to the relative sensitivity of these 2 criteria of autonomic blockade.

X-ray observations on dogs following explosive decompression to 30 mm Hg BUFORD H BURCH* AND FRED A HITCHCOCK *Lab of Aviation Physiology, Ohio State Univ, Columbus*

A series of 12 dogs were explosively decompressed to a terminal pressure of 30 mm Hg and maintained at this pressure for 2 minutes. Chest x-rays were taken 2 to 5 seconds after the explosion and subsequently at 30-second intervals. Partial collapse of the lungs occurred in all dogs shortly after the reduction of the pressure. This collapse of the lungs is believed to be due to vaporization of the intrapleural fluid, producing a condition which we have called 'vapo-thorax'. When these dogs were returned to normal atmospheric pressure the lungs expanded, completely filling the thorax, indicating that there was no pneumothorax or rupture of the lungs. Vapor usually appeared in the right heart 30 seconds after the ex-

plosive decompression and in the left heart about one minute later Vapor is usually found in the right heart before it is noted elsewhere When vapor was present in the left heart the dog usually died Marked dilatation of the heart occurred in all dogs We believe that blocking of the coronaries by vapor is an important cause of death It is also possible that the presence of vapor in the right heart may, by producing circulatory vapor lock, be a significant factor in the precipitation of ventricular failure Two dogs survived In one there was no vapor visible by x-ray present in the heart The other surviving animal had considerable vapor in his right heart and showed cardiac dilatation which persisted for about a week

Permeability of the urinary bladder to radioactive phosphorus, mercury and gold L E BURGESS,* D T ROLFE,* P F HAIN AND E L CAROTHERS * *Dept of Physiology and Cancer Research Lab, Meharry Med College, Nashville, Tenn*

Female dogs weighing 8-15 kg were anesthetized by intravenous sodium pentobarbital The ureters were doubly ligated at the bladder, severed, and the proximal ends cannulated to collect the urine flow A catheter was introduced into the bladder and the latter was emptied and washed several times with a warmed physiological salt solution Two to 5 m μ of radioactive H_3PO_4 , $HgCl_2$ and colloidal Au in warm physiological salt solution were allowed to flow slowly through the catheter into the bladder Analyses for P^{32} , Hg^{203} and Au^{198} were made at hourly intervals for a period of 6 hours on samples of blood taken from the external jugular vein and on samples of urine simultaneously collected from both ureters The blood showed a marked uptake of P^{32} during the first hour which reached its peak between the fourth and fifth hours The uptake of Hg^{203} by the blood was found to be very low during the first hour but increased rapidly and reached its peak between the second and fourth hours By the end of the sixth hour, a considerable absorption of both phosphorus and mercury had occurred from the radioactive materials instilled into the bladder, as revealed by analyses of the blood The rate of excretion of P^{32} and Hg^{203} by the kidneys, as shown by analyses of the ureteral urine, increased with the rise in blood levels of P^{32} and Hg^{203} Work in progress appears to indicate some slight absorption of colloidal gold by the urinary bladder

A new view of relation between pressure and flow in a vascular bed, and validity of 'peripheral resistance' ALAN C BURTON, J T NICHOL,* F GIRLING,* AND WM JERRARD * *Dept of Biophysics, Univ of Western Ontario Med School, London, Ontario, Canada*

The non-linear relation between pressure and flow of blood in vascular beds has been variously

explained as due to the distensibility of the vessels, changing the resistance with the pressure and to the anomalous viscosity of blood, changing with the velocity of flow Neither of these is the important factor Physical theory and experiment show that the equilibrium of the small vessels is unstable If the pressure within them falls below a critical value, dependent of their size and on the tension in the wall, they close abruptly and completely At pressures well above this 'critical closing pressure,' direct observation shows that the small vessels act like almost rigid tubes Under vasomotor tone the critical closing pressure may be above 100 mm Hg (spasm) Experimentally obtained curves of resistance vs pressure show an almost constant resistance until the critical pressure is approached, when it rises quite abruptly to infinity The rise is more abrupt with Ringer's solution than with blood These results re-establish the usefulness, in haemodynamics, of the 'resistance' when combined with a knowledge of the 'critical closing pressure' and also give a new view of the nature of spasm

Bile cholesterol, bilirubin, and physical constants in the rat SANFORD O BYERS, MEYER FRIEDMAN AND FRED MICHAELIS * *Harold Brunn Inst, Mount Zion Hospital, San Francisco, Calif*

An external bile fistula was created in 23 albino rats by inserting a catheter of plastic tubing into the bile duct and externalizing the free end of the tubing through the abdominal wall Each animal was restrained by a special technique, allowed water or 0.8% saline *ad lib*, and the entire 24-hour biliary output collected for determination of cholesterol bilirubin and physical constants The average concentration of free cholesterol (substantially equivalent to total cholesterol) was 12.7 mg/100 cc the average 24-hour total output was 1.83 mg Bilirubin was produced in total quantity of 1.2 mg/24 hours (av 7 rats) The average volume of flow per 24 hours was approximately 15 ml, or 5 ml/100 grams body weight Secretory pressure was 22-24 cm of bile of average specific gravity 1.011 The pH (glass electrode) varied from 7.9 to 8.5, average 8.3

Human tolerance and responses to high positive pressure breathing CLARENCE C CAIN, JOHN A WOOD AND DAVID I MAHONEY (introduced by J W HEIM) *Aero Med Lab, Air Materiel Command, Wright-Patterson Air Force Base, Dayton, Ohio*

An experimental study was conducted to determine the time tolerance of a group of individuals breathing oxygen at various positive pressures from 30 to 60 mm Hg at room temperatures of 65°F and 95°F The heart rate, venous pressure, pulmonary ventilation and relative cardiac output as measured by the ballistocardiogram, were deter-

mined for certain pressures. The end point of these time tolerance studies was symptoms of impending syncope. A fairly narrow time range of tolerance occurred with the high pressures of 50 and 60 mm Hg. This range widened considerably at 30 and 40 mm Hg approximating time tolerances of 1 min at 60, 7 min at 50, and 15 min at 40 mm Hg. When breathing on 30 mm Hg pressure, the time was extended to between 1 and 2 hours and the exposures were terminated due to fatigue rather than impending syncope. However, at the same pressures at 50,000 feet it was necessary to terminate the exposures after 10 minutes on 2 subjects. At pressures of 40 mm Hg there occurred an average increase in heart rate of 50% and a reduction in cardiac output of approximately 42%. The venous pressure increased but not in linear relationship to the increase in mask pressure. These responses are in accord with the accepted view that impending syncope is due to circulatory stress. The pulmonary ventilation on a group of 7 subjects at ground level breathing oxygen at a positive pressure of 30 mm Hg increased to 150% of normal during the first 5 minutes and decreased to 130% of normal at the end of 20 minutes. The ventilation did not return to normal on any of the subjects tested while exposed to 30 mm Hg breathing pressure.

Relatively light doses of sesame oil on oxygen consumption and role of adrenal cortex THOMAS B. CALHOON* AND CLIFFORD A. ANGERER *Dept of Physiology, Ohio State Univ., Columbus*

We have previously reported (*Anat Rec Suppl* 105:142, 1949) the effects of large doses of sesame oil on the oxygen consumption of 'normal' goldfish and that these effects were antagonized by adrenocortical extract. Recent studies with light doses of sesame oil have shown that the previous results are open to a different interpretation. Oxygen consumption determinations were made weekly on 2 groups of goldfish over a period of 7 weeks, using a respirometer developed in this laboratory. All fish were fasted from 12-18 hours prior to use to render them in a 'basal' state. A group of 12 fish were given 1 ml 0.0002 cc sesame oil/gm of body weight/every other day for a 7-week period. At the end of the second week the mean metabolic rate increased 12% compared with controls. This was followed by a sharp drop in oxygen consumption until the 7th week when the mean value was 40% below normal. A second group of 12 fish were injected 1 ml with 0.0002 cc sesame oil and 0.00036 cc Upjohn's Adrenocortical Extract/gm body weight every other day for a 7-week period. At the end of the second week the mean oxygen consumption decreased 35% from normal. This was followed by a leveling of these values at ca -37%. Although the weights of all fish fluctuated greatly, the mean weight of fish given sesame oil alone increased 3%

at the end of the 7th week. Fish given adrenocortical extract plus sesame oil increased 26% at the end of this period.

Experimental analysis of electrical responses of inferior colliculus to sound and cutaneous sensibilities BERRY CAMPBELL *Dept of Anatomy, Univ of Minnesota Med School, Minneapolis*

Responses evoked in the inferior colliculus and associated structures by condenser discharges through a piezo-crystal near the ipsilateral or contralateral ears have been recorded by micro-electrodes using a cathode ray oscillograph. Additional data on the responses of these centers to spinal nerve stimulation is presented. Correlation of structure and electric potential of cell groups and tracts is made on the basis of histological examination of the needle tracts. Monkeys, cats, rabbits, and rats have been used in the experiments. The potential evoked by a contralateral click in the inferior colliculus consists of 2 components, one a pick-up from the underlying nucleus of the lateral lemniscus responding at 4 msec, and a local potential commencing at the base of the colliculus and rising at a speed of from 0.5 to 1.0 msec/sec to the dorsum. Analysis of the sign changes and spike form of this potential indicates that it is conducted as an upward rising positive-negative wave. The distribution of the potential of the lateral lemniscus shows that the cells, upon activation, form the center of a strong bipolar field, the dorsal side positive, the ventral negative. This potential is marked throughout the colliculus and the underlying tegmentum, but spiking, and the highest voltage is seen at the nucleus. Contralateral clicks evoke much less activity. Stimulation of contralateral spinal nerves evoke potentials in the inferior colliculus. These decrease the response of the inferior colliculus to auditory activation.

Flight response of drosophila to variations in temperature L. E. CHADWICK *Medical Div., Army Chemical Center, Md*

Data are presented to define the variations in wing-beat frequency of flies as a function of age and temperature.

Positive pulse stimulation of anterior sigmoid and precentral gyri: electric current threshold dependence on anesthesia, pulse duration, and repetition frequency WILLIAM W. CHAMBERS, GEORGE M. AUSTIN AND JOHN C. LILLY (introduced by MARGARET SUMMALT McCOUCH) *Dept of Anatomy, Neurosurgery and Biophysics, Univ of Pennsylvania, Philadelphia*

In determining current thresholds (J) for positive pulses (durations (PD) from 0.1 ms - 500 ms) at frequencies (PRF) from 1/60 to 9000 pulses/sec, trains (5 sec duration, 1/min) are sent through an

Ag-AgCl-saline pore electrode (10 mm diameter) on a cortical motor zone in 3 series of animals. For each stimulus the threshold current value and pulse waveform are determined by observation by means of a series resistor, a direct-coupled amplifier, and a cathode-ray oscilloscope. The animals' movements were observed by visual means. Exposed cortex and anesthesia (dial 4 mg/kg) were used in the first series (I) (5 cats and 3 monkeys), exposed cortex and none but local anesthesia in the second series (II) (2 monkeys), and a closed cranium and only local anesthesia in the third (III) (1 monkey). Horsley-Clark fixation was used in I, skull edge fixation in II and an implanted electrode in III.

The resulting curves of J versus PRF for constant PD were reproducible for several hours. In I and II the 'rheobase' was 1.1 ma at 10 ms at all PRFs, at 10 ms above 500 cps and at 0.1 ms above 1000 cps. A low PRF (1/60-15/sec) plateau (12 ma at 0.1 ms, 3-6 ma at 10 ms) is connected by a uniformly falling curve to the high PRF 'rheobase'. In contrast in III, on the 1st day, the low PRF plateau is connected to the high PRF plateau by a 'valley' (minimum at 10 ms, 200 cps and 0.65 ma, at 0.1 ms, 200 cps and 2.0 ma), in the 2nd through the 3rd day this curve changes from the valley-type to the I and II type. In the same animal, on other side, cutting out upper cortical layers caused immediate flattening of the valley. There was little if any impairment of voluntary use of responding part on either side at any time during 8 days of observation.

Influence of pulmonary proprioceptive reflexes during electrophrenic respiration. PAUL O. CHATFIELD* AND STANLEY J. SARNOFF *Depts of Physiology, Harvard Med School, and Harvard School of Public Health, Boston, Mass*

During periodic stimulation of a phrenic nerve to produce descent of the diaphragm (electrophrenic respiration), one obtains an inhibition of spontaneous respiration which is clinically useful since it eliminates the conflict between the patient's spontaneous breathing and the imposed respiration. It has been shown that this inhibition can be brought about by vagal reflexes alone or secondarily by hyperventilation. In the present study, by recording muscle action potentials from the left (unstimulated) leaf of the diaphragm and from inspiratory intercostal muscles in dogs during application of electrophrenic stimulation to the right phrenic nerve, it was possible to ascertain the conditions of electrophrenic respiration which facilitate the desired inhibition of spontaneous respiration. It was found that inhibition depends on the integrity of the vagi (previously shown), that inhibition of spontaneous respiration is facilitated by a) an increase in rate of electrophrenic

respiration, b) prolongation of inspiration and c) an increase in the depth of inspiration. A changed state of excitability of the respiratory center (as from rebreathing) may cause previously inhibited spontaneous respiratory discharge to resume. Under many conditions, even when spontaneous respiration was not completely inhibited, there was synchronization of spontaneous and electrophrenic respirations. The prompt suppression of spontaneous respiration seems much more easily obtained with an active diaphragmatic contraction than with positive pressure breathing. The results are discussed in the light of recent unitary analysis of pulmonary proprioceptors and their practical significance pointed out.

Studies on pharmacology of tetraethylthiuramdisulfide (Antabuse) and the 'Antabuse-Alcohol' syndrome in animals and man. GEORGE P. CHILD, EUGENE BARRERA,* WALTER OSINSKI,* ZACK RUSS* AND GEORGE HEMSTED* *Depts of Physiology and Pharmacology, Neurology and Psychiatry, and Medicine, Albany Med College, Albany, N Y*

The duration to alcohol sensitization by Antabuse was tested in rats. The sensitization reached a maximum 6-12 hours after giving an oral dose of Antabuse and it lasted for 10-15 days. The duration and degree of effect were determined largely by the dose of Antabuse given. The ascending paralysis produced by large doses of Antabuse in animals could not be alleviated by prostigmine and was suggestive of the paralysis caused by spinal cord interneuron blocking drugs. This was tested by giving white rats convulsant doses of strychnine. The Antabuse did not protect the rats from the cord stimulating effect of strychnine. Humans who are under treatment for chronic alcoholism were given a test dose of alcohol before and after the administration of Antabuse was started. Among the signs and symptoms of the Alcohol-Antabuse syndrome are hypotension, tachycardia, hyperpnea, ECG changes, descending erythema, feeling of warmth, diaphoresis, thirst, gastric hypermotility, nausea, confusion, blurred vision, vertigo, and above all the amazement on the part of the patient that such minute amounts of an alcoholic beverage can make one feel so peculiarly. The temporal and intensity relations of these effects will be presented. At present the best antidote for this syndrome is intravenous ascorbic acid. The effect of Antabuse alone is discussed.

Use of intravenous ethanol in treatment of a case of opiate addiction, and other experimental therapeutic tests with intravenous ethanol in humans. GEORGE P. CHILD, FRANK CLARE* AND JOHN FARRELL* *Depts of Physiology and Phar-*

macology, Neurosurgery, and Surgery, Albany Med College, Albany, N Y

The pharmacologic properties of intravenous ethanol as an analgesic, euphoric antipyretic and vasodilator have been used by us with good effect in the treatment of phantom limb pain, peripheral vascular disease (to test the ability of the vessels to dilate), pyrexia and in the management of intractable patients. Another use, which deserves particular emphasis because of its significance if verified by further work, is in the treatment of opiate addiction. Three patients have, thus far, been under treatment. Two of the patients had very painful lesions and were receiving a considerable quantity of opiate. During the period of alcohol administration it was possible to reduce the opiates and even eliminate them for a day or two without producing withdrawal symptoms. Because of the lesions, however, it was felt that the opiates should not be withheld. The other patient had been receiving natural and synthetic opiates for approximately 3 years. After surgical treatment for her lesion, attempts were made to cure her of addiction without success. She also had diabetes and hyperthyroidism. When the 5% intravenous alcohol (Abbott) was started all opiates were discontinued. On each of 6 successive days she was given from 1- $\frac{1}{2}$ l of 5% alcohol in divided doses with a complete cure of addiction. The only withdrawal effects noted were mydriasis, mild diarrhea and a pyrexia of 101° for one day. Although this one case has thus been cured of addiction the result was so spectacular as to warrant publication.

Effect of depletion of extracellular electrolytes on the water intake in dogs LOUIS J CIZEK,* ROBERT E SEMPLE,* K C HUANG* AND MAGNUS I GREGERSEN *Dept of Physiology, College of Physicians and Surgeons, Columbia Univ, New York City*

The experiments were designed to test whether or not the degree of cellular hydration determines the fluid intake. Following a 2- to 3-week control period during which observations were made on levels of serum chloride, plasma protein, plasma volume, thiocyanate space, water intake and output, the animals were depleted of extracellular chloride and sodium by the intraperitoneal glucose procedure. The dogs were then maintained on a low salt diet for a period of one to 3 weeks, throughout which the serum chloride, plasma volume and thiocyanate space remained at low levels. The dogs were lethargic immediately after the salt depletion but this lethargy wore off gradually in a day or two. Within 48 hours the water intake exceeded the control intake. Urine excretion likewise increased. The increase in water intake varied from 33 to 85%. One dog, however, refused to eat

after salt depletion. Nevertheless his fluid intake and urine output were greater than when the same animal in normal electrolyte balance was placed on a starvation regime. With intravenous sodium chloride and restitution of salt to the diet, water intake, plasma volume, plasma protein etc returned to control levels. These findings are not in accord with the concept that the degree of cellular hydration determines the fluid intake.

Thalamic loci of afferent stimulation in the cat SYDNEY M COHEN (introduced by FRED A METTLER) *Dept of Neurology, College of Physicians and Surgeons, Columbia Univ, New York City*

Upon electrical stimulation of afferent nerves (face, limbs) or tactile stimulation of skin, electrical activity (50-200 uv, 5-40 msec duration) is recorded with needle microelectrodes in the ventral posterior thalamic nucleus. The responses to face afferent activity have the shortest duration. Contralateral stimuli produce greater activity than do ipsilateral. Only forelimb stimulation elicits activity in a small region of the rostro-medial part of the nucleus. Elsewhere, activity is elicited by afferents from hind- and forelimbs or face, though the responses differ in amplitude and duration. Potentials referable to pick-up of incoming activity are observed over a wider region of the thalamus. Cervical section of lateral columns does not affect thalamic activity, but section of dorsal columns abolishes thalamic responses to limb afferent impulses. Electrical stimulation of one nucleus produces no response in the other. Nor does division of anterior, middle or posterior commissures alter responses of either nucleus. Cross connections between nuclei are therefore lacking. Latency of thalamic response remains nearly constant with increasing afferent stimuli. However, the time difference between arrival of afferent activity in the nuclei of the dorsal columns and the onset of the thalamic response is 10-20 msec greater for the forelimb activity than for that of hindlimbs.

Effect of intraluminal hydrostatic pressure on water and sodium movement across intestinal wall of dogs S T COLEMAN, R F JONES AND M J FOGELMAN (introduced by ROBERT W LACEY) *Dept of Exper Surgery, Southwestern Med School of The Univ of Texas, Dallas*

In an attempt to determine the causes for the collection of fluid in an obstructed intestine the effects of various intraluminal pressures on water and sodium exchange were studied in Thiry-Vella loops of trained unanesthetized dogs. Forty ml of a 1% aqueous solution of D₂O were placed into the Thiry-Vella loops and subjected to maintained pressures of 10, 20, 30 and 40 cm of water for 90 minutes. Samples for Na and D₂O determinations

were withdrawn by catheter from the lumen at graded intervals. The heavy water analyses were made by the falling drop method and the sodium determinations by flame photometry. At the termination of the experiments the gut was emptied with a catheter and the remaining volume measured. The rates of movement of water into and out of the intestine were calculated by the method of Visscher (*Am J Physiol* 142: 550, 1944). There was no positive correlation between the intraluminal hydrostatic pressures and the rates of movement of water and sodium across the intestinal wall. Eleven additional experiments using a constant initial volume of plain water and pressures varying from 10–40 cm of water also failed to show a correlation between intraluminal pressure and net rate of movement of water across the intestinal wall.

Effect of X-irradiation on intestinal motility

ROBERT A. CONARD (introduced by HARVEY M. PATT) *Biological Div., Argonne National Laboratory, Chicago, Ill.*

In vivo studies have been carried out measuring kymographically contractions of the longitudinal muscle of rat jejunum immersed in constant temperature Tyrode's solution. During X-irradiation with doses of 100 r (250 kv, 100 r/min), and above there is a typical pattern of intestinal activity produced. After a lag of about one minute there is an increase in tone accompanied by an augmentation of contraction which continues for several minutes after the irradiation is terminated. Tonus gradually returns to the pre-irradiation level (with 100 r in about 4–6 minutes). Larger doses of irradiation in general produce a higher and longer rise of tone. Shielding studies and *in vitro* irradiation of isolated segments of intestine reveal that the contraction response is largely due to a direct effect on the intestinal tract. Atropine and nicotine strongly inhibit the response while physostigmine augments and prolongs it. Sympathetic drugs have little or no effect. Immediate vagotomy has no appreciable influence while vagotomy of a week's duration gives an augmented response to x-ray. The antihistaminic benadryl has no effect. These data suggest that the x-ray response is largely due to a direct effect on the intestinal tract, specifically, on its parasympathetic elements.

Bicarbonate concentration of pancreatic juice during experimental acidosis

S. S. CONLY,* J. O. CRIDER AND J. E. THOMAS *Dept. of Physiology, Jefferson Med. College, Philadelphia, Pa.*

Unanesthetized dogs with permanent gastric and duodenal fistulas fitted with metal tubes were used, and the pancreatic juice collected during intravenous administration of secretin. The normal curve of bicarbonate secretion was determined for

each animal. The concentration of bicarbonate was plotted on the ordinate and the rate of secretion on the abscissa. With rapid rates of secretion the bicarbonate concentration appears to approach a maximum asymptotically. Acidosis was produced by the intragastric administration of 500 cc of a 2% ammonium chloride solution through the gastric fistula at a rate of about 125 cc/hr. Two or 3 days of such administration lowered the CO_2 combining power of the blood plasma from a normal of 22–24 mm/l to 12–16 mm/l. The bicarbonate content of pancreatic juice as evoked by secretin was determined for 2 or 3 days following the administration of ammonium chloride. After this time the blood CO_2 had returned to normal and the bicarbonate of the pancreatic juice to normal. During the period when the blood CO_2 was lowered the CO_2 content of the pancreatic juice was lowered for a given rate of secretion but the curve has the same general form as before the period of acidosis. The bicarbonate content of the juice for a given rate of secretion was lower the second day than it was on the first day following the administration of the ammonium chloride and this at a time when the blood CO_2 was approaching its former normal level.

Effect of electrical polarization on oxygen consumption of nerve

CLARENCE M. CONNELLY* AND DETLEV W. BRONK *Dept. of Biophysics, Johns Hopkins Univ., Baltimore, Md.*

It is well known that the membrane potential of resting nerve is maintained by oxidative metabolism. However, little or no information exists with respect to the effect on the oxygen consumption of nerve of an imposed electrical polarization. An attempt has been made to determine the degree of interaction between the mechanism maintaining the membrane potential of frog nerve and the reaction system that consumes oxygen. Two types of experiment have been performed, using a polarized platinum electrode in contact with the nerve to determine changes in the rate of oxygen utilization. In the first type of experiment, anodal or cathodal polarization was applied to a resting nerve in the region of the oxygen-recording platinum electrode. The changes in the rate of oxygen utilization resulting from polarization (0.25–2.0 microamperes, 1–10 minutes) were strikingly small, being considerably smaller than, or at most of the order of, 1% of the resting rate. The sensitivity of the measuring system was in this case determined in terms of the steady-state extra-oxygen consumption resulting from 1 impulse/sec. In the second type of experiment, the region of nerve immediate to the oxygen electrode was blocked with excess potassium ion. During a period in which local anodal repolarization of the nerve relieved the block, it was found that the conduc-

tion of impulses (100/sec, 50 sec) did not result in detectable extra-oxygen consumption

Influence of diet composition on alarm reaction to acute stress P. CONSTANTINIDIS (introduced by H. SELYE) *Institut de Medicine et Chirurgie experimentales Université de Montreal, Montreal, Canada*

Rats gavaged with isocaloric and isovitaminic amounts of high-amino-acid and high-carbohydrate diets were exposed to various types of acute sublethal stressors (cold, muscular exercise, formaline, urethane, colchicine) for short periods of time. It was found that in every instance the dietary intake significantly modified the ensuing alarm reaction. Thus, in the case of every stressing agent except colchicine, adrenal enlargement, cortical lipid discharge and lymphatic karyoclasia were maximal on a high-amino-acid and minimal on a high-carbohydrate intake. The response to colchicine was conditioned in the reverse fashion. Chemical estimations of adrenal cholesterol, adrenal ascorbic acid and liver glycogen were subsequently performed in animals exposed to cold for 1, 6, 15 and 30 hours. During the first 15 hours the rats fed a high-amino-acid diet showed a considerably greater cholesterol and glycogen depletion than those on a high-carbohydrate diet, while at 30 hours of cold the difference between the 2 nutritional groups decreased. There was no difference in the ascorbic acid response between groups at any interval. The influence of protein on the pituitary-adrenal system under resting conditions has remained controversial up to date. It has been shown by Selye (*J Clin Endocrinol* 6:117, 1946) however, that high protein diets aggravate certain pathological changes provoked by prolonged exposure to stress or anterior pituitary principles. The present data demonstrate that the adrenal response to acute, short-lasting stress is also conditioned by the amount of protein or carbohydrate in the diet.

Observations on the etiology of explosive decompression injury E. L. COREY AND E. G. LEWIS * *Univ. of Virginia Med. School, Charlottesville*

The explosive (0.64 sec) decompression of rats (250) to 21 mm Hg was found to be innocuous even when frequently repeated (20 exposures), provided recompression was immediate (> 1 sec). Mortality was observed to be directly related to post-decompression time under reduced pressure, and all rats succumbed when this interval was prolonged to 40 seconds. Animals placed in an atmosphere of nitrogen succumbed within a comparable period (63.5 sec). However, such procedures as pre-oxygenation and resuscitative measures (insufflation with air, O₂, CO₂, O₂-CO₂) failed to produce striking reductions (av. 25%) in mortality. Aeroembolism was never observed within the vessels (jugular,

mesenteric, renal, femoral) of animals under 21 mm Hg, although present *in vitro* in rat blood at this pressure. Alterations in autonomic activity (vagotomy, atropine, mecholyl) reduced mortality only 22%. Hence aeroembolism and endogenous autonomic activity appeared to represent minor determinant factors. On the other hand, anesthetics (cold, ether, evipal, pentothal) enabled all animals tested (29) to survive the normally lethal time-interval under reduced pressure. Indeed this period might be lengthened by as much as 100% without fatality in anesthetized rats. It was noted that the convulsive dyspnea characteristic of normal animals under such conditions was absent. On the basis of these experiments it was considered that intrapulmonary gas expansion *per se*, aeroembolism, and endogenous autonomic nervous influences do not constitute primary etiologic agents in explosive decompression injury, but that anoxic anoxia, with resultant convulsive dyspnea, represents the major factors producing lethal injury under the conditions cited.

Diuretic effects of sodium salts of organic acids in patients with cardiac and nephrotic edema

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Following a period of bed rest, a 1.4 M solution of disodium succinate-fumarate was infused intravenously to a patient with cardiac edema at the rate of 1.3 ml/min, giving a total dose of 2.8 ml/kg body wt. This led to a diuresis of 12.9 ml/min (average) for a period of 325 minutes (control = 1.3 ml/min). The total volume of urine during this period was 4.2 l. The next day the patient showed no pitting edema. The thiocyanate space was reduced from 27 l (34.5% of body wt) to 19 l (25% of B.W.). The patient remained edema-free for the duration of her hospital stay (one week). The rate of Cl and Na excretion increased parallel with the diuresis. During the 325 minutes the patient excreted 275 mEq Cl (2.2 mEq infused) and 708 mEq Na (635 mEq infused). A similar infusion was given to a patient with longstanding rheumatic heart disease and terminal renal insufficiency (probably of obstructive origin) exhibiting progressive oliguria, uremia, ascites and pitting edema limited chiefly to the lower extremities. A diuresis of about 3 l was obtained without significant improvement in the clinical condition. Two infusions to one nephrotic patient with hypoproteinemia, severe ascites and pitting edema led to a diuresis of 4 l for a period of about 400 minutes both times. At the end of both experimental periods the patient showed Na retention. Another nephrotic patient failed to give any diuretic response. This patient proved to have depressed

clearances of inulin and para-amino hippurate. More detailed studies are in progress.

Relation between heat balance and physiological strain in walking men clad in a ventilated impermeable envelope F N CRAIG *Medical Div , Army Chemical Center, Md*

Measurements of metabolic heat production and of heat removed by ventilation were made on men enclosed in an impermeable garment and walking on a level treadmill at 3 miles/hr in a room at 85° F. Ventilating air was introduced at the 4 extremities and the top of the head by means of an internal manifold, and left through an opening at the waist. The heat removed was calculated from increase in temperature and moisture content of the ventilating air. Inspired and expired air were kept separate from the ventilating air with a gas mask. Metabolism was determined by analysis of expired air. Heat balance was altered by varying the flow rate, temperature and moisture content of the ventilating air. The physiological strain was measured in terms of an index obtained by adding sweat production in kg/hr, the rise in rectal temperature in degrees C/hour, and one hundredth the heart rate at the end of a 30-minute period. In 28 tests the physiological index ranged from 2-5. The heat stress, defined as the metabolic rate in calories/hour minus the heat removed by ventilation, ranged up to 300 calories/hour. The index increased at the rate of one unit of strain ± 0.5 unit/100 calories of heat stress. This formulation provides a measure of heat stress free of a heat storage term depending on body temperature measurements, and so permits their use in an index of strain.

Some effects of oxine, carbostyryl and quinoline on isolated, living bullfrog nerves FREDERICK CRESCITELLI *Dept of Zoology, Univ of California, Los Angeles*

The role of metal-containing enzyme systems in numerous cellular reactions is recognized. Such enzyme systems may be inhibited by agents which combine with the metal. To test for the possible participation of a metal-enzyme system in the mechanisms which maintain the nerve resting potential and in the reactions which determine impulse conduction, the effects of 8-hydroxyquinoline (oxine) on bullfrog nerve have been examined. Oxine is a powerful chelating agent for a number of metals. When a 0.003M solution of oxine in Ringer fluid is added to a segment of nerve, block of the A fibers occurs in about 30 minutes. This block is reversible if the nerve segment is washed promptly, but if exposure to the oxine is continued for 10 or more minutes, restoration of conduction is incomplete or absent. In addition, oxine produces an elevation in the nerve resting potential of about 0.5 mv. It is

doubtful, however, whether either of these effects can be attributed to interference with a metal-enzyme within the nerve. This conclusion is based on the fact that 2-hydroxyquinoline (carbostyryl) and quinoline, both poor metal chelators, are also capable of producing nerve block, although concentrations of 0.006-0.008M are required. Moreover, quinoline is also capable of eliciting slight positivity. Carbostyryl has no measurable effect on the resting potential. The similarity of action of all 3 substances, only one of which is a good chelator, suggests some mode of attack other than through a metal-enzyme system.

Depression of capillary responses to epinephrine by ferrous iron J M CRISMON *Dept of Physiology, Stanford Univ , Stanford, Calif*

Mazur and Shorr identified the hepatic vasotropic factor (VDM) as ferritin (*J Biol Chem* 176:771, 1948). They attributed its action to the protein rather than the iron because injection of apoferritin depressed terminal vascular response to epinephrine as effectively as ferritin. Laurell (*Acta Physiol Scand* 40 Supp 46, 1947) reports plasma Fe globulin up to 250 μ g Fe/liter. This could furnish Fe in sufficient amount to permit injected apoferritin to reach terminal vascular smooth muscle as ferritin. The action of Fe on epinephrine sensitivity was tested on the Zweifach rat mesoappendix preparation by injecting FeSO_4 in doses calculated to saturate iron-carrying globulins in plasma. Intravenous Fe^{++} in doses of 91 μ g/kg raised the epinephrine threshold of the capillary bed in exteriorized rat mesoappendix 3- to 15-fold, a response greater than that obtained by 0.5 ml doses i.v. of saline extract of anaerobic liver or of edema fluid from rat legs after release of 4-hour tourniquets. Thus, Fe^{++} produced vaso-depression without added specific protein. The depressed responses in the vessels of the rat mesoappendix produced by Fe^{++} and by vasodepressor material from ischemic liver and muscle were both reversed by intravenous injections of rutin in propylene glycol in doses of 2 mg/kg, but rutin exposed to metallic iron lost its ability to increase capillary sensitivity to epinephrine. The increased sensitivity to epinephrine after rutin may depend upon competition of rutin with epinephrine destroying enzymes for Fe^{++} (WELCH, A D *Am J Physiol* 108:360, 1934).

Pathogenesis of plasma transfusion reaction WILLIAM H CROSBY AND MARIO STEFANINI (introduced by DAVID RAPPORT) *Ziskind Labs (Hematology section) of the Joseph H Pratt and New England Center Hospitals and the Dept of Medicine, Tufts College Med School, Boston, Mass*

Certain patients develop febrile reactions following transfusion of compatible whole blood. These reactions occur after plasma transfusion

but not after washed red cells (Dameshek, W and J Never In press) Patients with paroxysmal nocturnal hemoglobinuria, in addition to chill and fever, also develop a hemolytic crisis Since the hemolytic factor in paroxysmal nocturnal hemoglobinuria is probably identical with a coagulation accelerator, (Crosby, W II and W Dameshek In press) it was suspected that the plasma transfusion reaction involves the clotting mechanism The behavior of the known clotting factors was studied during such reactions 1) The platelet count fell sharply just preceding the chill 2) Clotting time became shorter Clot retraction became incomplete 3) Prothrombin activity was slightly decreased (70%) after the reaction 4) Prothrombin consumption increased at the time of the chill, thereafter slightly decreased 5) Serum coagulation accelerator activity diminished sharply during the reaction but thereafter increased above the original value At this point, in patients with paroxysmal nocturnal hemoglobinuria, the hemolytic reaction began 6) Plasma labile factor was increased (135%) at the time of the reaction Serum labile factor varied inversely with the accelerator activity 7) Fibrinogen was halved during the reaction 8) Fibrinolytic activity became intense The fibrin in clotted whole blood disappeared in an hour Fibrinolytic activity appeared with the first subjective evidence of reaction, reached a peak one hour after the chill and 3 hours later disappeared Plasma transfusion reaction involves a severe disturbance of the equilibrium of the coagulation mechanism The precipitating cause of the reaction has not been established The drop in platelets suggests that the clinical symptoms may be due to platelet embolism

Renal hemodynamic effects of Neosynephrine in man ARCHER P CROSBY, JR, JOHN KAPP CLARK AND HAROLD G BARKER (introduced by JAMES E ECKENHOFF) *William Pepper Lab, Dept of Medicine and Harrison Dept of Research Surgery, Univ of Pennsylvania Hospital and School of Medicine, Philadelphia*

The clinical observation of oliguria in several postoperative patients treated with Neosynephrine for the maintenance of blood pressure following hypotensive states led to measurements of renal plasma flow (PAH clearance), glomerular filtration rate (endogenous creatinine clearance), urine flow, blood pressure (direct arterial) and pulse rate in 5 normal subjects before and after the intravenous administration of 3-5 mg of Neosynephrine HCl Uniformly in all 5 patients blood pressure rose, pulse was slowed, renal blood flow was decreased and glomerular filtration and urinary output were essentially unchanged These data indicate that Neosynephrine results in renal

vasoconstriction, located chiefly in the efferent arteriole, with an increase in renal peripheral resistance greater than that of the body as a whole and a resultant shunting of blood away from the kidney to other vascular beds Although no change in urinary volume was demonstrated in these normal individuals, in shock, where decreased renal blood flow may be a primary factor in causing oliguria, this drug may delay recovery of renal function

Cerebral blood flow and oxygen metabolism following head-up tilt in hypertensive patients after sympathectomy CHARLES W CRUMPTON, JOHN H MOYER, HERBERT WENDEL, HENRY A SHENKIN, WILLIAM A JEFFERS AND JOSEPH H HAFKENSCHIEL (introduced by H S WIGODSKI) *Robinetle Foundation, Med Clinic, Hospital of the Univ of Pennsylvania, Harrison Dept of Surgical Research, and Dept of Pharmacology, Med School, Univ of Pennsylvania, Philadelphia*

Cerebral blood flow and oxygen uptake were measured in 14 post-sympathectomy patients, before and 20 minutes after 20° head-up tilt in order to evaluate the role of sympathectomy in the effects of semi-erect posture on the cerebral circulation of patients with primary hypertension The mean responses of the patients studied 3 weeks to 2 years after bilateral thoracolumbar sympathectomy were compared with data obtained similarly in 5 normotensive and 16 unoperated hypertensive patients Mean effective cerebral arterial pressure and cerebral vascular resistance were reduced with tilt after sympathectomy Cerebral blood flow was unchanged The oxygen content of the internal jugular venous blood was reduced and cerebral arteriovenous oxygen difference increased Cerebral oxygen uptake increased but not significantly ($p = 0.06$) The other changes were significant ($p < 0.02$) A greater mean decrease in effective cerebral arterial pressure during tilt was noted in the post-sympathectomy patients as compared with the normotensive and hypertensive group response The post-sympathectomy patients appeared to differ in respect to cerebral blood flow with tilt, showing a decrease, whereas a majority of normotensives showed an increase Otherwise, the data showed no evidence that the post-sympathectomy group of patients differed from the unoperated hypertensive group in the cerebral hemodynamic and metabolic adjustment to the 20° head-up tilt

Action potential of the Purkinje tissue of the ox heart HOWARD J CURTIS AND DAVID TRAVIS * *Dept of Physiology, Vanderbilt Univ Med School, Nashville, Tenn*

Certain of the false tendons of the ox heart are composed of strands of Purkinje cells surrounded by a thick layer of connective tissue. Histologically the cells appear to be separate from each other, but the myofibrils appear to be continuous from one cell to the next. These strands were dissected out at a local slaughter house a few minutes after the death of the animal, placed in ice Ringer's fluid, and taken to the laboratory. On warming, they seemed to be in excellent condition. These strands exhibit an injury potential of several millivolts. In recording between a killed end and an active region it is found that on stimulation the injury potential falls approximately to zero and stays there for some time and then suddenly recovers. In spite of the fact that histologically there appear to be a number of strands of cells in each bundle, the response is almost invariably all or-none. The action potential recorded between two electrodes on active tissue consists of a monophasic response followed by a T wave. It is concluded that the Purkinje system of the heart is a functional syncytium, and that physiologically Purkinje tissue is qualitatively identical to cardiac muscle.

Factors affecting acid secretion and cytological structure of mouse stomachs incubated *in vitro*

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Conditions have been found which greatly increase the rate of acid secretion by mouse stomachs *in vitro*. The conditions include incubation for one hour at 3200 mm Hg pO₂ and distention of the stomach to 75 cm H₂O. Optimal concentration of glucose is 15-30 mM/l, and lactate or pyruvate partially replaces glucose as an adequate substrate. No other adequate substrates have been found. Secretion occurs without extrinsic stimulation, but it is further increased by carbaminoylcholine or histamine. The optimal concentration of carbaminoylcholine is 0.1 mg %, and higher concentrations inhibit secretion. CO₂ in addition to that produced by the stomach is unnecessary. The cytoarchitecture of all cellular elements of the stomach is perfectly preserved, and the parietal cells are much larger and more granular than those in unincubated stomachs. Parietal cells of unincubated stomachs when stained with hematoxylin and eosin do not appear to contain canaliculi, but the parietal cells of stomachs secreting *in vitro* under optimal conditions contain large canaliculi which can be seen to envelope the nuclei of the cells and lead to the lumina of the glands. The frequency of occurrence and size of the canaliculi are directly correlated with the rate of acid secretion.

Effect of chronic underfeeding upon electrolyte concentrations of the plasma, brain and muscle of rats VIRGINIA D. DAVENPORT (introduced by LOUIS S. GOODMAN) *Dept of Physiology, Univ of Utah College of Medicine, Salt Lake City*

Plasma, brain and fat-free muscle of rats have been analyzed for sodium, potassium, chloride and water. The rats have been fed a standard diet *ad lib*, or they have been fed restricted amounts of the standard diet or synthetic diets high or low in sodium. Rats underfed with the synthetic diet high in sodium received the same quantity of sodium as those eating the standard diet *ad lib*. In all instances, regardless of the sodium intake, the plasma sodium concentration of underfed rats rose from a control level of 142 mEq/kg to a maximum of about 148 mEq/kg. The plasma chloride concentration rose concomitantly, but there were no changes in the plasma potassium or water concentrations. The sodium and chloride concentrations of the muscles of chronically underfed rats increased, the potassium decreased, and there was no change in the water content. However, the muscle sodium concentration of rats chronically underfed the low-sodium diet fell below the control level despite the fact that the plasma sodium concentration was high. Changes occurring in the brain electrolytes were smaller in magnitude. With the exception of those in rats underfed the low-sodium diet they were also opposite in direction.

Ninhydrin reaction with amines DEAN F. DAVIES* AND HENRY A. SCHROEDER *Hypertension Division, Dept of Internal Medicine, and the Oscar Johnson Inst, Washington Univ School of Medicine, St Louis, Mo*

Of the various theories regarding the humoral factors in hypertension, one with some experimental basis involves the production of pressor amines by an ischemic kidney. Investigation of this theory has been limited because of inadequate analytical techniques for identification of pressor amines in biological fluids. The present study deals with the techniques and results obtained in the first parts of a project designed to study amine metabolism in normal and hypertensive subjects. The reaction of ninhydrin with amines has been recognized for 35 years but has not been used. Four approaches are being made: 1) Spectral absorption characteristics of the reaction between amines and ninhydrin, 2) separation of amines from amino acids in blood and urine, 3) isolation and identification of naturally occurring amines, and 4) quantitative estimation of plasma amine concentrations in normal and hypertensive subjects. Spectral absorption curves have been obtained for the reaction between ninhydrin and methylamine, isoamylamine, arterenol, trypt

amine, tyramine, phenylethylamine, ethylamine, butylamine and histamine. Each of these compounds gave 2 peaks of maximal absorption, one ranging between 400 and 420 m μ , the second from 560-600 m μ . All amines had approximately the same limits (0.04-0.12 mv) of spectrophotometric determination between which standard curves were essentially straight. Guanine, guanidine hydroxylamine, epinephrine and choline gave no color. Since amino acids give a color with ninhydrin, extraction methods for quantitative separation of the bases from the acids are being studied and identification of individual amines by paper chromatography will follow.

Effect of high potassium on respiration and demarcation potential of a small locally-perfused area of cerebral cortex. P. W. DAVIES AND R. G. GRENNELL. *Dept. of Biophysics, Johns Hopkins Univ., and the Psychiatric Inst., Univ. of Maryland, Baltimore.*

Using the technique of local perfusion, combined with an occlusion-type oxygen electrode, described 2 years ago at these meetings, the oxygen consumption of a small area (approx. 2 mm diam.) of the cat's cerebral cortex has been measured. With the aid of a capillary pipette electrode the demarcation potential of the region has also been observed. The perfusion media were modified Krebs solutions containing Knox gelatine, glucose, bicarbonate buffer, but no phosphate. The standard solution, with all the ions in their normal concentrations, was capable of maintaining normal oxygen consumption and brain wave activity of the perfused area for about 2 hours, after which, especially if the perfusion pressure was high, local edema made measurements of oxygen consumption by the occlusion technique rather difficult. The colloid osmotic agent is essential in keeping respiration up (Grenell and Davies, this meeting). Solutions high in potassium were made up by replacing a corresponding amount of NaCl. Upon perfusing with high potassium (142 mM) the rate of oxygen consumption rises, reaches a level more than twice the normal value, and then declines to an approximately normal level in about 10 minutes after starting the perfusion. During this time the demarcation potential of the area falls uniformly and rapidly, reaching an asymptotic level about 10 mv below that of a distant unperfused cortical point. Curves showing the behavior at other concentrations of potassium are given. For example, when the potassium concentration is 50 mM, the respiration rises to an intermediate level, but tends to maintain itself there.

Auditory action potentials in response to clicks. H. DAVIS, C. FERNANDEZ* AND D. R.

McAULIFFE* *Central Inst. for the Deaf, St. Louis, Mo.*

Acoustic transients were produced by passing square or exponentially rising electrical pulses through a PDR-10 receiver. They were delivered to the ear of a guinea pig through a closed coupler that included a 62 mm length of plastic tubing. Strongly damped trains of waves at frequencies of 300, 2500 and 8000 cps could be identified in the aural microphonic. These frequencies correspond to peaks in the frequency response of the receiver and coupler. The pattern of action potentials was complex, but was nearly the same from holes in different turns of the cochlea. Three groups of nerve impulses correspond to the 3 frequencies mentioned. One or another could be largely suppressed by 1) local injury or electro-coagulation at the appropriate turn, or 2) by slowing the rise of the electrical wave (eliminating the 8000 cps wave), or 3) reducing the intensity (eliminating the 300 cps wave). At moderate intensity if the initial wave of the transient was rarefaction the several waves of action potentials (disregarding the late waves that apparently arise in the cochlear nucleus) were nearly synchronous, in condensation they were less synchronous and the latencies were in general longer, particularly for the 300 cps group. The method offers opportunity for measuring the latency of response of the cell bodies of the primary neurons (where these potentials apparently arise) as a function of intensity, polarity and wave form of the 'click'. Preliminary results will be presented.

Method for rapid measurement of intrarenal and other tissue pressures. JOHN C. DAVIS, JR.* AND H. G. SWANN. *Dept. of Physiology, Univ. of Texas School of Medicine, Galveston.*

Tissue pressures are measured by thrusting into the tissue a 20-gauge needle having several holes bored in its shaft. The needle is connected with a device for recording pressure, 250 mm Hg of pressure is then suddenly imposed upon the system. The elasticity of the pressure device then rapidly forces a small amount of saline-heparin (about 10 cm.) into the tissue until equilibrium is reached with the pressure of fluids in the tissue. The point of equilibrium is recorded, thus giving an objective measure of tissue pressures. The final equilibrium depends neither upon the volume of fluid forced into the tissue nor upon the pressure initially imposed upon the fluids in the needle. The results obtained by this method agree closely with those found by other observers for intramuscular and subcutaneous pressures. The intrarenal pressure, as measured in the renal medulla, was ascertained in studies on dogs under sodium pentobarbital anesthesia or after decerebration or in unanesthetized dogs with an ex-

planted kidney and contralateral nephrectomy. The pressure was found to average about 25 mm Hg and to range from 10-58 mm.

Effects of growth hormone on insulin hypersensitivity and glucose tolerance of hypophysectomized dogs R C DE BODO, M KURTZ,* A ANCOWITZ* AND S P KIANG * *Dept of Pharmacology, New York Univ College of Medicine, New York City*

Previous work has shown (*Federation Proc* 8 32, 1949) that while a potent adrenal cortical extract in massive doses exerted a marked 'anti-insulin' action in hypophysectomized dogs, the characteristic insulin response seen in normal dogs was never obtained. Therefore we investigated the possibility that lack of one of the anterior pituitary hormones might be at least partly responsible for the insulin hypersensitivity of hypophysectomized dogs. A purified preparation of growth hormone (Armour) was administered, 1 mg/kg daily, to hypophysectomized dogs manifesting a maximal insulin hypersensitivity (more than 60 times normal). Two doses of growth hormone, given in an 18-hour period preceding the insulin experiment resulted in a very marked 'anti-insulin' action, in that the blood sugar fell only slightly but after 4 hours it still had not returned to its pre-injection level. After daily administration of growth hormone for several days the exaggerated insulin response disappeared completely and the hypophysectomized dogs were even more resistant than normals when very large doses of insulin were used. In addition to insulin resistance, the hypophysectomized dogs treated with growth hormone had abnormally high post-absorptive blood sugar levels and diabetic glucose tolerance curves. Neither glycosuria nor acetoneuria was noted. A marked lipemia was observed. The dogs show a very rapid increase in weight although their food allotment was not increased. Nitrogen, water and electrolyte balance studies are in progress.

Electrolyte and water excretion in the hypophysectomized dog R C DE BODO, D P EARLE, JR, I L SCHWARTZ,* S J FARBER* AND E D PELLEGRINO * *Depts of Pharmacology, Medicine and Physiology, New York Univ College of Medicine, New York City*

The purpose of this investigation was to determine whether hypophysectomized dogs, while manifesting severe disturbances of carbohydrate and protein metabolism, reveal concurrent abnormalities in electrolyte and water excretion. The following observations were made on hypophysectomized dogs after maximal insulin hypersensitivity (more than 60-fold increase over normal) had developed (*Federation Proc* 7 116, 1948) and fasting produced hypoglycemic shock

1) The dogs responded to salt deprivation (sodium intake roughly 2 mEq daily for 3 weeks) in a normal fashion in that urinary excretion of sodium and chloride became negligible and there were only minor decreases in serum sodium. 2) The dogs' tolerance to acute administration of potassium appeared normal. During intravenous infusion of potassium at a rate of 0.5 mEq/min, potassium excretion increased promptly approaching or equalling the filtered load. 3) The excretion of water administered by stomach tube was very markedly delayed. When a water load of 40 ml/kg body weight was given only a small fraction was excreted in 3 hours (5-15%). 4) Glomerular filtration rate and renal plasma flow were reduced confirming earlier work of others. Glucose-T_m was also reduced. It is concluded that the hypophysectomized dog is able to conserve sodium and excrete potassium normally at a time when water excretion is impaired and the organic metabolism is gravely disturbed.

Absorption of phenol following application of 2% phenol in calamine and 'campho-phenique' to skin of experimental subjects WILLIAM B DEICHMANN, R RUEDEMANN* AND T MILLER * *Division of Pharmacology, Depts of Physiology and Pharmacology, and of Dermatology and Syphilology, Albany Med College, Albany, N Y*

One to 4 doses of 1 gm of phenol each were applied at 2-hour intervals to the skin (back, chest and legs) of 19 experimental subjects in the form of either 50 gm of 2% phenol in Calamine Lotion or 21 gm of Campho-Phenique liquid. This preparation contains 4.75% phenol and 10.86% camphor in aromatized mineral oil. Individuals donned clothing immediately after each application and did not remove them until termination of a 24- or 48-hour period of exposure. Blood phenol levels were followed for 3 days, using the method of Deichmann and Schafer (*Am J Clin Path* 12 129, 1942). 'Free' blood phenol rose from a control of about 0.15 to approximately 0.4 mg % after either one, two, three or four doses, each equivalent to 1 gm of phenol. This indicated that under the conditions employed the 'free' blood phenol level was not increased by repeated doses. 'Conjugated' blood phenol rose from about 0.35 to 1.0 mg % after application of one dose of 1 gm of phenol in either preparation. Each additional dose induced a higher peak of the 'conjugated' fraction. The highest mean concentration of 'conjugated' phenol of 1.92 mg % was obtained in the 4 subjects receiving 3 1-gm doses of phenol in Calamine. These results show that phenol is rapidly absorbed from the skin from these dilute preparations. The bulk is rapidly detoxified, as evidenced by a prompt rise in the 'conjugated' fraction. Application over a period

of 6 hours of 4 1-gm doses of phenol, as 2% phenol in Calamine or as Campho-Phenique Liquid, to the chest, back and legs did not raise the 'free' blood phenol more than a single application. It is quite possible that the clothing played a role in retarding the absorption of phenol. There were no signs of local irritation or systemic intoxication.

Distribution of muscle fibers in a single motor unit J S DENSLOW AND OLWEN R GUTENSOHN * *Kirksville College of Osteopathy and Surgery, Kirksville, Mo*

Experiments have been done in a study of the geographic arrangement of the group of muscle fibers in a single motor unit. Attempts to isolate single motor fibers to the peroneus longus (cat) have been made by sectioning all motor nerves to the lower extremity, except the nerve to the peroneus longus, and by splitting 7 L anterior root under a dissection microscope until given bundles contain one fiber to the peroneus longus. The identification of single fibers is made by a number of methods. When it appears that a given bundle contains but one fiber to the peroneus longus, pairs of recording electrodes are placed on the muscle and a 'map' of the location, pattern, etc. of the muscle action potential on the surface of the muscle is made. It has been observed a) that the action potential of a single motor unit may appear all over the surface of the muscle, although concentrated in certain areas, it may be found in a narrow strip in the long axis of the muscle or it may be limited to one corner of the muscle, b) the action potential may be mono-, di-, tri- or polyphasic, depending apparently on the orientation of the electrodes to active fibers and c) one pair of electrodes may pick up the action potential from one group of fibers in the unit while another pair, within 2-3 mm may pick up another. Experiments to determine the third dimension are now being done.

Physiological effect of plastic prosthesis for mitral valves in dogs G REHMI DENTON,* BENEDICT MASTRIANI,* CURTLAND BROWN, JR * AND HAROLD C WIGGERS *Dept of Physiology and Pharmacology, Albany Med College, Union Univ, Albany, N Y*

A prosthesis, made out of polyethylene, has been designed to replace mitral valves in dogs. Constructed without moving parts, designs have been produced to fit various study problems such as a) the effects of an essentially normal replacement for mitral valves, b) the situation with varying degrees of mitral stenosis and little regurgitation, c) the reverse, with no stenosis and marked regurgitation, or d) varying degrees of both stenosis and insufficiency. After 14 operations the technic for satisfactory insertion of these valve

substitutes was established. We now have several healthy dogs who show no ill effects 4-5 months after insertions of substitute mitral valves. Electrocardiograms were taken before, during and at repeated intervals following valve insertion to estimate the degree of cardiac damage entailed. Heart sounds and murmurs have been followed constantly and have been optically recorded. Fluoroscopic study of the heart and lungs has been carried out at frequent intervals. Recognizing that insertion of these valves is relatively safe and that certain degrees of stenosis and/or insufficiency can be tolerated, extensive cardiovascular and respiratory data are being collected on control dogs, prior to creating varying degrees and types of valvular incompetence by insertion of 'substitute mitral valves'. A stress test has been devised to measure the pulmóno-circulatory efficiency of dogs before and after insertion of substitute heart valves. The possible application of this procedure to the correction of valvular defects in the human is apparent.

Central summation following contralateral stimulation of tarsal chemoreceptors V G DETHIER (introduced by ARTHUR J DZIEMIAN) *Dept of Biology, Johns Hopkins Univ, Baltimore, Md*

When chemoreceptors on the leg of the blowfly are stimulated by certain sugars, the fly responds by extending its proboscis. Addition to sugar of a sufficient concentration of electrolyte or non-carbohydrate organic compound inhibits the response to sugar. When one leg is exposed to a threshold concentration of sucrose and the contralateral leg simultaneously to a subthreshold concentration of unacceptable compound (e.g. an alcohol), proboscis extension occurs. As the concentration of alcohol is increased, a point is reached where the response to sucrose is inhibited. This rejection threshold is identical with that obtained by exposing a single leg to mixtures of sucrose and alcohol. If one leg is exposed to a mixture of threshold sucrose plus one half threshold alcohol and the contralateral leg to varying concentrations of alcohol alone, a rejection is obtained when the sum of the alcohol concentrations stimulating each leg approximates that which is required to cause rejection when applied to a single leg. These results indicate 1) that the tarsi contain at least 2 types of chemoreceptors, one of which can be stimulated by sugar and initiates proboscis extension, the other of which can be stimulated by unacceptable compounds and prevents extension of the proboscis, or if the proboscis is already extended, causes its withdrawal, 2) that inhibition of response to sugar is a central phenomenon, 3) that within the CNS, possibly in the segmental ganglion, impulses from the

same type of receptors in the contralateral legs are summed

Acid phosphatases of chicken bone marrow H F DIERMEIER* AND V F LINDEMAN *Dept of Zoology, Syracuse Univ, Syracuse, N Y*

The results reported here on the acid phosphatases are the first of a comprehensive study of the enzyme systems of bone marrow. The method of Fisk and Subba Row (*J Biol Chem* 66 375, 1925) was used to determine inorganic phosphorus released after incubation of a water extract of marrow with the substrate for 15 minutes at 37°C. Results are expressed as μg (γ) of inorganic phosphorus released/100 mg of heat coagulable protein/min. Two acid phosphatases were found to be present. One, a phosphomonoesterase, with an optimum pH of 5.5 released 185.5 γ from disodium-monophenylphosphate. Mg or Mn ions had no effect on the activity in concentrations of 10^{-2} to 10^{-4} M but inhibited the activity approximately 50% when the concentration reached 10^{-2} M. Storage of the extract at 1°C for 5 hours did not decrease the activity of the extract. After 24 hours storage at 1°C 80% of the original activity remained. Dialysis for 18½ hours against distilled water reduced the activity 25%. Dialysis for 19 hours against 0.85% NaCl reduced the activity 35%. The activity is not restored by the addition of Mg ions. This enzyme did not actively attack adenylic acid, glucose-1-phosphate, glycerophosphate (52% α), or fructose diphosphate. A pyrophosphatase was also found to be present. It exhibits a broad pH optimum between pH 4.6 and 5.1. At optimum pH and optimum substrate concentration (0.004 to 0.005 M), inorganic phosphorus is released from sodium pyrophosphate at a rate of 292.66 γ , and from adenosinetriphosphate at a rate of 261.33 γ .

Refractory period and excitability of isolated auricle and papillary muscle of the cat J R DI PALMA AND A V MASCATELLO* *Dept of Medicine, Long Island College of Medicine, Brooklyn, N Y*

A specially designed square wave dual pulse stimulator was used. The muscles were suspended in oxygenated Locke's Solution at a pH of 7.3. Electrodes were of Ag coated with AgCl. Strength-duration curves were determined on 22 auricles and 19 papillary muscles at a temperature of 37.5°C. For the auricle the mean rheobase was 0.8 ma (S.D. \pm 0.14) and the mean chronaxie was 4.7 ms (S.D. \pm 1.7). The papillary muscle had a mean rheobase of 0.3 ma (S.D. \pm 0.25) and a mean chronaxie of 3.5 ms (S.D. \pm 2.0). Thus the papillary muscle was rather more excitable than the auricle. This was confirmed in other experiments. The absolute refractory period was determined over a range of rates of from 35 to 250

per min and at temperatures from 24° to 39°C. Both auricle and papillary muscle showed a shortening of the refractory period as the rate of stimulation increased. This decrease in refractory period followed an exponential curve. A decrease in temperature of the bath increased the refractory period. Between the temperatures of 27° and 36°C this relationship was a linear one. In all comparable instances the refractory period of the auricle was shorter than that of the papillary muscle. An interesting finding was that under certain conditions the auricle and papillary muscle showed summation to two successive stimuli. In the case of papillary muscle at a temperature of below 27°C even incomplete tetanus could be demonstrated. No relationship between refractory period and excitability was found.

A study of alkali hematin prepared from hemin HAROLD L DOBSON,* JOSEPH H GAST* AND JAMES A GREENE *Depts of Biochemistry and Medicine, Baylor Univ College of Medicine, Houston, Texas*

In an effort to obtain a simple reproducible alkali hematin standard, 18 samples of hemin were compared. These samples were prepared by the method of Fischer, from human blood, to study the effect of variations in temperature, amount of blood added, and stirring on the yield and purity. After carefully washing and drying, but before recrystallization, these samples were compared analytically and spectrophotometrically. The color intensity of the solution of hemin in 0.1 N sodium hydroxide, measured on a Klett-Sumner-son photoelectric colorimeter with filter no. 54 (maximum transmission 525-530 m μ), was fully developed within 5 minutes at room temperature and was stable over a period of at least 3 months. The optical density was reproducible. The calculated hemin content of an alkalinized sample of whole blood of known hemoglobin concentration was arbitrarily accepted as the alkaline hematin standard. In comparison studies on the purity of the samples there was a 12.1% difference between the iron and alkaline hematin analyses, with the iron giving the higher values. This difference may be eliminated by adding a small amount of human plasma to the alkaline hematin determination. Spectrophotometric studies indicate that there is a change in absorption over the first nine hours after the hemin was put into solution, after this period of time the spectrum remained stable. Other absorption spectrum studies indicate that alkaline hematin, prepared from hemin, changes with the addition of fresh human plasma.

Adrenal cortex and water metabolism in the golden hamster ROBERT L DORSEY AND W R BOSS (introduced by ROBERT GAUNT) *Dept of Zoology, Syracuse Univ, Syracuse, N Y*

Two hundred-thirty fasted adult normal or adrenalectomized male hamsters were administered 5% of body weight of normal saline or distilled water by stomach tube. Normal animals receiving a single dose of distilled water or saline required an average of $2\frac{1}{2}$ and 4 hours respectively to excrete 50% of the fluid administered. Four hourly doses of water or saline retarded the 50% excretion time to 7 hours for water and 5 hours for the saline. Three half-hourly doses of water produced water intoxication and four doses killed 75% of the animals. Small doses (0.25 mg) of DCA protected against water intoxication and did so more effectively than 2.25 mg of ICA. When animals adrenalectomized 18 hours were hydrated with a single dose of water it required 11 hours for the 50% excretion time. Saline drinking solution was refused by adrenalectomized hamsters, but maintained life and the diuretic response to water when given by stomach tube. DCA (0.25 mg/day) maintained both body weight and water diuresis. Water diuresis tests exceeded normal in animals maintained on very small doses of DCA (0.025 mg/day) plus saline. The hamster differs markedly from rats and mice in its relative inability to excrete water rapidly, in its susceptibility to and symptoms of water intoxication, in the greater diuretic activity of DCA and saline both in normal and adrenalectomized animals, and in its naturally low water exchange.

Pulmonary 'capillary' pressure as an index of left atrial mean pressure in dogs JAMES W. DOW AND RICHARD GORLIN (introduced by GEORGE W. THORN) *Dept. of Pediatrics, Children's Medical Center, the Medical Clinic, Peter Bent Brigham Hospital, and the Depts. of Pediatrics and Medicine, Harvard Med. School, Boston, Mass.*

In 18 dogs, weighing 8-18 kg, each previously anesthetized with Nembutal, no. 7 or no. 8 Courmand catheters were inserted under fluoroscopic control 1) via a jugular vein into the pulmonary artery and advanced to occlude a terminal branch in order to measure pulmonary 'capillary' pressure, 2) via a femoral artery through the left ventricle into the left atrium. Phasic and mean pressures were recorded in these areas by Sanborn electromanometers. Alterations in pulmonary blood volume and flow and changes in respiratory excursion were effected by 1) rapid intravenous infusion of 10% dextrose in saline, 2) severe graded anoxia (0-6%), 3) experimental atrial septal defect, 4) respiratory outflow obstruction and mild positive pressure breathing. The average difference in mean pressures in the pulmonary 'capillaries' and left atrium was negligible under the above conditions, although the range varied from 0 to 4 mm Hg in 49 observations. These experi-

ments demonstrate that pulmonary 'capillary' mean pressure reliably equals left atrial mean pressure at rest and during major circulatory and respiratory variations. It is believed that either the gradient from pulmonary capillary to left atrium approximates the error of measurement of true pulmonary capillary pressure, or the pressure gradient is so small as to be undetectable by the recording methods utilized in this study.

Prototypal curves of dye concentration for cardiac output by injection method PHILIP DOW AND W. F. HAMILTON *Dept. of Physiology, Univ. of Georgia School of Medicine, Augusta*

The validity of the determination of cardiac output by a single rapid injection of an intravascular dye depends upon graphical recognition of recirculation of the dye. This is often difficult when flow is sluggish and intrathoracic blood volume is large. Previously published attempts to circumvent this limitation by 'theoretical' treatments have neglected one or another of the essential determinants. The varying velocity of parts of a single stream, the different lengths of path traversed, the relationship between volume and flow and the discontinuous filling and partial emptying of both sides of the heart must all be accounted for. Therefore an empirical treatment has seemed a more promising approach to the problem of discovering uniform characteristics which would permit use of the earlier, presumably less distorted, portions of the curves. Current studies of several extensive series of experiments taken from departmental files have yielded evidence of significant uniformities among curves from extremely different cardiovascular conditions. The relationship between peak time and appearance time exhibits a strong central tendency (average ratio 1.5) and its variation appears to be random rather than systematic. Employment of a logarithmic time scale improves the symmetry of rise and fall, but the scatter is much less satisfactory. Further manipulations, and comparisons with direct Fick results are in progress.

Effect of ACh on simultaneously recorded ECG and cardiogram of the heart of *Venus mercenaria* FENWORTH DOWNING,* A. L. DUNN* AND A. R. MCINTYRE *Dept. of Physiology and Pharmacology, Univ. of Nebraska College of Medicine, Omaha*

Simultaneous kymographic recordings by both a direct writing ECG and mechanograph and by a C-R tube were made possible by the use of a Transducer (RCA 5734) arranged in such a manner that each systole deflected both the RCA 5734 plate-shaft and a direct writing mechanograph. A silver-silver-chloride tip on an enamelled No. 40 copper wire served as one electrode and also connected one end of the isolated heart to the

Transducer and thence to the writing lever. The characteristic ECG consists of a positive deflection of approximately 100 microvolts of 0.1-0.5 seconds duration followed by negative deflection of 500-900 microvolts. Wide variations in these figures were found in 'normal' hearts. The activity normally arises in the atrium but reversal of ECG phase and direction of contraction is seen following drugs especially Ergotrate which may cause arrest in systole with electrical 'silence'. The isotonic contractions expressed as work may exceed 15,000 gm/cm/24 hr. ACh reduces the ECG-emf more than the mechanograph amplitude. Observations of the ECG renders possible the detection of 0.000025 gamma ACh/ml. Very low concentrations of ACh may slow the heart rate without changing the systolic amplitude and without affecting the ECG. Full technical details and experimental data will be published.

Peptidase activity of paraffin-embedded tissue

W. L. DOYLE, *Dept of Anatomy, Univ of Chicago, Chicago, Ill*

The preparation of accurately thin, serial microtome sections of frozen tissues for quantitative histochemical studies on enzymes requires special and inconvenient procedures. Except for certain notably resistant esterases, the manipulative advantages of paraffin embedding have been discarded out of regard for the lability of most enzymes. The denaturing action of fixatives prior to embedding can be avoided by quick-freezing followed by dehydration *in vacuo* in the frozen state (Altmann-Gersh technique). The stability of a relatively labile peptidase to subsequent heating is shown as follows. Frozen-dried rabbit appendix was infiltrated with paraffin at 65°C *in vacuo* for 40 minutes. The paraffin was subsequently extracted with benzene at 45°C for twenty minutes. These times and temperatures exceed normal requirements. With respect to the hydrolysis of alanyl-glycine the treated tissue not only showed no loss of activity but also gave better aqueous extracts due to removal of fat. The method has promise for other enzymes usually considered too labile to permit embedding in paraffin.

Slope of the CO₂ curve obtained by breathing into the rapid infra-red gas analyzer. A. B. DuBois,* A. Soffer,* R. C. Fowler* and W. O. Fenn. *Dept of Physiology and Vital Economics, Univ of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

The gas analyzer of R. C. Fowler gives a photographic record of the CO₂ concentration as a function of time during a single expiration. This curve of expiration in 17 normal subjects shows a rapid S-shaped rise lasting 1 to 1.5 seconds abruptly changing to a plateau that gradually

risks with time. The plateau ascends more steeply with work and hyperventilation. No plateau was reached in subjects with severe emphysema. In one emphysematous subject, normal expiration gave 6% CO₂, full expiration 10% CO₂. However, in normal subjects end readings of deep and shallow expirations delivered in the same time showed differences in percent CO₂ which are small compared to changes in the plateau with time. Thus, the ventilation to bloodflow ratio was not much altered at different lung volumes, although small differences defy observation. The average slope of the CO₂ plateau in resting normal subjects is 0.11% CO₂/sec (± 0.03). This is less than expected for overall CO₂ output. Diffusion of CO₂ from blood to alveolar air is greater during normal inspiration than expiration, being several times greater for a single deep breath. This cannot be explained without postulating inordinately large changes in blood flow or a blood and tissue reservoir of CO₂ at alveolar concentration as a source of the extra CO₂ appearing during inspiration. Such a reservoir also diminishes the expiratory CO₂ slope by acting as the mathematical equivalent of an additional or 'phantom' residual air.

Influence of pantothenic acid on response of adrenalectomized and intact rats to stress

MARY E. DUMM* and ELAINE P. RALLI. *Labs of the Dept of Medicine, New York Univ College of Medicine, New York City*

The response of adrenalectomized and intact rats to stress has been evaluated by 1) determining the capacity of the rats to swim in water at 18°C, 2) following the total number of white blood cells and lymphocytes for 6 hours after swimming for 25 minutes at 25°C and for 8 hours after the injection of ACTH, and 3) determining the blood sugar of rats (fasting and non-fasting) after swimming 25 minutes at 25°C. Intact rats receiving experimental diets varying only in their pantothenate content, swam 62 ± 12 minutes on 4 mg of pantothenate daily, 29 ± 4 minutes on 0.1 mg of pantothenate, 24 ± 4 minutes on 4 mg of pantothenyl alcohol, and 17 ± 2 minutes on the dog chow diet which supplies about 0.1 mg of pantothenate daily. Adrenalectomized rats swam 38 ± 6 minutes on 4 mg of pantothenate daily and 19 ± 2 minutes on 0.1 mg of pantothenate. Both in the adrenalectomized and intact rats the leucocyte and lymphocyte responses to swimming and to ACTH were modified by the presence or absence of pantothenate in the diet. Following swimming, the average blood sugars of non-fasted, adrenalectomized rats, receiving the 4 mg pantothenate diet, fell from 119 ± 4 to 90 ± 4 mg %, compared with a decrease from 107 ± 6 to 69 ± 4 mg % by the adrenalectomized rats on 0.1 mg of

pantothenate daily, and a decrease from 101 ± 5 to 71 ± 9 mg % by the pantothenate deficient adrenalectomized rats. In the fasted adrenalectomized rats on the 4 mg pantothenate diet the average blood sugar fell from 65 ± 5 to 53 ± 4 mg % compared with a decrease from 71 ± 3 to 43 ± 3 mg % by the rats on the 0.1 mg pantothenate diet, and a decrease from 59 to 46 mg % by the pantothenate deficient rats. These results suggest that calcium pantothenate influences the ability of both intact and adrenalectomized rats to withstand stress.

Factors involved in evaluation of pulmonary edema at autopsy STANLEY H. DURLACHER, WILLIAM G. BANFIELD* AND A. DOROTHY BERGNER*
Pathology Section, Medical Div., Army Chemical Center, Md.

The ratio of lung to body weight has been determined in rabbits immediately and at intervals after sacrifice by a variety of procedures. Methods of sacrifice have included exsanguination, air embolism, electrocution, ether, rabbit punch, pentobarbital sodium by various routes and intracardiac saturated magnesium sulfate solutions. Histological examination of the lungs was made in all instances. The ratio of lung weight to body weight increased after death by all methods except exsanguination. The greatest increase occurred in animals sacrificed with pentobarbital sodium or magnesium sulfate solutions when introduced into the blood stream rapidly and in such fashion that high concentrations were present in the pulmonary vasculature after death. Under these conditions the lung weight to body weight ratio was 2 to 3 times greater after a 3-hour interval than was the ratio found in animals killed by the same materials administered by other routes. Increase in lung weight was due largely to accumulation of proteinaceous fluid within the alveoli, although a small portion of the increased weight was due to clotted blood remaining in the pulmonary blood vessels. When quantitative estimations of the degree of pulmonary edema present at autopsy are desired, rapid exsanguination is the most satisfactory method of sacrifice. In instances where this procedure is not feasible, air embolism should be used.

Metabolic response of epileptic patients to adrenaline A. DURY, J. W. HOLLER* AND C. SMITH*
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The effect of a single i.m. injection of 0.4 ml. aq. adrenaline (1:1000) upon the plasma potassium, glucose, absolute number of eosinophiles, and other blood constituents was measured in epileptic and Addisonian patients, and normal adults.

The percentage change in these constituents from the overnight-fast level was calculated for the periods of 4, 20, 60, and 120 minutes after an injection of adrenaline. The mean change of the blood glucose in normal adults was +44% and +47% after 20 and 60 minutes, respectively, and at pre-injection level after 120 minutes. The mean change of the blood glucose in Addisonians was +27% and +22% and in the epileptics was +27% and +25% after 20 and 60 minutes, respectively. The mean change in the plasma potassium in normal adults rose slightly after 4 minutes, was lowest after 20 minutes and at pre-injection level and slightly above after 60 and 120 minutes. In the epileptics and Addisonians the mean plasma potassium levels were below preinjection means 4, 20, and 60 minutes after adrenaline injection. Maximum change in the Addisonians was -20%, and in the epileptics -8%. The mean change in circulating eosinophiles after adrenaline injection decreased at a much slower rate and to a lesser extent in the epileptic group than the normal adults. Placebo injection of physiological saline in epileptics did not induce any change in these blood constituents. These data suggest that the mobilization of glucose into the blood and the metabolism of potassium in epileptic patients are comparable to Addisonians.

Physiologic responses of certain animals and isolated preparations to mixtures of snake venoms and egg yolk MURRAY DWORETZKY AND P. BOQUET (introduced by F. C. MANN)
Inst. of Experimental Medicine, Mayo Foundation, Rochester, Minn. and Pasteur Inst., Paris, France

Each of 8 different snake venoms when incubated with 40% egg yolk produced a substance extremely toxic for rabbits, cats and guinea pigs, but less toxic for dogs. Injections of equal amounts of the egg yolk emulsion alone were without toxic effect. The snake venoms were added to the mixture in nontoxic doses or they were neutralized by antivenom or inactivated by boiling the mixture after incubation. For convenience this material has been called the D-L substance since it was first described by Delezenne and Ledebt. Its effects on the animals mentioned strikingly resemble those caused by large doses of histamine or by anaphylactic shock. However, the D-L substance is not counteracted by benadryl or epinephrine. Furthermore, it causes immediate marked relaxation of the isolated guinea pig ileum when it has been contracted by histamine, acetylcholine or cobra venom. These findings have led to the conclusion that the D-L substance is not histamine. Mixtures of certain snake venoms incubated with other materials such as lymph, homogenized milk, homogenized rabbit muscle

or brain did not produce a toxic substance. The effects on the blood pressure and respiration of the rabbit and cat were indistinguishable from those following clamping of the common pulmonary artery. It has been demonstrated by vinyl acetate casts made of the pulmonary vascular system of cats and rabbits that death following the injection of the D-L substances resulted from occlusive spasm of the secondary pulmonary arteries.

Coronary vasodilation following intracoronary injections R. W. ECKSTEIN, J. CHAMBLISS,* J. DEMMING* AND K. WELLS * *Dept of Medicine, Western Reserve Univ., Cleveland, Ohio*

Total left coronary flow was measured in anesthetized heparinized open-chest dogs under artificial respiration. Blood was led from a carotid artery into a constant pressure chamber through a Shipley recording rotameter and thence into the common left coronary artery which was cannulated through the left subclavian artery. Injections were made through the rubber tube between the rotameter and the coronary cannula. It was accidentally found that rapid injections of blood, saline, or plasma in amounts of 0.1-0.2 cc through small needles results in large increases in blood flow, lasting from 5-30 seconds not attributable to pressure or viscosity changes. Likewise, when blood in a syringe is rapidly injected into large amounts of blood in a beaker, the blood in the beaker develops a potent vasodilator. The activity of this material steadily decreases upon standing during a 30-45 minute period, after which time activity is absent. There is evidence that this vasodilator is a product of the mechanical destruction of red cells. Its effect is not abolished by atropine and its behavior is not that of histamine. Spectrographic analysis shows that it is probably ADP or ATP and is identical with 'Früh gift'.

Adrenal cortex and survival of rats after x-irradiation ABRAHAM EDELMANN *Brookhaven Natl Lab., Upton, N. Y.*

Protecting the adrenals of male rats by means of lead shields placed about the glands significantly increased the survival incidence following 850 r of x-irradiation (160 KVP, 10 ma, 0.15 mm Cu). The x-irradiation (650 r) shortens the lives of adrenalectomized rats from an average of 8 days to 4 days. Substitution therapy in the adrenalectomized rat is effective inasmuch as 3 cc/day of a whole gland adrenal extract (Upjohn) or 5 mg/day of desoxycorticosterone acetate resulted in 20 and 40% mortalities respectively at 15 days after 600 r, whereas 90% of the adrenalectomized controls were dead at this time. It is thought that the adrenal cortical response to x-irradiation is similar to that following any other stress.

Blood flow distribution in the heated dog N. E. EDERSTROM *Dept of Physiology, St. Louis Univ School of Medicine, St. Louis, Mo.*

Dogs anesthetized with pentobarbital were warmed gradually by lamps until a lethal temperature was reached. Blood flows were recorded from the foot pads and tongue by means of the photoelectric plethysmograph. With an increase in rectal temperature blood flows in the foot pads decreased until they reached about one-half the normal as the lethal point of 45°C was approached. In contrast, blood flows in the tongue increased with the rise in body temperature, reaching about double the normal level at 43°C, and then declined abruptly between 43 and 44°. The foot pad surfaces, although shielded from direct heating, increased in temperature despite the drop in blood flows. Calculation of the thermal equilibria indicate an increased convection by the blood stream due to a greater A-V temperature difference. The tongue surface increased in temperature as flow increased, reaching a peak simultaneously with blood flows at 43°. Surface temperatures in both areas fell as blood flow declined at the approach of lethal body temperatures. Blood pressure, heart rate, respiratory rate increased with the rise in body temperature, but declined near the lethal level. These observations indicate that in hyperthermia the blood of the dog is shunted to the heat dissipating area provided by the tongue, and away from the skin surface and consequent exposure to the hot environment. This redistribution of the blood in the non-sweating dog is therefore opposite in direction to that occurring in a sweating man.

Urine amine levels in normotensive and hypertensive subjects EDWARD F. EDINGER, JR.* AND NORMAN S. OLSEN *Hypertension Division, Dept of Internal Medicine, and the Oscar Johnson Inst., Washington Univ School of Medicine, St. Louis, Mo.*

The presence of pressor substances in urine has been described previously by several workers. These substances have been thought to be amines. For this reason the amine content of urine from normotensive and hypertensive subjects has been studied. Using a modification of Richter's pierate method for amines, freshly voided morning specimens of urine were analyzed. Creatinine concentrations were also measured and urine amine-creatinine ratios calculated. It was found that the urine specimens from normal individuals contained significant quantities of free amine, measured in terms of isoamylamine. The urine amine-creatinine ratios of normal non-smokers fell within a narrow range, whereas smokers tended to have higher ratios. Certain hypertensive patients had low urine amine levels with reduced

amine creatinine ratios as compared with the normal controls

Oximetric determinations of cardiac output in man at rest and mild exertion JAMES O. ELAM*, WILLIAM SLEATOR*, W. N. ELAM* AND H. L. WHITE *Lab. of Thoracic Physiology and Dept. of Physiology, Washington Univ. School of Medicine, St. Louis, Mo.*

Changes in arterio-venous oxygen difference with the increased cardiac output of mild exertion in normal subjects were studied by means of the modified oximetric method of Matthes previously described (*Federation Proc.* 7: 104). Arterial and mixed venous oxygen saturations were recorded while the subject breathed air and rebreathed the helium-alveolar air mixture. The red and infrared light transmissions of the histamine-flushed pinna were recorded separately, with occlusion to 220–280 mm Hg to obtain bloodless values. Oxygen saturations were deduced from the transmission data by means of a calibration curve previously established by means of Van Slyke analyses. Greatest deviation of oximetric value from Van Slyke value was $\pm 4\%$. Hemoglobin determinations were used to obtain oxygen contents from saturation values. Arterio-venous oxygen difference and oxygen consumption were determined after the subject had been sitting at rest for 30 minutes and at 5-minute intervals 10 minutes after exertion was started. Repeated cardiac output determinations while each subject was in a given steady state showed mean deviations from their means which averaged 4–2% for 48 measurements on 4 subjects. Increase of oxygen consumption up to 20% above the resting value was sometimes accompanied by a decrease in arterio-venous oxygen difference. When oxygen consumption was increased more than 20% above the resting value the arterio-venous oxygen difference usually increased. Except in one experiment, the percentage increase in arterio-venous difference was less than that in cardiac output.

Some responses of the ear to high frequency sound DONALD H. ELDRIDGE, JR. (introduced by E. A. PINSON) *Aero Med. Lab., Air Materiel Command, Wright-Patterson Air Force Base, Dayton, Ohio.*

Davis *et al.* (*OSRD Final Report on Temporary Deafness Following Exposure to Loud Tones and Noise*, September 1943) showed that exposure to pure tones (speech frequency range) produced temporary deafness principally in the octave above the exposure tone. This observation is shown to hold for audible tones at frequencies above 6000 cps, provided the sound level is not high enough to overload the ear. Subjects exposed

to tones from a siren (6–40 Kcps) at 150–158 db ref. 0.0002 dynes/cm² reported hearing unusual sounds not present in the sound field and subsequently showed elevations of the threshold for pure tones at frequencies considerably below the exposure tone. Moderate background noise combined with varying amounts of frequency and intensity modulation made necessary experiments designed to separate the factors producing this elevation threshold. A possible mechanism for the production of the low frequency hearing loss was indicated when subjects were exposed to a very pure tone of 9200 cps and 140–146 db produced by an aluminum rod in longitudinal vibration. The subject heard one or more of the subharmonics of the tone well enough to match them to a pure tone presented to the opposite ear. The first subharmonic radiated from the subject's ear and was heard by other observers. The measured level of the radiated subharmonic was 60–80 db below the exposure tone.

Alterations in renal hemodynamics and excretion of electrolytes J. R. ELKINTON, A. P. CROSLLEY, JR., *H. G. BARKER,* AND J. K. CLARK **Harrison Dept. of Research Surgery, School of Medicine Hospital, Univ. of Pennsylvania, Philadelphia.*

Following control periods renal dynamics were altered in normal human subjects by 3 procedures: 1) infusion of concentrated salt-poor albumin, 2) injection of Neosynephrine, and 3) tilting 60° from the horizontal position. The glomerular filtration rate (GFR) was obtained from clearances of mannitol, inulin, or endogenous creatinine. The renal plasma flow (RPF) was measured with paraaminohippurate as effective flow by clearances in the Neosynephrine experiments and in the others as total flow by the direct Fick method with a catheter in the renal vein. Following concentrated albumin when the RPF rose markedly but the GFR was unchanged, the excretion rates of Na and K increased while that of Cl was essentially unaltered. Following Neosynephrine when the RPF fell moderately without change in the GFR, the excretion rates of Na, K and Cl fell, and that of Na returned to the control level when the RPF did likewise. On tilting when both the RPF and the GFR fell, the excretion rate of Na decreased markedly, and failed to return to control levels when the RPF and the GFR were restored, excretion of K and Cl decreased in equivocal amounts. These data indicate that, under the conditions of these experiments and within the limitations of the methods, sodium excretion appears to be determined by changes in tubular transfers rather than by changes in glomerular filtration, and is most closely correlated with changes in renal blood flow and filtration fraction. The dissociation seen during recovery periods in the tilt experi-

ments supports the fact that the correlation does not necessarily result from a simple causal relationship

Increased fragility of erythrocytes during experimental hypertension M M ELLIS, M D ELLIS* AND G C RAU * *Dept of Physiology and Pharmacology, Univ of Missouri Med School, Columbia*

Chronic hypertension was induced in adult dogs by encapsulating first the right kidney and some 14 days later the left kidney with plastic as described by Rau (in press) A 5-cc blood sample was drawn from the jugular vein of each dog every 7-10 days throughout the 60-day period of the test, about 30 minutes after the i p injection of 32.5 mg/kg of sodium pentobarbital (Nembutal) The blood was diluted immediately 1:100 with 0.71% NaCl solution buffered to pH 7.4 with disodium phosphate The blood mixture was incubated in pyrex test tubes for 22 hours in an electrically operated water bath at 37°C Haemolysis was read with a spectrophotometer The blood pressure rose from normals around 120 mm Hg to 150-180 after encapsulation of right kidney, held or fell slightly until the left kidney was encapsulated, and then rose to 198 or better The amount of haemolysis at the end of 22 hours incubation increased with a slight lag, as the blood pressure rose When the blood pressure held or fell before the encapsulation of the second kidney the 22-hour haemolysis decreased but did not return to the normal level Following encapsulation of both kidneys the 22-hour haemolysis again increased as the blood pressure rose, remaining well above normal to the end of the experiment some 30 days after the last operation These increases in 22 hour haemolysis varied from 100 to 270% in terms of the normal values Comparable results were obtained using other strengths of saline

Dose response data in man following orally administered or injected lipo-adrenal cortical extract FRED ELMADJIAN (introduced by HUDSON HOAGLAND) *Worcester Foundation for Experimental Biology, Shrewsbury, Mass*

Lipo-adrenal cortical extract (Upjohn) was given to human subjects in doses of 20 cc, 10 cc, 5.0 cc, and 2.5 cc intramuscularly and orally Changes from premedication levels were noted in the rate of urine excretion, creatinine, 17-ketosteroids, uric acid, potassium and sodium excretion as well as changes of blood lymphocytes and eosinophils at intervals during a period of 5 hours after the administration of the extract Considering the overall adrenal cortical responses noted in these dosage ranges the effectiveness of the orally administered extract proved to be comparable to that of the injected extract Of the adrenal cortical indices determined urine volume and eosinophil counts were more proportional to the dosage than

the sodium, potassium and 17-ketosteroid excretions Uric acid excretion showed the least relative response to the quantitatively increasing dosage The directions of the changes obtained in the adrenal response measures for orally administered extract were the same as those produced by intramuscular injection of the extract with all of the indices except that of sodium In all dosage ranges employed the intramuscularly administered extract clearly showed a decrease in the sodium excretion especially in the period from the 2nd to the 5th hour after the injection, while the orally administered hormone indicated an increase in the sodium excretion in the same period

Action of valves of the beating mammalian heart with simultaneous records of ECG, vibrations and sounds (motion picture) HIRAM E ESSEX, H L SMITH* AND E J BALDES *Mayo Clinic and Mayo Foundation, Rochester, Minn*

The behavior of the pulmonic, aortic, mitral and tricuspid valves of isolated beating mammalian hearts will be shown Attention will be given to the sphincteric action of the auriculo-ventricular ring which constricts in systole and dilates in diastole It will likewise be shown that the mitral valves are inactive when fluid is drawn from the ventricular cavities and that auricular pressure is not necessary for opening the valves since they open freely when the auricular walls have been incised and retracted The mitral and tricuspid valves will be excised and the effect on the cardiac sounds will be demonstrated

Spreading cortical depression CARL F ESSIG* AND WADE H MARSHALL *Intramural Research Branch, National Inst of Mental Health and Lab of Physical Biology, Exper Biology and Medicine Inst, National Inst of Health, Bethesda, Md*

A cat or monkey preparation of exposed cortex maintained under physiological conditions rarely produces spreading depressions We have developed 3 almost certain methods of producing the reaction 1) radical internal dehydration, 2) cooling the surface of the cortex approximately 10°C, and 3) exposure to room air for several hours A 4th but uncertain method is some unknown traumatic factor in surgical procedure An occasional monkey preparation will yield the reaction within the 1st hour after exposure to room air Failures of reactions are correlated with highly negative cortical base line voltages The chilled cortex (monkey) shows 4 definite components D1, D2, D3, and D4 D1 jumps the central sulcus, D2 follows the cortex or pia membrane D1 and D2 velocities are approximately 0.03 mm/sec D3 velocity is about 0.0053 mm/sec, and D4 velocity is about 0.0035 mm/sec The D1, D2, and D3 reactions are obtained after prolonged exposure to room air but at 1.5-3 times the above velocities

In both cooled and room air exposed preparation D4 is not always seen. The reactions develop gradually to the same pattern that can be obtained in 10 minutes by cooling the cortex. The initial stages of development of the phenomena can be seen within 15 minutes after exposure to room air.

Plasma and erythrocytic ion concentrations in infant diarrhea J N ETTELDORF* AND R R OVERMAN *Divisions of Pediatrics and Physiology, Univ of Tennessee College of Medicine, Memphis*

Determinations of plasma Na, K, Cl, Ca, CO₂ content, protein, erythrocyte Na, K, Cl, blood pH, NPN, and hematocrit were made on the following groups of infants: 81 normal, 26 with diarrhea before treatment, 40 with diarrhea receiving parenteral NaCl, sodium lactate, blood and/or plasma, and glucose, 95 with diarrhea receiving the above treatment plus KCl (Solutions administered contained 5.6 to 16.0 mEq/l of K). In normal infants average values were similar to those of normal adults except the erythrocyte Na was higher (19.4 compared to 15.3 mEq/l), hematocrit lower (35.6%), and plasma protein lower (6.3 compared to 7.6 gm%). The following observations were made on patients before treatment: a) Plasma: reduced K (4.6–3.8 mEq/l), Na (141–133 mEq/l), CO₂ (54–37 vols %), pH (7.40–7.36), normal Cl; b) Erythrocytes: reduced K (102–95 mEq/l), Cl (64–60 mEq/l), elevated Na (19.4–20.2 mEq/l); c) Blood: NPN elevated (30–44 mg%). In treated infants receiving no K, plasma Na was higher (136 mEq/l) but K lower (3.3 mEq/l) than the untreated group, erythrocyte Na was definitely elevated (23.2 mEq/l) but K and Cl unchanged. CO₂ content and pH were normal but NPN remained high (39 mg%). The average plasma K of all infants receiving K was not significantly higher than that of the preceding group, erythrocyte Na was less elevated (21.4 mEq/l). Otherwise the 2 groups of treated infants were similar. A series of diarrheal patients are being investigated to whom larger doses of KCl are being administered.

Studies on the physical properties of bone F GAYNOR EVANS AND MILTON LEBOW (introduced by ERNEST GARDNER) *Dept of Anatomy and Engineering Mechanics, Wayne Univ, Detroit, Mich*

The tensile strength and modulus of elasticity were determined for 103 samples of human bone from 9 femora of 3 Negro females and 5 white males. All but 2 bones were from dissecting room cadavers. In contrast to most earlier studies of this nature, the samples were tested under direct tension. The tests were made in a Baldwin-Southwark Universal testing-machine calibrated to an accuracy of 2%. The deformations were measured by a Porter-Lipp extensometer over a one inch

gauge length. Samples were obtained from the compacta of the antero-lateral, antero-medial, postero-lateral and postero-medial quadrants of the proximal, middle and distal thirds of the shaft. An attempt was made to standardize the size of the samples. A fairly complete series of samples was obtained from the femur of a 26-year-old and a 45-year-old Negro female and a 64-year-old white male. In these 3 bones the samples from the lateral half of the shaft had a higher average tensile strength than those from the medial half. This difference was most consistent and conspicuous in the proximal third of the shaft. Where the sample series was complete the average tensile strength of all quadrants of the middle third of the shaft was the greatest. No consistent relationship between tensile strength and age of the individual was noted. Similar studies on other bones will be reported.

Pyruvate metabolism in rabbit bone marrow

JOHN D EVANS (introduced by DAYTON J EDWARDS) *Dept of Physiology, Cornell Univ Med College, New York City*

Previous *in vitro* studies have shown that marrow cells suspended in Ringer-bicarbonate at pH 7.3 exhibit a respiratory quotient of 0.84 in the absence of glucose. If glucose is added to the medium the R.Q. of marrow is raised to 0.95. In the present study it is observed that when pyruvate is added to marrow, active utilization occurs and the R.Q. is elevated to 1.11. At a concentration of 1 mg of pyruvate/ml of marrow suspension 40% of the pyruvate disappears in 4 hours, but despite this rapid disappearance the rate of oxygen consumption is not increased. In 36 experiments the average rate of pyruvate utilization is 5.7 μ g/mg of cell protein/hr. There is an average production rate of 3.1 μ g of lactic acid/mg/hr and an average evolution of 3.2 μ l of respiratory CO₂/mg/hr. These figures represent a pyruvate:lactate:carbon dioxide ratio approximating 2:1:4. It is concluded from the aerobic experiments that marrow preferentially metabolizes added glucose and pyruvate, thereby producing high respiratory quotients which approximate the theoretical values for those substances. Anaerobically, pyruvate is converted to lactate in approximately a 1:1 ratio. The amount of pyruvate utilized in the absence of oxygen averages 4.2 μ g/mg/hr.

Fibrinolysokinase activity in tissues JOHN H FERGUSON AND JESSICA H LEWIS *Dept of Physiology, Univ of North Carolina, Chapel Hill*

Tissue substances capable of activating pro-fibrinolysin to active fibrinolysin have been described by Astrup and Permin, Permin, and Taghon. Various dog tissues were fractionated into 7 fractions, of different particle size, by differential centrifugation and each of these frac-

tions was tested for fibrinolysokinase activity on dog serum profibrinolysin. The 37 different tissues studied were divided into three groups: *Group I*, those tissues having one or more fractions which showed appreciable kinase activity, *Group II*, those tissues having one or more fractions which showed minimal kinase activity, and *Group III*, those tissues from which no fractions showed kinase activity. Kinase activity was usually concentrated in Fractions IV (Claude's large granules) and VI (Claude's microsomes). Fraction VII, the saline soluble portion, never showed kinase activity and, in fact, usually contained some antifibrinolysin. Tissues included in *Group I* were lung, uterus (pregnant and non-pregnant), pancreas, gall bladder, lymph gland, vein and urine. Fractions IV and VI of lung were chosen for special study. Kinase activity could not be extracted with common organic solvents. The kinase was unstable, disappearing rapidly at 37°C or higher, slowly at room temperature, and very slowly at 0°C. Freezing and storage at -20° resulted in a marked initial loss of activity, with little change thereafter. The activation of profibrinolysin with the lung fractions was found to be slow in comparison to that produced by staphylokinase but apparently the same enzyme precursor was activated in each case. Whole serum and plasma treated with these fractions showed little fibrinolytic activity in comparison with partially purified profibrinolysin.

Comparison of the A-A gradient determined by different methods BENJAMIN G. FERRIS, JR. (introduced by WILLIAM H. FORBES) *Dept of Physiology, Harvard School of Public Health, Boston, Mass.*

Alveolar-arterial (A-A) gradients were determined by the method described by Riley and Lilienthal (*Am J Physiol* 147:191, 1946) and compared with values obtained by Dill and Penrod's method (*J Applied Physiol* 1:409, 1948). The latter were obtained by subtracting the sum of the arterial pO_2 , pCO_2 , pH_2O , and pN_2 from the prevailing barometric pressure. In certain patients Bohr's method of graphic integration was used to determine the diffusion coefficient for oxygen, and from this procedure the Bohr A-A gradient was obtained from the final ΔpO_2 . The similarity between the Riley-Lilienthal and Dill-Penrod methods was shown in normal persons, where the mean A-A gradient was 9 mm Hg (Riley-Lilienthal), and 10 mm Hg (Dill-Penrod). When these 2 methods were compared in cases with emphysema, pulmonary vascular disease, and cirrhosis of the liver, this close similarity was not as pronounced. The Dill-Penrod method yielded a 5 mm Hg higher value. In this group, the final ΔpO_2 in the Bohr integration resulted in an A-A gradient

intermediate between these 2 values. Further comparison of the Dill-Penrod and Riley-Lilienthal methods in other patients showed a slightly better agreement. The Dill-Penrod values were still 3 mm Hg higher on the average than the Riley-Lilienthal values. Possible reasons for these discrepancies were presented and it was pointed out that the Dill-Penrod method, although apparently less accurate, does give one much useful information with only a single analysis of arterial blood.

Influence of tonic contracture upon the composition of mammalian skeletal muscle, especially upon its electrolyte content ERNST FISCHER AND HELEN V. SKOWLUND * *Dept of Physiology, Med College of Virginia, Richmond*

The electrolyte content of left and right identical normal rabbit muscles shows little difference. The chloride content of different anatomical muscles varies more than 15%, while the values for potassium and sodium differ about 5% and 10% respectively. However, if one calculates the K and Na concentrations existing inside the muscle fibers, assuming that chloride is restricted to the tissue spaces, rather uniform K and Na values are found for different muscles. The early contracture of neurological origin developing in tenotomized gastrocnemii, which can be prevented by denervation, decreases K content much more than can be explained by the relative increase in connective tissue caused by the higher rate of atrophy. Na enters the muscle fibers to a large extent and replaces the lost K. The muscle membranes become rather permeable for Cl, and finally about 20% of the total Cl is apparently located inside the fibers. Experiments with the radioisotope K^{42} revealed that contracture increases considerably the permeability of the muscle membranes for K. Besides the changes in electrolytes, early contracture causes a decrease in total protein concentration, a deterioration of the specific muscle proteins myogen and mvosin, and a decrease in the aldolase and adenosinetriphosphatase activity quotient of the isolated myogen and myosin respectively.

Internal water exchange in normal and hypotensive dogs M. J. FOGELMAN AND P. O. 'B' MONTGOMERY (introduced by CARL A. MOYER) *Dept of Exper Surgery, Southwestern Med School of The Univ of Texas, Dallas*

A study was undertaken to determine the rates of exchange of water between the vascular and extra-vascular spaces using D_2O as a tracer. The studies were designed so that the differential rates of water movement were determined in each dog both under normal conditions and following subjection to a constant hypotensive level induced by bleeding. D_2O was determined by a

modification of the falling-drop method on purified samples of lyophilized whole blood. Plasma volume was determined with T-1824. A known amount of D_2O in isotonic saline (approximately 0.5 cc/kg/bw) was injected intravenously and heparinized arterial blood samples drawn at frequent and close intervals for periods ranging from 40-90 minutes. Time concentration curves were constructed and the equations for these curves derived according to the method of Flexner, *et al* (*Symposia on Quant Biol* 13: 88, 1948). The calculations reveal that in the normal dog approximately 72% of total blood water moves extravascularly/min. Following subsection to a constant hypotensive level of approximately 75 mm Hg mean arterial pressure an average of 41% of total blood water passes across the vascular membrane/min. Further calculations reveal that in the normal dog approximately 23% of total body water moves across the vascular membrane in each direction/min, and that in the animal subjected to hypotension approximately 12% of the total body water moves across the vascular membrane/min. The reduction in exchange rates of D_2O following hemorrhage are tentatively ascribed to the reduction in the functional diffusion area induced by the loss of blood. The significance of these results and their relationship to water and electrolyte movement in various conditions will be discussed.

Application of a technique for sampling respiratory gases in non-cooperative subjects C A FORSANDER (introduced by C WHITE) *Dept of Physiology, Univ of Pennsylvania School of Medicine, Philadelphia*

By applying an oral suction technique developed for the collection of samples of alveolar air it has been possible to obtain estimations of the CO_2 tension of the mixed venous blood entering the lungs, within 6-12 seconds of breathing mixtures of CO_2 and oxygen, and without interfering with the respiratory pattern. Reproducible levels of CO_2 equilibrium have been found at 6, 15, 21 and 24 seconds. In a basal subject breathing 10/min, the CO_2 tension of the alveolar air after one normal breath of gas mixture was 46 mm Hg, after 2 breaths 48.8 mm Hg, 3 breaths 49.5 mm Hg, 4 breaths 53 mm Hg. The rise in CO_2 tension after 12 seconds is interpreted as being due to recirculation. Before 12 seconds the rise is due either to delay in mixing of the inspired gas with the residual air, or may reflect the volume of the pulmonary blood flow. These results are demonstrated if the deadspace air is removed before introduction of gas mixtures and before sampling.

'Resonance' of muscle to central stimulation

A A FOSTER (introduced by HANS LOWEN-

BACH) *Dept of Anatomy, Duke Univ, Durham, N C*

It has been previously demonstrated and we have confirmed the gross fact that frequencies below approximately 17 stimuli/sec applied to the proper point in motor cortex of a dog, under sodium pentobarbital (Nembutal), produce extension of the contralateral hind limb. Frequencies from 17-21 s p s produce flexion. Frequencies just below 34 s p s produce extension and good flexion results from 34-37 stimuli/sec. Grossly these phenomena indicate resonance of flexors to 17 and 34 stimuli/sec. The distal branch only of the nerve to that double muscle, the semitendinosus, was cut and a neurogram taken distal to that cut. The sensory responses to the twitching of the extensor portion followed one to one up to 17 s p s but became irregular thereafter. Just below 34 the response was again regular but only to alternate stimuli. These responses again failed to pass 17 as the stimuli passed 34. The extensors apparently can follow up to 21 or one half 42 stimuli/sec. These 'drop out points' rise and fall slightly with rise and fall of intensity of stimulation. This indicates a relaxation oscillator type of action. In general tetany, extension is the rule due to the greater strength of the extensors. It is therefore concluded that the normal pattern is extension but between 17 and 21 s p s the extensors are not responding to every stimulus while the flexors still are and so the latter prevail over their antagonists. The flexors enjoy a similar advantage in the band just above 34 stimuli/sec.

Effect of lithium on the renal excretion of potassium JAMES G FOULKS,* GILBERT H MUDGE* AND ALFRED GILMAN *Dept of Pharmacology, Columbia Univ College of Physicians and Surgeons, New York City*

The renal excretion of electrolyte during the infusion of isotonic lithium chloride was studied in dogs under barbiturate anesthesia. Cations were determined by direct flame photometry. The volume distribution of lithium varied with the rate and duration of the lithium infusion, but uniformly exceeded the extra-cellular fluid volume. The calculated lithium space was 30 to 60% of the body weight. The proportion of filtered lithium excreted in the urine was relatively constant over a wide range of serum lithium concentrations (1-25 mEq/l), lithium clearances averaged 30% of the filtration rate (range 18-48%). The excretion of lithium was not altered significantly by mercurial diuretics. The infusion of lithium consistently elevated rates of potassium (K) excretion. At serum lithium concentrations of 4-5 mEq/l, K clearances of 50-80% of filtration rate were generally observed. K clearances in excess of filtration rate were promptly obtained when

lithium infusion was combined with a moderate osmotic (S_2O_3) diuresis. Lithium induced increments in the rate of K excretion are interpreted as representing tubular K secretion, since they are markedly reduced by mercurials. Simultaneous infusion of lithium and K failed to reveal evidence of competitive excretion.

Measurement of ventilatory dead space in subjects with uneven pulmonary ventilation WARD S FOWLER (introduced by J H COMROE, JR.) *Dept of Physiology and Pharmacology, Univ of Pennsylvania, Graduate School of Medicine, Philadelphia*

Measurement of ventilatory dead space requires identification of alveolar gas. The N_2 concentration—expired volume curve of gas expired after one inspiration of O_2 by normal subjects and patients with pulmonary disease—shows three phases. N_2 concentration successively 1) equals inspired concentration, 2) changes in an S-shaped fashion, 3) is rectilinear. Previous studies of normal subjects indicate that phase 3 is alveolar gas, following dead space wash-out (phases 1 and 2). In certain patients, the intersection of phases 2 and 3 is less definite, since the slope of the last part of the S-curve changes little and approaches the slope of phase 3. When such patients inspired O_2 followed without interruption by air, the sign of slopes of phases 2 and 3 were dissimilar, clarifying the point of intersection. The expired volumes corresponding to the intersection point were similar with inhalations of O_2 alone, or O_2 air. Such studies indicate that after maximal inspiration expired volume required for patients with pulmonary disease to wash out the dead space is not greater than normal, and that a valid alveolar concentration can be obtained for measurement of ventilatory dead space in patients if their expirations show a third phase which is linear, even though sloping (i.e. expired alveolar gas composition varies).

Mechanical impedance of surface of human body and its relation to elastic properties of skeletal muscle ERNST K FRANKE (introduced by H M SWEENEY) *Aero Med Lab, Air Materiel Command, Wright-Patterson Air Force Base, Dayton, Ohio*

In order to investigate the mechanical behavior of the human body surface when brought in contact with various vibrating structures, measurements were made with a small, sinusoidally vibrating piston applied to the body surface. From the vibratory forces exerted by the piston on the body, measured by means of a variable reluctance pressure gauge, the mechanical response of the body was calculated in terms of impedances, a procedure widely used in acoustics. It is shown that the results justify establishing a simple

harmonic vibrating system with lumped constants as a model of the mechanical behavior of the body surface. The constants of this model are calculated and their meaning is discussed. In order to investigate the nonlinear characteristics of muscle, some further measurements were made to determine the stiffness constant as a function of the pressure used to apply the piston. From the results obtained, the equation of motion of a hammer hitting the body surface was established. In this way it was possible to compare the results of the impedance measurements with measurements of contact time and rebound obtained by a ballistic method recently published by E Simonson *et al* (*J Applied Physiol* 1:512, 1949).

Propagation of surface waves over the human body ERNST K FRANKE, HANS OESTREICHER AND WOLF W VON WITTERN (introduced by H M SWEENEY) *Aero Med Lab, Air Materiel Command, Wright-Patterson Air Force Base, Dayton, Ohio*

A well established wave pattern appears on a body surface, such as that overlying the rectus femoris, when the surface is in firm contact with and driven by a small piston (diameter 1 in.) at frequencies near 100 cps. This pattern is easily observed and photographed by stroboscopic light. The wavelength was determined by measuring, through several wavelengths, the change of phase of the deflection as a function of distance from the center of the piston. For this purpose the vibrations were detected by a capacitive device which used the body as one electrode and a small disc at a suitable distance as the other. This arrangement did not disturb the wave propagation. Changes of capacity due to the vibrations are converted into electrical signals by operating the condenser in a high frequency resonant circuit. The propagation velocity was found to be constant at about 160 cm/sec in the frequency range 15–150 cps. When the amplitude of the exciting piston is held constant, 1) at any frequency the wave amplitude varies inversely with the square of the distance, and 2) at any distance the wave amplitude varies inversely with frequency. Theoretical considerations concerning the wave propagation in viscous-elastic media give a general picture of the mechanism of the waves and their relation to the elastic properties of the medium. Such considerations show also that the waves are essentially shear waves in this frequency range.

Descriptive analysis of vasomotor responses in footpad of the dog F E FRANKE, W C RANDALL, J W COX,* W F ALEXANDER* AND A B HERTZMAN *Depts of Physiology and Anatomy, St Louis Univ School of Medicine, St Louis, Mo*

Reflex vasoconstrictor responses in the footpad

of the dog as recorded by the photoelectric plethysmograph were compared with those responses induced by direct faradic stimulation of the sympathetic chain. In many constrictor responses, a decrease in amplitude of the volume pulse preceded the reduction in volume of the part by 0.5 to 5.0 seconds. In some responses, the decrease in volume pulse and blood volume occurred almost simultaneously. The frequently observed striking differences in onset of these two phases of the vasoconstrictor responses strongly suggest the operation of separate arteriomotor and venomotor mechanisms. After cessation of a strong stimulating current, the vascular constriction persisted for as long as 20-30 seconds before relaxation of the vessels first became apparent. Recovery was marked first by an increasing amplitude of the volume pulse followed by a progressive rise in volume of the part. In all cases the recovery was slow, and in many instances following constriction to complete obliteration of the pulse, the total recovery period extended over 3-5 minutes. The latent period from the time of stimulation to the appearance of the first phase of the constriction was approximately 0.5 to 1.0 second longer in the reflex responses as compared with the response to direct stimulation of the chain, and in both instances was several times as long as in the somatic reflex arc.

Effect of physical factors on radiosodium clearance in dogs FREDERICK R. FRANKE,* JOSEPH B. BOATMAN,* ROBERT S. GEORGE* AND CAMPBELL MOSES *Addison H. Gibson Lab., Univ. of Pittsburgh School of Medicine, Pittsburgh, Penna.*

The clearance of radiosodium from injected sites under varying conditions has been studied in a series of 31 experiments on dogs under pentobarbital sodium anesthesia. Injections of 0.25 to 1.0 cc. of saline carrying approximately 5.0 microcuries of Na^{24} were made subcutaneously and intramuscularly. The decline of activity over the injected area was measured with a shielded thin window Geiger-Mueller tube coupled to an autoscaler. Decline of activity was expressed as slope constant (clearance constant) based on change in activity per unit time. Control observations have been made and clearance constants under various conditions have been determined. The mean intramuscular clearance constant in a series of 27 determinations was 0.0473, the mean subcutaneous clearance constant of 5 experiments, 0.0071. Variations in muscle clearance have been produced by local application to the injected site of ice-salt mixtures and radiant heat. Alteration of the clearance constant to almost zero was seen with external interruption of venous return by applications of an abdominal pressure cuff. The addition

of hyaluronidase to subcutaneous injections produced an increased clearance constant of almost sixfold over the subcutaneous control. Preliminary studies support the view that clearance of radio-sodium from intramuscular sites may be made a quantitative test for detection of early congestive failure.

Direct measurement of I^{131} thyroid gland uptake and turnover in relation to thyroid function in normal and pathologic states A. STONE FREEDBERG, DAVID L. CHAMOVITZ AND ALVIN L. URELES (introduced by H. L. BLUMGART) *Medical Research Labs., Beth Israel Hospital and Dept. of Medicine, Harvard Med. School, Boston, Mass.*

The successful development of a quantitative method (FREEDBERG, URELES AND VANDILLA, *Federation Proc.* 8:50, 1949) for the measurement of thyroid I^{131} radiation has permitted more exact study of thyroid gland function in respect to I^{131} metabolism in man. Utilizing this 4 Geiger-Mueller tube method, uptake and turnover of I^{131} in the thyroid gland following oral doses of 150 μc , carrier-free, have been studied in 171 patients. Evaluation of thyroid function was made by clinical criteria, basal metabolic rate, serum cholesterol and, in some instances, serum protein-bound iodine. In 32 normal euthyroid subjects (Group A) the 24-hour uptake averaged 29% (S.D. 4.8), in 27 euthyroid patients with nodular goiters (Group B), 36% (S.D. 11.0). In 17 patients (Group C), euthyroid for 5 to 39 (av. 19) months after I^{131} therapy for hyperthyroidism, the 24-hour uptake averaged 38% (S.D. 11.3). The differences between the means of Groups A and B, and A and C are statistically significant. In 48 thyrotoxic patients (Group D) the 24-hour uptake averaged 69% (S.D. 13.1). In 7 patients with mild hypothyroidism the 24-hour uptake averaged 18% (range 13-23), in 10 patients with marked hypothyroidism, 8% (range 4-13). The biologic half-life (turnover) in Group A was 7.5 (S.D. 0.9) days, Group B, 7.5 (S.D. 0.5), Group C, 4.7 (S.D. 0.8) and Group D, 5.7 (S.D. 1.1). In 18 patients, persistently hyperthyroid after I^{131} therapy (Group E), the turnover was 4.3 (S.D. 1.1) days. The differences between the means of all groups, except A and B, and C and E, are statistically significant. The influence of various drugs and pathologic states are described.

Comparative study of ascorbic acid levels in gastric secretion, blood, urine and saliva JOSEPH T. FREEMAN* AND ROBERTA HAFKES BRING *Depts. of Medicine and Physiology, Woman's Med. College of Pennsylvania, Philadelphia, Penna.*

Ascorbic acid determinations of fractional gastric specimens, whole blood, saliva and urine

(24-hour) were made on 100 apparently normal individuals. The purpose was to establish a standard for comparative studies of intrinsic gastrointestinal diseases. All determinations were on individuals fasted 15 hours and with a low vitamin C intake for the preceding 24 hours. Roe and Kuether's method was used (*J Biol Chem* 147:399, 1943). The average gastric vitamin C was 1.04 mg %, range 0.04-3.9 mg %. The average whole blood C was 1.00 mg %, range 0.26-2.1 mg %, salivary content was 0.10 mg %, range 0-0.36 mg %, and the urine was 1.62 mg % with a range of 0-9 mg %. The average 24-hour urine excretion was 15.00 mg with a range of 0-80 mg. Allowing a deviation of 0.05 mg, there was a correlation between blood and gastric vitamin C in only 4 cases, in 59 cases the blood ascorbic level was higher, while in 33 the gastric ascorbic level was higher. No apparent relationship was noted between the blood gastric ascorbic content and free or total acidity. The same conclusion holds for these 2 measurements when correlated with the salivary vitamin C content and the 24-hour urinary output of vitamin C. The evaluation of all possible correlations of these figures in normal individuals suggests that ascorbic acid in gastric juice may be a function of gastric cells. Application of these results is already being made in various types of abnormal conditions.

Functional regeneration after nerve root transplantation in the guinea baboon. L. W. FREEMAN, J. C. FINNERAN* AND L. R. RADIGAN*. *Labs of Surgical Research, Indiana Univ School of Medicine, Indianapolis, and Labs of Physiology and Surgery, Yale Univ School of Medicine, New Haven, Conn.*

The vigorous capacity for regeneration demonstrated by spinal nerve roots has led to a preliminary investigation of the possibilities of nerve root transplantation. The male Guinea baboon characteristically maintains a relatively constant penile erection. After sacral nerve root denervation intradurally this power is lost. Bilateral lumbar sympathectomy in two preparations has failed to alter penile erection. In five preparations anastomosis of the coccygeal (1), third lumbar (1) or fourth lumbar (3) nerve roots to the sacral innervation has been followed in a period of approximately 8 months by a return of erectile ability. Stimulation experiments in one animal definitely established the neural continuity of the anastomosed nerve roots. Anatomic specimens of all have shown neural continuity.

Chemical changes occurring during symphyseal relaxation. EDWARD H. FRIEDEN* AND F. L. HISAW. *Biological Labs, Harvard Univ, Cambridge, Mass.*

Relaxation of the pelvic symphysis of the

guinea pig is accompanied by a considerable change in the weight of the symphysis. This weight increase, which in extreme cases may be 4- or 5-fold, appears to be due largely to infiltration of plasma, together with some extravasation of whole blood. The available histological evidence suggests alteration in the state of the ground substance as well as changes in the collagenous matrix of the tissue. In a search for clues to the mechanism of the action of relaxin, comparative chemical and enzymatic analyses have been made of control and relaxed (pregnant rabbit serum or purified relaxin) guinea pig symphyses. Assays for phosphatase and glucuronidase reveal no differences significant enough to implicate these enzymes in the relaxation process. Tissue analyses of the distribution of N, hydrolyzable reducing groups, and hydrolyzable hexoseamine indicate little, if any, recognizable effect upon a polysaccharide or mucoprotein substrate. The most significant finding has been that the ratio of collagen N to total insoluble N decreases during relaxation. Collagen, as determined by alkali extraction or tryptic digestion, constitutes from 60-80% of the total residue protein of the unrelaxed symphysis. In the relaxed animal, the range is 40-70%, in comparable experiments the range of overlap is small, and the decrease in collagen may be correlated with the extent of relaxation.

Purification and properties of relaxin. EDWARD H. FRIEDEN* AND F. L. HISAW. *Biological Labs, Harvard Univ, Cambridge, Mass.*

The problem of the preparation and purification of relaxin has been re-examined. Aqueous saline or acid extracts of pregnant sow ovaries contain relaxin in combined form, as evidenced by the fact that the physical and physiological properties of the active fraction vary with the extent of purification. The combination appears to be a relatively labile one, and may readily be split by the addition of alcohol. Subsequent purification is effected by a combination of isoelectric and ethanol precipitations. The most active fractions will induce good symphyseal relaxation in the guinea pig at a level of about 10 micrograms, corresponding to a purification of 500-1000-fold compared with the original fresh tissue. There is evidence that this preparation represents the hormone in nearly pure form. Purified relaxin is a relatively low molecular weight protein, as indicated by dialysis studies and positive reactions to common protein reagents, and is quite stable. It contains 12.7% N and 10.5% reducing sugar upon hydrolysis, about half the latter is hexoseamine.

Histochemical differentiation of substrate specific alkaline phosphatases in tissue of the rat. JONAS S. FRIEDENWALD AND GERTRUDE D. MAENGWYN-DAVIES*. *Wilmer Ophthalmological*

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Histochemical differentiation of substrate specific alkaline phosphatases in tissue of the rat. JONAS S. FRIEDENWALD AND GERTRUDE D. MAENGWYN-DAVIES* *Wilmer Ophthalmological*

Inst, Johns Hopkins Univ and Hospital, Baltimore, Md

In order to study the tissue localization of various substrate specific alkaline phosphatases, we have applied an adaptation of Gomori's technique (Proc Soc Exper Biol & Med 42: 21, 1939)

to fresh frozen tissue sections. In such sections many enzymes tend to diffuse. Good localization was obtained when working with a substrate solution half saturated for sodium acetate, and fully saturated for calcium phosphate. After incubation the acetate was washed out with a water-alcohol-calcium chloride mixture, and the enzymatically deposited calcium phosphate converted to cobalt sulfide. These studies were undertaken with the following substrates: glycerophosphate, glucose-1-phosphate, fructose 6 phosphate, hexose diphosphate, calcium β -naphthylphosphate, and muscle adenosine-triphosphate. All substrates were employed at the same molarity. Evidence for the substrate specificity of a number of alkaline phosphatases will be presented on the basis of the effects of different inhibitors and activators, and of variations in diffusibility and organ distribution

Transfer of proteins tagged with radioactive iodine

from plasma into lymph HERBERT D. FREEDLANDER, PARKER BIRROD, HOWARD J. CURTIS AND GEORGE R. MENEZLY (introduced by WALTER E. GARREY) Radiobiology Unit of Thayer Veterans Administration Hospital and Dept of Physiology and Medicine of Vanderbilt Univ School of Medicine, Nashville, Tenn

Small quantities of dog plasma were tagged with I-131. When introduced i.v. into dogs this protein disappears relatively slowly during the first few hours of highest concentration. The rate of fall in concentration varies from dog to dog. From 15-40% may disappear from the circulation within 2-3 hours. The tagged protein soon appears in the lymph. Within 15-20 minutes, sizeable amounts of radioactive protein can be collected from the thoracic duct lymph. In the paw the slow flow of lymph makes the time for collecting measurable quantities longer than is the case with the thoracic duct lymph, however, within 30 minutes a considerable amount can be measured in lymph collected from the hind leg. The concentration of the labelled material in the lymph increases with time so that in this fluid the concentration approaches 25-50% that of the plasma in about 2-3 hours. The amount of labelled protein/mg of protein nitrogen in the paw lymph differs from that found in the lymph collected from the thoracic duct. This difference is not striking in all animals examined, but where it occurs the tagged protein/mg of protein nitrogen is greater in the lymph collected from the thoracic duct. These

results suggest that the protein in this experiment is transferred across the capillary membrane more rapidly than previously thought for plasma proteins in the capillaries of the gut and liver as well as in the excretories

Absence of anti-ulcer factor from urine of patients with duodenal ulcer AL H. F. FRIDMAN, Dept of Physiology, Jefferson Med College, Philadelphia, Pa

A diet was formulated which consisted essentially of an enzymatic digest of purified protein fortified with tryptophane. Supplements of purified dextrose, lipids, vitamins and salts were added as needed. (1) This diet proved ulcerogenic for mice. The animals lost weight progressively, developed diarrhea with bloody stool, and assumed a posture suggestive of peritoneal irritation. The maximum survival time on this diet was 12 days. At autopsy, the gastric mucosa was always found to have ulcerative lesions. These ranged in severity from mucosal erosions to apparently penetrating ulcers. Alucosal bleeding and the presence of free blood were often noted in the small intestine, usually in the duodenum and terminal ileum. (2) Addition to this diet of crude extracts of urine from healthy men protected female mice, but not male mice, from developing gastrointestinal ulcerations and prolonged their survival time. The maximum survival time for female mice was 23 days and for male mice 12 days. (3) Crude extracts of urine from men with duodenal ulcer did not exert any protective action against ulcer development in either male or female mice, and failed to prolong life (maximum survival time, 12 days). (4) Protection against ulcer development and prolongation of survival time (up to 46 days) were noted when crude extracts of hog duodenal mucosa were added to the diet. Crude extracts of hog duodenal mucosa, purified extracts of hog intestinal mucosa ('enterogastrene'), and crude extracts of pork muscle were without anti-ulcer effects. Crystalline vitamin B₁₂ was also without effect. (5) Studies on the nature of the anti-ulcer factor and the effects of various fractions of normal male urine on normal and castrate female mice are continuing.

Effect of ultrasound on nervous tissues

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Ultrasound was applied to peripheral nerve, grayish ventral nerve cord and spinal cord of eel-frog. Ultrasound was without effect on excitability, wave form of the spike potential or propagation velocity of peripheral nerve, even after prolonged exposures. The exposed grayish ventral nerve cord exposed to ultrasound exhibited a reduction of spontaneous activity after several seconds exposure and recovered its original activity about one minute after the ultrasound was

turned off Frogs positioned so that ultrasound was incident on the dorsal surface over the lumbar enlargement evidenced paralysis of the hind legs after 4.3 seconds exposure (at room temperature) and produced paralysis after 7.3 seconds exposure (at $1^{\circ}\text{--}2^{\circ}\text{C}$) Histological examination of the sciatic nerves showed extensive degeneration of nerves and examination of the spinal cord showed marked pathology of the lower motor neurones Temperature measurements indicated that peripheral nerve and crayfish ventral nerve cord exhibited a maximal rise of $1^{\circ}\text{--}2^{\circ}\text{C}$ The spinal cord of intact frogs exhibited temperature rises as great as 40°C By using frogs cooled to 1°C and reducing the ultrasound exposure to two 4.3-second pulses interrupted by 4-minute cooling off period, it was demonstrated that temperature rises did not exceed 15°C and that paralysis of the hind legs occurred during the second 4.3-second exposure Similar experiments on frogs (room temperature) indicated paralysis upon exposure to ultrasound pulses of 0.080 second, delivered at a rate of 2.0/sec and no paralysis upon exposure to sound pulses of 0.010 second delivered at a rate of 20/sec, yet the latter procedure produced a higher cord temperature than the former

Characteristic response of isolated frog skin potential to neurohypophyseal principles and its relation to transport of sodium and water
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Previous experiments of one of us have indicated a close correlation between the potential difference and the sodium transport across the isolated frog skin The present experiments which deal with the effect of neurohypophyseal principles on Na transport, water transport and P D across the isolated skin of *Rana esculenta*, bear out very clearly the above mentioned relationship The apparatus used has been described previously (USSING *Acta Physiol Scandinav* 17:1, 1949) Na influx and outflux were determined with Na^{24} , and the water uptake was calculated from the radioactivity measurements Commercially prepared fractions of vasopressor and oxytocic principles, as well as whole powdered posterior lobe of the blue whale, markedly increased the P D This increase in P D varied inversely with initial P D, but within wide limits was nearly independent of hormone dose Both with and without hormone the influx and outflux of Na showed a nearly linear increase with increasing P D The influx was always higher than the outflux and the net Na influx increased with increasing P D After addition of the hormones, however, a higher P D was obtained for a given Na influx Thus, after

hormone addition, more work has to be done to transport Na actively against the electrical force Increase in net uptake of water from the outside solution occurred after addition of the hormones

Blood flow in transilluminated membranes after stasis and under pathological and traumatic conditions (motion picture)
 GEORGE P. FULTON AND ROBERT P. AKERS (introduced by LELAND C. WYMAN) *Dept of Biology, Boston Univ, Boston, Mass*

Motion picture records were edited to demonstrate the characteristics of the circulation in the transilluminated retrolingual membrane of the frog and the cheek pouch of the hamster under pathological and traumatic conditions, and after stasis In hamsters with advanced tumors of carcinogenic origin, the blood appears thin with relatively few erythrocytes, but vast numbers of leucocytes apparent even on the walls of the arterioles Numerous white cells are also seen in hamsters with a staphylococcus infection In both cases erythrocytes are seen at times in groups separated by plasma and white cells, resembling sludged blood However, individual components of the group can be separated readily by manipulation of the vessel wall with a microprobe Pictures taken with reflected light at low power ($\times 54$, $\times 90$) suggest sludged blood, but those taken with transmitted light at higher power ($\times 200$), accompanied by microprobing, indicate that the erythrocytes are not agglutinated The circulation is shown in animals under severe traumatic conditions Erythrocytes are unagglutinated in prolonged stasis as shown by microprobing of vessels packed for several days with noncirculating red cells and by the escape of the erythrocytes as individual corpuscles from vessels severed with a microknife Platelet accumulation is shown following release from venous occlusion in the cheek pouch Groups of platelets break off continuously from the accumulating mass and circulate Additional platelets adhere at the same point In larger vessels, blood flow forms complex channels through the adherent mass of platelets

Food deprivation and sexual reflexes
 W. HORSLEY GANTT *Pavlovian Lab, Phipps Clinic, Johns Hopkins Univ, Baltimore, Md*

Sexual conditional reflexes (crs) were formed in 3 male dogs (Poco, age 7, Chester, 4, Choptank, 4) These Chesapeake Bay dogs formed sexual crs quickly (to stimulation of the external genitalia) and retained them for at least 7 months without reinforcement All male dogs do not form overt sexual reflexes under laboratory conditions In some dogs where there is no motor sexual cr (erection), a cardiac component gives evidence of sexual cr formation (ROBINSON AND GANTT *Bull Johns Hopkins Hospital* 80:231, 1947) The

Synergism of thromboplastin extracts from brain and lung CLIFFORD F GERBER* AND ERNEST W BLANCHARD *Research Labs, Schieffelin and Co, New York City*

When saline extracts of brain and lung are combined the resulting mixture exhibits greater thromboplastic potency than either of the original extracts. The same synergism is obtained if a saline extract is prepared from a mixture of the two tissues. Identical potencies are obtained in all proportions of the tissues, from 70% brain plus 30% lung to 30% brain plus 70% lung. The synergism was given by brain and lung mixture from the several species used and by dried tissues as well as fresh. There are time and temperature factors for the full development of the synergism indicating that a definite interaction occurs. Extracts of the two tissues were made separately. Each extract was then subjected to ether extraction. The ethereal solutions were added to the heterologous tissue extract. The lung saline extract plus brain ethereal solution showed almost complete restoration of activity. The brain saline extract was unaffected by the addition of lung ethereal solution. This experiment indicated that neither tissue is a complete thromboplastin and that excess lipid is supplied by the brain extract to the lung extract.

Further observations on metabolic fate of chloramphenicol (Chloromycetin) ANTHONY J GLAZKO, WESLEY A DILL* AND LORETTA M WOLF* *Research Laboratories, Parke, Davis and Company, Detroit, Michigan*

Earlier reports from this laboratory have shown that chloramphenicol is converted in the body to a microbiologically inactive monoglucuronide derivative which is excreted in the urine and bile along with small amounts of unchanged chloramphenicol. Further studies have been made on the metabolic fate of this glucuronide in the rat, using material isolated from human urine. Studies with isolated intestinal loops in the rats indicate that the glucuronide as such is poorly absorbed from the intestinal tract. Parenteral administration results in the appearance of chloramphenicol as well as inactive nitro compounds in the urine, probably due to enzymatic hydrolysis of the conjugate in the tissues prior to absorption. Similar activation has already been demonstrated *in vitro* with β -glucuronidase. Antibiotic activity is also found in the caecum and large intestine of the rat after administration of the glucuronide derivative, due to the hydrolytic action of bacterial enzymes. A portion of the liberated chloramphenicol is then reabsorbed. This process may contribute to the maintenance of effective antibiotic levels in the blood and tissues, providing a cyclic mechanism for return of a portion of the metab-

olized drug to the body in an active form. In addition to the activation process, large quantities of aromatic amines are produced by bacterial reduction of the nitro group in the intestinal tract of lower animals.

The effect of clothing on the physiological adjustments of human beings to sudden change in environment N GLICKMAN, T INOUE,* R W KEETON, S E TELSER* AND M K FAHNESTOCK* *Dept of Medicine, Univ of Ill College of Medicine, Chicago, and Dept of Mechanical Engineering, Univ of Ill, Urbana*

Physiological adjustments to sudden changes in ambient environment of 7 subjects wearing standard summer weight clothing (SC1) were compared with those of 10 subjects wearing a 90% cotton full length union suit (US). Exposure for one hour in the comfortable room (CR1) at 24.4°C with water vapor (VP) of 9.2 and 24.4 kilodynes/cm² was followed by one or two hours in the hot room (HR) at 37°C with water vapor of 41.1 kilodynes/cm², then re-entry into the comfortable room (CR2) for one hour. All data were analyzed statistically. The SC1 with its several layers of material compared with the single layered US effected only slight differences in the physiological adjustments of the subjects, these occurred primarily during the first 10 minutes of exposure to the changes in environment. The buffering effect of clothing was evident on entering the HR and on re-entering CR2, upon entering the HR the MT, and pulse rate increased less for the SC1 group than for the US group and decreased less upon re-entering CR2. Subsequent changes in these and other factors were similar for both groups, except that during the second hour in the HR the MT, and T_{re} increased more for the SC1 group. Greater differences of ambient temperature undoubtedly would have shown the effect of clothing more clearly. Evaporative weight loss, thermal sensations, clothing temperatures (MT_{cl}), gradients between MT, and MT_{cl}, and the rapidity and magnitude of certain adjustments are discussed.

Osmotic behavior of isolated nuclei LESTER GOLDSTEIN AND CLIFFORD V HARDING (introduced by L V HEILBRUNN) *Dept of Zoology, Univ of Pennsylvania, Philadelphia*

Nuclei from the oocytes of the frog, *Rana pipiens*, were isolated by hand, and the osmotic behavior of these large nuclei was then studied by following the volume changes after immersion in various solutions. Nuclei from full-grown oocytes and from small, unpigmented oocytes were compared. The nuclei from oocytes of both stages were found to be freely permeable to salts and sugars, similar results with the large nuclei have recently been reported by Callan. The rates of penetration of Ca-free frog Ringer's solution and 1.5 molal

sucrose solution across the membranes of the younger (smaller) nuclei were found to be greater than across the membranes of the older (larger) nuclei. Egg albumin, however, did not penetrate the membrane and it was found possible to obtain an isotonic solution. We can therefore determine the colloid osmotic pressure of the nucleoplasm at the two different stages of oöcyte development. Results indicate that the colloid osmotic pressure of the nucleoplasm within the smaller oöcytes is significantly greater than in the larger oöcytes. In comparing nuclei of small and large oöcytes, the observed difference in rates of penetration of salt and sugar solutions is probably due, at least in part, to this difference in colloid osmotic pressure, and it may also be due in part to permeability differences. This latter possibility is now being investigated.

Myocardial glucose, lactate and pyruvate metabolism of normal and failing hearts studied by coronary venous catheterization in man. WALTER T. GOODALE, ROBERT E. OLSON* AND DONALD B. HACKEL* *Peter Bent Brigham Hospital, Dept of Medicine, Harvard Med School, and Dept of Nutrition, Harvard School of Public Health, Boston, Mass*

Studies on 9 subjects revealed significant differences between arterial and coronary venous blood concentrations of glucose, lactate and pyruvate, reflecting extraction and presumptive utilization of these metabolites by the myocardium. In terms of coronary arteriovenous difference, extraction of each metabolite was related directly to its own arterial level. The slopes obtained by plotting extraction against arterial level for each metabolite compared closely with those obtained in intact dogs by similar techniques, except that glucose values in man showed much less scatter. At average non-fasting arterial glucose, lactate and pyruvate levels of 103, 7.5 and 1.3 mg % respectively, the average coronary arteriovenous differences were 10.7, 2.7 and 0.4 mg % respectively. Oxidation of this quantity of substrate could account for 90-100% of the simultaneous oxygen extraction. At low fasting arterial levels, glucose, lactate and pyruvate extractions were small, suggesting a dependance of the myocardium upon other substrates during fasting. Three patients with multivalvular disease in congestive failure with low cardiac outputs, showed low coronary venous oxygen saturations of 10, 17 and 18% (normal 29-38%, 7 cases). Myocardial glucose, lactate and pyruvate utilization was normal, indicating a maintenance of a normal aerobic oxidation pattern. Related studies on similar cases with Bing *et al* showed that left ventricular blood flow/100 gm of myocardium was normal or slightly increased, with increased total flow by virtue of cardiac hypertrophy. Thus there

appears to be no defect in oxidative energy production, but rather a failure to convert this energy to effective mechanical work.

Changes in muscle phosphorylase and hexokinase activities produced by inanition in the rat. E. S. GORANSON, NANCY M. DANOFF AND ELIZABETH F. PURDIE (introduced by E. A. SELLERS) *Dept of Physiology, University of Toronto, Toronto, Canada*

It has previously been reported that the muscle phosphorylase activity of rats increased after a short period of starvation and that this increase was reversed by refeeding (K. LUNDBAEK AND E. S. GORANSON *Acta physiol Scandinav* 17: 280, 1949). More recent studies show a reversal of this increase in phosphorylase activity on refeeding with carbohydrate but not on refeeding with an isocaloric fat diet after a period of inanition. A reversal of activity was also observed one hour after the simultaneous administration of glucose and insulin but not after the injection of insulin alone. Glucose per se, one hour after its injection into fasted rats, had a variable effect in reversing the activity of the enzyme. While an increase in muscle phosphorylase activity was observed after a period of inanition, starvation produced a significant lowering in muscle hexokinase activity. The possible significance of these changes will be discussed.

The adrenal gland and hemopoiesis in the rat. ALBERT S. GORDON AND SAM J. PILIERO* *Dept of Biology, Washington Square College of Arts and Science, New York Univ., New York City*

Female rats (140-170 gm) were adrenalectomized and maintained on 1% saline given as drinking water. Hematologic determinations were made at 7-10 day intervals. Reductions in red cell numbers and hemoglobin values occurred within a few days and became maximal at about 3 weeks. This was followed by a gradual return of the red cell and hemoglobin values to almost normal levels by approximately 60 days. Reticulocytosis and decreased red cell fragilities accompanied the anemia. Sedimentation rates increased slightly and total and differential white cell counts were unaffected by adrenalectomy. The various hemic alterations induced by adrenal removal could be prevented by administration of adrenal cortical extracts. To evaluate further the role of the adrenal in blood formation, adrenalectomized and control rats were subjected to the stress of lowered barometric pressures (311 mm Hg) for periods up to 21 days. Adrenal removal did not impair the ability of the rat to respond with increased red cell, hemoglobin and reticulocyte values to this stimulus. Bone marrow myelograms of the pressure-exposed adrenalectomized rats were no different from those of the controls.

Following a 3-week rest period, however, greater numbers of granulocytes and decreases in the numbers of nucleated erythroid cells with a resulting lowered erythroid-myeloid cell ratio became apparent in the marrows of the adrenalectomized rats. It is concluded that although adrenalectomy in the rat results in alterations in the red cell production and/or destructive mechanisms, the adrenal is not essential for the response to the erythropoietic stress imposed by lowered barometric pressures.

Pyrogen fever in rabbits effects of adrenalectomy and thyroidectomy RONALD GRANT, J DOROTHY HIRSCH AND BARBARA B HIRSCH (introduced by FRANK W WEYMOUTH) *Dept of Physiology, Stanford Univ, Calif*

Effects of prior adrenalectomy and thyroidectomy on changes in rectal temperature (RT), ear temperature (ET), respiratory rate (RR) and oxygen consumption (OC) following i.v. injection of pyrogens, have been determined. Normal fever includes two phases of rising RT associated with lowered ET and RR. An interpolated phase of stable or declining RT is associated with increased ET and RR. The first rise in RT is aided by a brief increase in OC not usually due to shivering. Adrenalectomy (accessories not removed, animals kept on DCA) had no marked effect on any of the changes listed, but shivering seemed to participate more in increasing OC. At room temperature (22°) thyroidectomized animals are hypothermic, the initial rise in RT is normal, but the secondary rise absent. Shivering is marked and stimulation of OC prolonged. ET and RR are low initially and show no marked changes. Thyroxine medication largely restores the normal responses. At 36° heat defense mechanisms are active, pyrogen causes normal changes in ET and RR and a weak biphasic fever. No shivering occurs. Most of the differences after thyroidectomy can be attributed to the low metabolic rate. It is concluded that neither adrenal nor thyroid secretions are essential intermediaries in fever but that absence of either gland causes increased participation of shivering. In thyroidectomized animals this is attributed to hypothermia. In adrenalectomized animals it may be due to removal of inhibition normally exerted by epinephrine.

Influence of carotid body removal upon the polycythemic response to discontinuous anoxia WILSON C GRANT *Dept of Physiology, Columbia Univ College of Physicians and Surgeons, New York City*

Carotid bodies were destroyed and/or aortic nerves sectioned with the right vagus in rabbits. Chemoreceptor removal was demonstrated by lack of NaCN response, marked depression of arterial O₂ saturation and pO₂, and absence of

hyperpnea when exposed to low O₂ mixtures. Loss of the carotid bodies was verified histologically. The carotid bodies were responsible for the great bulk of chemoreceptor activity in the unanesthetized rabbits since their removal alone abolished the NaCN response. The arterial O₂ saturation of an animal with unilateral carotid removal breathing low O₂ was halfway between that of the normal and bilaterally operated. No lasting hypertension was observed. Anoxia was produced in a chamber maintained at 400 mm Hg (16,500 ft) for approximately 6 hours/day, 6 days/week. The average cumulative exposure was 150 hours. The denervated animal was accompanied in the chamber by a 'dummy operated' control. Three animals with carotid body and aortic nerve removal, as well as 5 with only carotid body removal, showed polycythemia. The hematocrit value rose 39 to 72 %, O₂ capacity 23 to 36%, R B C 23 to 62 % above pre-anoxic control levels and the reticulocyte peak was 4 to 12 %. Control values were re-established in about 40 days after removal from chamber. Values in the normal animals varied from zero to 10 % above control levels with no appreciable reticulocytosis. Incomplete carotid removal usually resulted in intermediate blood changes. Because of the lack of hyperpnea, a severe anoxic anoxia was produced without the acapnia present in normal animals. Preliminary investigation of the influence of carotid body removal upon the erythropoiesis following hemorrhage or cobalt administration yielded results similar to those from normal animals.

Observations on *in vivo* inaction of testosterone propionate by liver of white rat JOHN T GRAY-HACK* AND W W SCOTT *James Buchanan Brady Urological Inst, Johns Hopkins Hospital, Baltimore, Md*

A study is being made of the effects of various manipulations such as starvation, dietary deficiency, carbon tetrachloride poisoning and hypophysectomy on the ability of the liver of the castrate adult male rat to inactivate testosterone propionate. The animals were castrate for a period of at least 3 weeks before 10 mg pellets of testosterone propionate were implanted subcutaneously and intrasplenically. They were then subjected to the manipulations outlined above to test the testosterone propionate inactivating power of the liver under these conditions. The size of the prostate was used as an indicator of androgenic activity in the general circulation. Our results thus far indicate that the mechanism by which the liver inactivates testosterone propionate persists even in the face of apparently severe damage to this organ, this is contrary to the effect of liver damage on inactivation of estrogen by the liver.

Simultaneous studies of heart and kidney functions in human subjects D M GREEN, A D JOHNSON*, W C BRIDGES*, J H LIHMANN* AND F GRAY* *Dept of Medicine, Univ of Washington School of Medicine, Seattle, Wash*

Combined cardiac and renal functional measurements were made on 61 patients. The functions measured included cardiac output, mean blood pressure, glomerular filtration rate, renal plasma flow and extracellular fluid volume. The subjects were so selected as to present an extensive range of values for the functions studied. Little correlation was demonstrated between cardiac output and the other functions measured. Wide differences in filtration rate, plasma flow and extracellular fluid volume were observed among patients whose outputs were approximately equal. Renal plasma flow was found related to glomerular filtration rate. Both functions were inversely related to systemic blood pressure. When the effect of renal plasma differences was held constant by the partial correlation technique, the relation of filtration rate to blood pressure became nonsignificant. Extracellular fluid volume did not correlate well with any of the other functions measured.

Constancy of responses to intra-arterial injections of mechohyl in isolated blood-perfused extremity source and influence of constrictor substances produced by blood donor animal HAROLD D GREEN, J MAXWELL LITTLE, LEWIS T FRANKLIN* AND HOWARD H WAYNE* *Dept of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N C*

The blood flow responses to intrafemoral artery injections of mechohyl in a dog's hindleg, perfused from its aorta varied with spontaneous fluctuations of vasomotor tone and perfusion pressure (*Federation Proc* 8: 50, 1949). To prevent changes of perfusion pressure a constant-pressure pump was inserted between the aorta and femoral artery. In this preparation flow decreased progressively from around 10 cc to around 2 cc/100 gm/min in about 3 hours indicating marked vasoconstriction which was humorally mediated, since it was prevented by neither nerve section nor complete amputation with perfusion by blood from a separate blood donor dog. Splanchnic section, adrenalectomy or nephrectomy in the blood donor dog had little effect, pithing or prolonged spinal ischemia by elevation of the C S F pressure caused a momentary further decrease in leg flow coincident with the rise in mean arterial pressure in the blood donor dog. Within about 30 minutes the blood donor's mean arterial pressure fell to around 40 mm Hg and simultaneously the leg flow rose to or above the initial rate where it remained for several hours. The initial rate of leg

flow also could be restored temporarily by substitution of a new blood donor. The percentile responses to injections of mechohyl varied inversely with the control leg flow throughout these procedures. Since none of the above changes could be accounted for by the recorded alterations in blood viscosity the progressive reduction in control flow and increase in response to mechohyl must be due to a constrictor agent produced by the blood donor dog under the control of its nervous system in response to some stress induced by the perfusion system.

Effect of oral cinchona alkaloids on circulation of dogs with experimental renal hypertension INEZ GREENE* AND E P HIATT *Physiology Dept, Univ of North Carolina School of Medicine, Chapel Hill*

We have found that oral doses of quinine or quinidine have very little effect on the blood pressure of dogs with renal hypertension, which is in marked contrast to the previously reported depressor effect of these alkaloids on dogs with experimental neurogenic hypertension. Five dogs were made hypertensive by the application of clamps to both renal arteries according to the method of Goldblatt. Oral doses (15 mg/kg) of one of the alkaloids were given to each dog several times a day for 3 days and the effect on its arterial blood pressure and renal hemodynamics was measured. Blood pressure was recorded optically from a needle inserted into a femoral artery. Glomerular filtration rate was estimated by the renal clearance of creatinine and effective renal plasma flow was determined by the clearance of p-aminohippurate. The concentration of the alkaloids in the plasma was measured at corresponding intervals. At plasma levels which in normal or neurogenic hypertensive dogs cause marked vasodilatation, the dogs with renal hypertension showed practically no such effect. These experiments are considered to be additional evidence that the site of the vasodilator action of these drugs in this concentration range is on the neuromuscular junction and not directly on the arteriolar smooth muscle. The maintenance of blood pressure in these dogs with alkaloid concentrations up to 8 mg/l also indicates that cardiac function is not markedly depressed.

Parathyroids and vitamin D in mineral stress ROY O GREEP AND CLARY J FISCHER* *Harvard School of Dental Medicine, Boston, Mass*

Intact and parathyroidectomized young rats received for 4 weeks purified diets which embraced a wide range in calcium phosphate ratios and absolute quantities. Each mineral combination was also fed with no vitamin D to each category of animal. In intact and operated animals the blood levels of calcium and phosphate showed a marked

tendency to reflect the relative concentration of these substances in the diet. Excursions in blood levels were, in general, more extreme after parathyroidectomy, indicating a definite but limited parathyroid capacity to maintain a mineral homeostasis. In parathyroidectomized rats fluctuations in blood phosphate produced by diet reciprocally altered blood calcium concentration in an opposite direction. Conversely, changes in blood calcium had little or no effect on phosphate. In intact rats the magnitude of the blood calcium and phosphate-raising effect of vitamin D was greatest in mineral deficiencies and least, or negligible, in their excess. Parathyroidectomized rats responded to vitamin D more uniformly, except that with high phosphate intakes the blood calcium was actually reduced due to excessive elevation of blood phosphate. Vitamin D is not essential for growth of intact rats given an adequate calcium phosphate intake and may impair growth in mineral imbalances, given to parathyroidectomized rats with normal mineral intake growth was greatly improved. The skeleton was weakened by every dietary mineral deficiency. High calcium diets produced calculi in the bladder and ureters; high phosphate diets caused severe nephrolithiasis. Observations on tetany and bone ash will be reported.

Respiration of cerebral cortex in vivo, in the absence of glucose R. G. GRENNELL AND P. W. DAVIES *Psychiatric Inst., Univ. of Maryland and Dept. of Biophysics, the Johns Hopkins Univ., Baltimore, Md.*

With the aid of the oxygen electrode, and a technique which permits perfusion of a localized area of cerebral cortex (through cannulation of a pial arteriole), *in vivo* measurements have been made of activity and rate of oxygen consumption of the cortex of the Suprasylvian gyrus of the cat, in the presence and absence of exogenous carbohydrate. The perfusion fluids used were modified, bicarbonate buffered Krebs solutions, equilibrated with 5% CO₂ at 38° C. Either Knox gelatine or crystalline serum albumin was added to maintain the normal colloid osmotic pressure, and these standard solutions with glucose have shown themselves capable of maintaining the normal pre-perfusion metabolic level over periods of as much as 2 hours, although they failed to do so when the gelatine or albumin were omitted. The animals were under sodium pentobarbital (Nembutal) anesthesia, and the temperature of the exposed area of the brain was carefully maintained at 38° C. The following observations have been made: 1) Up to 2 hours (the longest period during which perfusion has been carried out thus far) in the absence of carbohydrate, there has been no drop in the rate of the local cortical oxygen consump-

tion. 2) Lack of glucose has thus far shown no appreciable effect on the spontaneous electrical activity of the cells in the local area. 3) That the local oxygen consumption and activity can be altered has been demonstrated by perfusion with various concentrations of Nembutal, citrate, potassium, etc. Nembutal in 0.5 mM concentration drops the respiration to about 75-80% of normal, and at a concentration of 10 mM, the oxygen consumption has reached zero. The 0.5 mM concentration causes marked depression of the local electrocorticogram. These experiments would indicate that the brain cells, at least of a local area of the cortex, can use something other than carbohydrate as a substrate.

Interrelationship of cardiac output, blood pressure, and peripheral resistance during respiration ARTHUR L. GROPPER AND ARNOLD H. WILLIAMS (introduced by JOHN R. SMITH) *Hypertension Division, Dept. of Internal Medicine, Washington Univ. School of Medicine, St. Louis, Mo.*

It is well known that cardiac output and blood pressure vary during respiration. Starr and Friedland (*J. Clin. Investigation* 25:43, 1946) showed that ballistocardiographic complexes enlarge during inspiration and diminish with expiration. They found that the outputs of both the left and right hearts varied, the latter to a greater degree. Right heart output increased and left heart output decreased (e.g., systemic blood pressure dropped) during inspiration, and vice versa. As the size of the BCG complex reflects the sum of right and left heart outputs it is probable that the variations of output are greater than those which the size of the complexes would indicate. In the control periods of some recent experiments in normotensive patients (WILLIAMS, A. H. and H. A. SCHROEDER, *Proc. Am. Physiol. Soc.* Sept., 1949) BCG complexes showed phasic changes of 25% whilst variations of blood pressure were small. Even 10% changes of left heart output would have produced greater variations of blood pressure than those obtained if no compensatory adjustments had been made in peripheral resistance. Studies are in progress to demonstrate the reciprocal interrelationships of left heart output, blood pressure and regional peripheral resistance during respiration in normotensive and hypertensive subjects.

Study of protein fractions of gastric juice obtained by electrophoretic separation ALLAN L. GROSSBERG,* S. A. KOMAROV AND HARRY SHAY *Samuel S. Fels Research Inst., Temple Univ. School of Medicine, Philadelphia, Penna.*

Protein material of canine gastric juice secreted in response to sham feeding was analyzed electrophoretically. Juice was collected directly into

phosphate buffer pH 6.1, the final pH never fell below 6.0, thus precluding the possibility of auto-digestion. This material was dialyzed against water at 0°C until salt free and then lyophilized. Electrophoresis of solutions of this material was performed according to standard procedures (e.g. LOWESWORTH, L. G. *Chem. Rev.* 30: 323, 1952). Upon electrophoresis 1 peak representing the major part of the protein material were consistently observed. One or 2 additional less prominent peaks were also occasionally observed. Two electrophoretically well-defined fractions having electrophoretic mobilities, $\mu = -3.5 \times 10^{-5}$ cm²/volt/sec and $\mu = -7.9 \times 10^{-5}$ cm²/volt/sec in pH 6.08 phosphate buffer, $r/2 = 0.1$, were obtained, but probably neither represents a chemical entity. These fractions contain mucoproteins of different nature, as indicated by liberation of hexosamine and uronic acid after acid hydrolysis, in characteristically different proportions. The lowest ratio of hexosamine to uronic acid was obtained in the fastest moving protein and was lower than in any of the specimens of sham feeding juice analyzed without fractionation. The highest ratio approximated values obtained from spontaneously secreted alkaline gastric mucus. The fractions of intermediate mobility also had intermediate values for the relative concentration of hexosamine and uronic acid. All fractions upon acidification exhibited peptic activity. The data are interpreted as further evidence for the existence of more than one mucoprotein in gastric secretion, as reported previously (GROSSBERG, KOMAROV AND SHAY *Federation Proc.* 8: 62, 1949).

Intraarterial pressures before and after aortic resection for coarctation in man. ROBERT F. GROVER,* HENRY SWAN, II* AND CLARENCE A. MAASKE. *Depts. of Physiology and Pharmacology, and of Surgery, Univ. of Colorado Med. Center, Denver.*

Simultaneous recordings of the electrocardiogram and intraradial and intrafemoral arterial pressures were made by means of resistance type strain gauges in 7 patients with coarctation of the aorta. A comparative analysis of these records indicated that the femoral-to-radial pulse pressure ratio and the magnitude of the onset-to-peak interval of the femoral pulse wave were the most significant criteria for establishing the existence of a coarctation. These same criteria have now been employed to evaluate the effect of aortic resection on the hemodynamic status of these patients. The average femoral-to-radial pulse pressure ratio prior to surgery was 0.18. When again determined an average of 6 weeks post-operatively, the average ratio had been elevated to 0.69, a significant alteration toward the normal of 1.0. In every case, the onset-to-peak interval

of the femoral pulse was reduced to within normal limits. These changes imply a marked improvement in the vascular dynamics of these patients.

Increased sodium entry into squid giant axons during activity at high frequencies and during reversible inactivation of cholinesterase. HARRY GRUNDIIST AND DAVID NACHMANSOHN. *Dept. of Neurology, Columbia Univ. College of Physicians and Surgeons, New York City.*

Squid giant axons are able to carry 300 impulses/sec for several hours. Maximum carrying rate for times up to 6 minutes lies between 350 and 400 impulses/sec. Recent association of the spike with transient increased permeance of sodium suggests that the higher the rate and duration of nerve activity, the greater should be the entry of sodium into the axoplasm. In a preliminary series of experiments, individual giant axons were exposed to continuously flowing artificial seawater containing a moiety of Na²⁴ while stimulated at 100, 200 and 300/sec up to 30 minutes. Sodium entry was calculated from the radioactivity of the extruded axoplasm. Net entry because of 15 minutes activity (subtracting entry into resting nerve) doubled (5 mV/gm to 11 mV/gm) by increasing rate of activity from 100 to 300 impulses/sec. Under the same conditions 30 minutes activity produced net entry 6.4 and 14.6 mV/gm. Axons inactivated by DFP or eserine under reversible conditions showed still larger net sodium entry in 15 minutes, approximately 10 and 15 mV/gm. Present concepts of ion transport across cell membranes will be discussed in the light of these experiments, and include the possibility that cholinesterase is associated with 'sodium pump' mechanism, that its combination with acetylcholine or esterase inhibitors may alter the dynamic ionic equilibrium by inactivating this mechanism.

Dissolution of fibrin clots in the presence of concentrated purified thrombin. M. MASON GUEST AND ARNOLD G. WARE.* *Dept. of Physiology and Pharmacology, Wayne Univ. College of Medicine, Detroit, Mich.*

Fibrin clots prepared by the action of thrombin on 0.5% fibrinogen solutions are later completely dissolved by the thrombin at 37.5°C. The rate of solution is a linear function of the log of the thrombin concentration plotted against the log of time. Thrombin in a concentration of 9,000 units/ml produces clotting and dissolution of the clot 90 minutes later. Antifibrinolysin (antiplasmin) has no effect on the rate of breakdown of the clot while soybean antitrypsin has only a slight retarding effect when present in high concentration. The clot dissolving property of thrombin is destroyed by heat at a rate equal to the destruction of the clotting activity of the thrombin.

Relation of the biological activity of progesterone to the extent of its conversion to pregnanediol

H S GUTERMAN *Dept of Metabolic and Endocrine Research, Med Research Inst, Michael Reese Hospital, Chicago, Ill*

The excretion of urinary pregnanediol was studied following the administration of progesterone to women. The results indicate that the conversion of progesterone to pregnanediol is influenced by the physiological state and more specifically by the corpus luteum and/or the placenta. Pregnanediol amounts to less than 20% of the administered progesterone in the non-pregnant state in the absence of corpus luteum activity (5-19%) and in those pregnancies associated with apparent decreased progesterone secretion which end in abortion (7-19%). More than 25% of the progesterone is converted to pregnanediol when the corpus luteum is active in the non-pregnant state (25-44%) and when the corpus luteum and/or placenta is functional in normal pregnancy (28-44%) and pregnancy retained in spite of symptoms of threatened abortion and apparent decreased progesterone secretion (27-60%). From these observations it appears that the conversion of progesterone to pregnanediol is increased in the presence of the functioning corpus luteum or placenta. The enhanced conversion pattern in pregnancies which are retained suggests that a metabolic factor is introduced by these endocrine structures which may have significance in maintaining the integrity of gestation.

Evidence for adrenergic sweating in man

HENRY HAIMOVICI (introduced by H B VAN DYKE)
Surgical Div, Montefiore Hospital, New York City

Dibenzamine, an adrenergic-blocking agent, was found to inhibit spontaneous sweating in man. This observation raised the problem of the presence of an adrenergic component in the nervous mechanism of sweating in man (HAIMOVICI *Proc Soc Exper Biol & Med* 68 40, 1948). In the present investigation, the anhidrotic effect of Dibenzamine was demonstrated by a colorimetric method in 31 subjects and by the electrical skin resistance in 12 subjects. The sudomotor response to adrenergic drugs was studied by intradermal injections. Epinephrine (0.1 γ to 0.3 γ) induced sweating in 84.0% of the subjects. Its sudomotor effect was consistently inhibited by Dibenzamine. Norepinephrine (0.1 γ to 0.3 γ) induced sweating in 74.0% of the subjects, while Isuprel (0.1 γ to 0.3 γ) provoked no response in 60.8% and delayed positive response in 32.1% of the subjects. The results obtained with norepinephrine and Isuprel suggest that sympathin E and the excitatory component of epinephrine are responsible for the sudomotor ability of the adrenergic systems. Intradermal

injections of epinephrine and acetylcholine or Mecholyl into the same point indicate that the secretory activity of the sweat glands is augmented both by cholinergic and adrenergic stimulation. These agents exert therefore a synergistic sudomotor action. Since sweating in man can be elicited by adrenergic agents and inhibited by an adrenergic-blocking agent, it is concluded that, in addition to the known cholinergic fibers supplying the sweat glands, there is also an adrenergic component in the nervous mechanism of sweating in man.

Absolute numbers of circulating eosinophil blood cells in dogs starved for three days

F HALBERG,* J R R BOBB AND M B VISSCHER
Dept of Physiology, Univ of Minnesota, Minneapolis

Thirty-five unselected dogs, previously dewormed, under standard housing conditions were used simultaneously in this study. The week preceding each experiment they were maintained on a diet consisting of $\frac{1}{2}$ pound meat, multivitamins, biscuits and water *ad libitum*. Eosinophils were determined in the counting chamber in blood withdrawn from the jugular vein, on the first day 4 samples from each dog at exactly 4-hour intervals (6 A.M. till 7 P.M.) and 2 daily on the following two days (6 to 7 A.M. and 6 to 7 P.M.). The data collected during the 60-hr fast—as well as those obtained from a second experiment carried out 2 weeks later on the same dogs, starved for 84 hours—did not support the concept of a regular or considerable eosinopenia (exceeding a -30% change) in the dogs starving for 60 hours or less (the only dog which showed a fall of 85% at the count after 60 hours of fasting died the same day from an infection). However, the results obtained after 84 hours of fasting averaged -51% of the starting value which under the conditions of our experiment may be considered as the time of manifestation of starvation eosinopenia in the dogs investigated.

Inactivation of gonadotrophins by liver and liver homogenates

B VINCENT HALL (introduced by AUSTIN M BRUES) *Univ of Illinois, Urbana, and Biological Division, Argonne National Lab, Chicago, Ill*

It is well known that gonadotrophic hormones rapidly disappear or lose their effectiveness after release or injection into an animal. To investigate the possible role of liver in the inactivation of gonadotrophins, implants of anterior lobes of rat pituitaries were made on 4 consecutive days either directly beneath the capsule of the spleen, into the abdominal cavity or subcutaneously in 72 immature female rats. The ovaries of the animals with subcutaneous implants were distinctly larger at 5 days than those receiving intrasplenic or

intraperitoneal implants. The ovaries of animals receiving intrasplenic implants were least developed. Incubation of chorionic gonadotrophins for 1 hour at 37°C with fresh rat liver homogenates resulted in a significant reduction in hormonal activity as tested by increase in size of ovaries and uteri of immature rats. Incubation for 1 hour with heat inactivated liver homogenates, or with saline solutions alone, will effect some inactivation of the chorionic gonadotrophins, but not to the extent resulting from incubation with fresh liver homogenates. The results suggest that the rapid disappearance of gonadotrophins in the organism may be largely due to inactivation in the liver.

Pattern of gas expulsion from human lungs during rapid decompression F G HALL, *Dept of Physiology and Pharmacology, Duke Univ School of Medicine, Durham, N C*

The pattern of gas expulsion from the lungs of each of 6 medical students subjected to rapid decompression from 225 mm Hg pressure to 565 mm Hg in an altitude chamber was recorded by an optical manometer. From the rheopneumograms thus obtained the velocities and volumes of gases expelled were determined for various rates of decompression. Results of these determinations are compared with the residual and reserve air determinations for the same individuals.

Mechanism of magnesium hypothermia in the rabbit V E HALL AND F A ELLIS * *Dept of Physiology, Stanford Univ School of Medicine, Stanford, Calif*

A dose of 25 mm/kg of magnesium chloride given intraperitoneally to unanesthetized rabbits at an environmental temperature of 28°C caused a body temperature fall of 0.32°C. Since the O₂ consumption was not significantly altered, the reduction in body temperature was attributable not to a reduction in heat production, but to increased heat loss by means of the polypnea and cutaneous vasodilatation, which have been found under these conditions by Heagy and Burton and by ourselves. The same dose given at an environmental temperature of 0°C caused a body temperature fall of 1.62°C. The O₂ consumption, which before injection was approximately double that at 28°C, was reduced following MgCl₂ administration by 29.9% (average of 10 experiments). Since we have shown that in cold environments MgCl₂ does not cause either polypnea or cutaneous vasodilatation, the fall in body temperature under these conditions appears to be due largely to a reduction in heat production, attributable to an interference with the nervous mechanism of defense against cold. The significance of these findings relative to the effect of magnesium salts on temperature regulation will be discussed.

Complete individuality of canine blood as the source of an urticaria-producing factor ANGIE S HAMILTON AND BERT MORROW (introduced by J E RHOADS) *Harrison Dept of Surgical Research, Univ of Pennsylvania Schools of Medicine, Philadelphia*

In the absence of erythrocyte incompatibility (HAMILTON *Am J Physiol* 151: 525, 1948) and/or non-specific factors (e.g. particulate matter or coagulant factors), the reaction to the individuality factor (IF) (FREEMAN, N E, AND A E SCHICKEN *Science* 96: 39, 1911) contained in heparinized or citrated plasma and serum is manifested by urticaria in infused recipients and by stinging of a bleb following intradermal injection in the presence of circulating dye (T-1824). Auto-serum fails to produce these phenomena in normal or hypersensitive dogs. A skin assay test for qualitative and semi-quantitative determination of the amount of IF contained in heparinized plasma or serum has been perfected. The test requires circulating dye, otherwise only hypersensitive dogs or so-called 'allergic' humans show qualitative differences in response to auto- and iso sera. Methods of handling blood affect the responses to iso sera. The content of IF is minimized by immediate separation of plasma from cellular elements at 4°C. It is increased by short contact with cellular elements *in vitro* at 37°C. Heparinized plasma gives stronger reactions than citrated plasma. Pooling is ineffectual. The IF is thermolabile, being significantly decreased by heating at 62°C for 30 minutes. Stress conditions in recipients and in donors have been studied. Recipient response to iso-sera is decreased by anesthesia and by severe illnesses. Moderate to severe blood loss immediately preceding blood sampling markedly decreased the responses of other dogs to the serum of the bled donor.

Relation between the size of the heart and the stroke index W F HAMILTON *Dept of Physiology, Univ of Georgia School of Medicine, Augusta*

In a series of dogs the heart size was calculated from the x-ray shadow and the stroke index was derived from the pulse contour. These two variables were compared during hemorrhage, epinephrine infusion, and during the action of acetylcholine, histamine, and other drugs. Balloons on catheters were passed down the vena cava to various sites and inflated. This procedure cut off various fractions of the venous return and tended to diminish the heart size and stroke volume. Increase in aortic pressure tends to decrease stroke volume and to increase heart size whereas increase in venous pressure and diastolic time tend to increase both stroke volume and heart size. Data are being accumulated with it in mind to work out interrelationships among these factors.

Comparative toxicity of chloral hydrate and chloral alcoholate PHILLIP V HAMMOND,* DONALD E STULKEN* AND WILLIAM A HESTAND *Lab of Animal Physiology, Dept of Biological Sciences, Purdue Univ, Lafayette, Ind*

According to popular belief the mixing of chloral hydrate with an alcoholic beverage results in the formation of chloral alcoholate, a product supposedly having greater hypnotic properties than the hydrate, the mixture being known as a 'Mickey Finn'. A comparative study of the toxicities of the two compounds was undertaken by determining their LD_{50} . Wistar rats and mice of the Hygienic and Swiss strains were used, the former being injected intraperitoneally, the latter subcutaneously with varying amounts of 4% solutions. In all experiments a total of 161 mice and 123 rats was used. LD_{50} was calculated by Wright's method. All surviving animals were discarded. In terms of gm/kg dosage in both species the toxicity of chloral hydrate proved greater than that of the alcoholate. The LD_{50} of chloral hydrate intraperitoneally in rats was 0.628 gm/kg, of chloral alcoholate 0.756 gm/kg. The LD_{50} of chloral hydrate subcutaneously in mice was 0.909 gm/kg, of chloral alcoholate 0.945 gm/kg. These results agree in principle with those of Adams who used intragastric administration (ADAMS, W L J *Pharmacol* 78:340, 1943).

Reaction to pain as determined by galvanic skin response JAMES D HARDY AND MANUEL FURER* *Depts of Physiology and Medicine, Cornell Univ Med College, New York City*

A study of the galvanic skin response as a reaction to graded standard pain intensities was carried out on 4 normal subjects. Pains were induced by intense thermal radiation and graded from threshold to 8 dols, in steps of 2 dols. The galvanic skin response was measured with chloridized silver electrodes attached to the palmar surface of the index and middle fingers. Three series of experiments were performed. 1) The subjects, in a relaxed state, were exposed daily over a 6-month period to pains presented in a fixed order of 0, threshold, 2, 4, 6 and 8 dols. The galvanic skin response to each pain was recorded. 2) The subjects were similarly studied in hot and cold environments. 3) Studies were made during and after events calculated to induce anxiety. The following observations were made. 1) In contrast to the uniform accuracy with which the pains were perceived, there were marked variations in the reaction to the pains. 2) In the first series of experiments the pains on daily repetition gradually lost their effectiveness in evoking a reaction. Several weeks were required for complete adaptation of the response and the time required for adaptation was different for each subject. 3) The magnitude of the galvanic skin response increased

in proportion to the intensity of the experimental pains. 4) In the second series of studies exposure of the subjects to very hot (50°C) or very cold (7°C) environments reduced or suppressed the galvanic skin response. 5) In the third series of experiments situations designed to increase the subjects' anxiety increased the galvanic skin response to the pains for a few days, but adaptation to the new situations was rapid. 6) The subjects could not predict the degree of their responsiveness to the pains or correlate it with estimates of their emotional states.

Phases of ectopic ventricular activity following experimental coronary occlusion A SIDNEY HARRIS *Dept of Physiology, Baylor Univ College of Medicine, Houston, Texas*

Ligation of the anterior descending coronary artery of the dog produces ventricular ectopic activity in phases related to time elapsed since occlusion. The first phase begins about 2 minutes and ends about 10 minutes after occlusion. This is a period of increasing frequency of idioventricular complexes and danger of early ventricular fibrillation. This phase can be eliminated by two-stage occlusion. The second phase follows and lasts about 4½ to 8 hours. It is characterized by absence or low frequency (0-5 per minute) of ectopic beats, with exceptions related to the anesthetic. Following ligation under pentothal sodium, ectopic frequencies up to 50 per minute have appeared 2½ to 4½ hours after occlusion. Following pentobarbital sodium significant ectopic frequencies have appeared 3½ to 4½ hours after occlusion. Following ether no animal out of 4 operated has exhibited increased ectopic frequencies earlier than 4 hours after occlusion and only one earlier than 4½ hours. Following morphine-barbital sodium occlusions and following pentobarbital sodium occlusions combined with upper thoracic sympathectomy no increased ectopic activity has occurred during the first 4½ hours. Second phase ectopic impulses are suppressed by barbiturate administered after they develop. The third phase develops most intense ectopic ventricular activity. Following its onset 4½ to 8 hours after occlusion the ectopic frequency increases rapidly to high levels. This activity follows occlusions under all anesthetics and after upper thoracic sympathectomy. It can develop soon after the administration of barbiturate, and persists if barbiturate is given after they develop.

Suppression of ventricular ectopic impulses accompanying acute myocardial infarction by diphenylhydantoin sodium and barbiturates A SIDNEY HARRIS AND ROBERT H KOKERNOT* *Dept of Physiology, Baylor Univ College of Medicine, Houston, Texas*

Experimental occlusion of the anterior descending artery of the dog's heart produces ectopic

ventricular tachycardia after a latency of $1\frac{1}{2}$ to 8 hours. This coincides with the duration of ischemia required to produce histological signs of necrosis. There is evidence that ectopic impulses originate in the boundary of the infarct. It is probable that some product of necrosis acting within the hyperexcitable boundary causes the discharges. In focal epilepsy the seizures are initiated by nerve cell discharges arising in the boundary of the brain lesion, and excised epileptogenic tissues are found to contain areas of acute necrosis. Because of these apparent similarities, drugs that prevent focal seizures are being tried as possible suppressors of ventricular ectopic rhythms. Diphenylhydantoin sodium (dilantin) given to unanesthetized dogs with ectopic ventricular tachycardia at its maximal intensity causes cessation of ectopic beats for a variable period and greatly diminishes ectopic frequency many hours if 200 mg/kg is given in divided doses within 3 or 4 hours. Later, with less intense ectopic drive smaller amounts suffice. In some animals effective doses of dilantin during the intense stage produce involuntary muscular movements, but not in all. Phenobarbital sodium, up to 60 mg/kg, fails to control ectopic activity when intense, but potentiates dilantin. After phenobarbital sodium, dilantin, 10 to 25 mg/kg, has produced lasting restoration of normal rhythm. Pentobarbital sodium potentiates dilantin also. Barbiturates increase the toxicity of dilantin, but the potentiation of ectopic impulse suppression offsets the increased toxicity.

Hypothalamic control of the secretion of adrenocorticotrophic hormone GEOFFREY W. HARRIS AND J. DE GROOT (introduced by ROBERT S. MORISON) *Physiological Lab, Cambridge, England*

In previous work (with the collaboration of Dr H. F. Colfer) it has been shown that emotional stress in rabbits is followed by a marked drop in the absolute number of blood lymphocytes, and that this response is abolished by hypophysectomy. In order to investigate the pathway between the brain and adeno-hypophysis which underlies this lymphopenic response, different regions of the hypothalamus and hypophysis have been electrically stimulated by the remote control method in unanesthetized, unrestrained rabbits. It was found that stimulation of the posterior region of the tuber cinereum or mammillary body resulted in a lymphopenia which was similar in time relations and magnitude to that following an emotional stress stimulus. Stimulation of other regions of the hypothalamus or hypophysis did not elicit the response. The lymphopenic response which followed an emotional stress stimulus in normal rabbits was abolished by lesions in the zona tuberalis (2 cases), and, in most cases, abolished or

diminished by transverse lesions in the posterior region of the tuber cinereum or in the mammillary body. Similar lesions in the posterior part of the pars distalis, pars intermedia, or lesions which interrupt the infundibular stem, were compatible with normal responses. The conclusion is drawn that anterior pituitary secretion of the adrenocorticotrophic hormone is under neural control via the hypothalamus and the hypophysial portal vessels.

Secretion of fat factor of the adrenal FRANK A. HARTMAN AND T. Y. LIU * *Ohio State Univ., Columbus*

Blood from the left adrenal of heparinized nembutalized dogs was assayed for the fat factor. Blood removed was replaced by blood from a second dog. The hormone was extracted from the plasma by means of ethylene chloride. It was assayed on adrenalectomized starved mice. Six of 14 dogs secreted very little fat factor. In the dogs that were secreting appreciable amounts of the hormone there was no change produced during 4-6 hours of the experiment. Injections of ACTH which produced marked increase in gluconeogenic hormone were ineffective on fat factor secretion.

Inability of desoxycorticosterone acetate to prevent the sodium excretion produced by mannitol diuresis R. B. HARVEY,* D. H. SIMMONS,* T. HOSHINO* AND M. B. VISSCHER *Dept of Physiology, Univ of Minnesota, Minneapolis*

Recent work of Pitts and others suggests that humoral mechanisms may play a role in the increased rate of sodium excretion occurring during mannitol diuresis. In our laboratory unanesthetized dogs were subjected to osmotic diuresis with 1.32 molar mannitol given intravenously at the rate of 0.2 cc/lb/min after having been on salt-poor (rice) diets for one to two weeks. Under similar conditions, but with administration of 1 mg/lb/day of desoxycorticosterone acetate (DCA) in sesame oil subcutaneously for 6 to 8 days previously, the experiments were repeated on the same animals. Determinations were made of urine and serum sodium concentrations, rate of urine flow, urine and serum total osmotic pressures (Hill-Baldes vapor tension method), and creatinine clearances before and after the injection of mannitol. It was found that the injection of mannitol increased the rate of sodium excretion, either in terms of moles/lb/min or as tubular rejection fraction of sodium, 50 to 300 times over control values. It was also observed that urine flow was increased up to 50% of glomerular filtration rate. The plasma sodium level fell an average of 17 mmol/liter yet the serum total osmotic pressure rose by an amount equivalent to as much as 30 mEq of sodium chloride per liter. None of the variables measured was significantly altered after the administration of DCA.

Radiophosphorus in mosquitoes C C HASSETT
(introduced by W H CHAMBERS) *Medical Div, Army Chemical Center, Md*

Data are presented showing paths of absorption of P^{32} and its distribution in the tissues of *Aedes aegypti*, as studied by radioautographs and by Geiger-tube counting technique

Mechanism of fibrous pepsin-albumin digestion
TERU HAYASHI AND GEORGE A EDISON *Dept of Zoology, Columbia Univ, New York City*

A mixture of pepsin and albumin buffered at pH 4.4 (mixture pH) when placed dropwise on the air-water interface of a Langmuir trough buffered at pH 4.0 (trough pH) forms a monomolecular film at several dynes pressure. Compression of the film forms a fiber which, when immersed in HCl at pH 1.5, undergoes autodigestion. No autodigestion occurs if the pepsin and albumin are placed separately on the same air-water interface. They must be in mixture prior to the formation of the film, showing that 1) the autodigestion is due to the fibrous pepsin rather than contaminating native pepsin and 2) that an interaction between pepsin and albumin occurs at the mixture pH of 4.4, this interaction being a necessary part of the enzymatic action of pepsin. Mixture pH's of 5.0, 6.0 and 6.6 are found to be progressively less effective in promoting autodigestion. A mixture pH of 7.2 shows no autodigestion. The interaction between pepsin and albumin may be shown by titration with saturated ammonium sulfate. The precipitate thus formed is measured photometrically by the amount of non-scattered light at 420 m μ passing through the suspension. The results show that at mixture pH's of 4.4, 5.0, 6.0, a complex of pepsin and albumin is formed, but that at pH 7.2, no complex formation occurs. It is concluded that 1) the autodigestible albumin-pepsin fiber is a stabilized enzyme-substrate complex and 2) the formation of enzyme-substrate complex is dependent on the establishment of linkages, probably salt linkages, between the pepsin and albumin.

Comparison of hemodynamics in pulmonary and systemic circulations in various diseases
FLORENCE W HAYNES AND LEWIS DEXTER*
Med Clinic, Peter Bent Brigham Hospital, and Dept of Medicine, Harvard Med School, Boston, Mass

By means of venous catheterization, pressures in the right auricle, right ventricle, pulmonary artery (PA), pulmonary 'capillaries' ('PC') and brachial artery have been recorded, as previously described, in normals and in patients with pulmonary hypertension (HT). A graph of progressive pressure changes through the lesser circulation compared with those accepted for the

systemic circulation emphasizes the following points. In normals the gradient between mean PA and 'PC' pressures is 4-10 mm Hg and the 'PC' pressure approximates the PA diastolic pressure. In contrast, the arterial-capillary gradient in the systemic circulation is many times that in the lung. In patients with pulmonary HT and normal 'PC' pressure, as in pulmonary vascular disease, the PA-'PC' gradient may reach 40 mm Hg and the PA diastolic pressure is usually well above the 'PC' pressure. This HT in the pulmonary circuit is in some respects analogous to arterial HT in the systemic circuit. In Eisenmenger's complex the gradient may equal that on the systemic side. In patients with elevated 'PC' pressure, as in mitral stenosis and left ventricular failure, the mean PA pressure is elevated either with a normal PA-'PC' gradient or, if the 'PC' pressure is excessive, with a widened gradient. This association of PA and 'PC' pressure elevations has no analogy of comparable degree in the systemic circulation. Since in none of the cases discussed has the pulmonary blood flow been increased above normal, an increased PA-'PC' gradient represents increased resistance. In systemic HT without cardiac failure, normal PA and 'PC' pressures exist.

Characterization of corticosteroids released from perfused cow adrenals OSCAR HECHTER *Worcester Foundation for Experimental Biology, Shrewsbury, Mass*

Previously, it was shown that perfused bovine adrenals respond to the addition of ACTH by a marked increase in the release and biosynthesis of corticosteroids (HECHTER, *Federation Proc* 8:70, 1949). In this study, the corticosteroids secreted by perfused preparations, in the absence and presence of ACTH, have been fractionated in a manner designed to estimate the number and character of the corticosteroids released. Adrenal perfusate extracts (HECHTER *et al*, *Arch Biochem*, in press) were fractionated by chromatography on silica gel. Under standardized conditions, the polarity of solvents requisite to elute reference steroids was determined by following the formaldehydrogenic steroid (FS) content of successive eluates. Desoxycorticosterone was removed from columns by benzene-ether mixtures (1:1-1:2), but not by benzene, whereas corticosterone was eluted by ether-ethyl acetate mixtures (1:1), but insignificantly by ether. In adrenal perfusates, wherein ACTH was not employed, two peaks of FS were observed in the benzene-ether eluate range. From color tests, it appears that one of these peaks may represent desoxycorticosterone. There is no or only a slight peak of FS in the ethyl acetate eluate range in such perfusates. In

preparations where ACTH was employed, in addition to the peaks previously described there was, in 2 or 3 experiments, the appearance of a new high peak of FS in the fractions eluted by ethyl acetate ether mixtures. This material released by ACTH has in one case been crystallized and appears to be 17-(α)-hydroxycorticosterone, as evaluated by mixed melting points and appropriate color tests.

Studies on biological and photochemical action of ultraviolet radiation at low temperatures F HEINMETS AND W W TAYLOR (introduced by ORR E REYNOLDS) *Depts of Biophysics and Bacteriology, Naval Med Field Research Lab, Camp Lejeune, N C*

There is a large number of studies concerning the fundamental mechanism of the action of the ultraviolet radiation on biological materials and chemical substances. For the liquid medium, it is difficult to differentiate between the primary and the secondary processes during the irradiation. This paper deals with studies of ultraviolet radiation on *B. action Coli* bacteria in the frozen state. The rate of inactivation depending on various experimental parameters has been determined. The photo reactivation of bacteria, which had been subjected to ultraviolet irradiation at low temperature, has also been studied. Various factors, such as temperatures, storing time after irradiation, etc. influencing the photo reactivation rate, have been investigated. At the same time, the photo-chemical decomposition of the tyrosine and alcohol soluble protein Zein has been investigated. As a decomposition measure, the changes of the absorption characteristics of the solution under study, have been used. There is evidence, that in the frozen state, the secondary chemical reactions are inhibited. This is revealed in the changes in the absorption characteristics of protein or amino acid solution, when compared with the experiments in the liquid phase. An attempt will be made to compare the photo-decomposition data with the biological inactivation results.

Vacuum Spark discharge with liquid-jet electrode as a radiation energy source for photochemical reactions and biological inactivations F HEINMETS AND W W TAYLOR (introduced by ORR E REYNOLDS) *Depts of Biophysics and Bacteriology, Naval Med Field Research Lab, Camp Lejeune, N C*

The Vacuum Spark has been used in the field of spectroscopy as a source of high energy photons. An experimental arrangement for studies of photochemical reactions and biological inactivations, by the vacuum spark discharge with one liquid-jet electrode, is described and the general instrumental set-up presented. The decomposition of

tyrosine solution is used to demonstrate the photochemical action of the vacuum spark discharge. The influence of various experimental factors, such as gas pressure, electrode potential, velocity of jet flow, etc. on the rate of tyrosine decomposition has been studied, and experimental data will be presented. The action of the vacuum discharge spark on various bacteria has been studied, and in certain experimental conditions, complete inactivation of the bacteria was obtained. Further, the influence of various experimental parameters on the inactivation rate of bacteria will be discussed. Experimental results of the bacterial inactivation and the photochemical decomposition of tyrosine will be compared and analyzed. Furthermore, the vacuum spark discharge and ultraviolet irradiation action on tyrosine solution have been studied on a comparative basis, and the resulting changes in the ultraviolet absorption characteristic analyzed for both cases.

A quantitative method for measuring pulmonary edema in the extirpated lungs of guinea pigs ALLAN HEMINGWAY *Dept of Physiology, Univ of Minnesota, Minneapolis*

The present methods now used in determining pulmonary edema usually consists of a gross examination of the extirpated lung and a visual inspection to determine qualitatively the presence of congested areas, petechia, fluid in the bronchi, the texture of the lung and its general color. The sole quantitative determination has consisted of a measurement of the lung weight. In order to obtain more quantitative information on determining the degree of pulmonary edema investigations have been made on chemical analytical methods for determination of those changes which have occurred as a result of the edema. A method has been devised whereby guinea pig lung is divided into four fractions: 1) insoluble protein nitrogen, the nitrogen remaining in the insoluble residue after grinding with sand and extracting with one per cent NaCl solution, 2) the pulmonary hemoglobin, determined spectrophotometrically from an aliquot of the saline extract but using a special extraction procedure for elimination of turbidity due to lipids, 3) the soluble protein fraction consisting of soluble protein nitrogen, other than hemoglobin nitrogen, in the saline extract, and 4) non-protein nitrogen of the saline extract determined by soluble nitrogen remaining after protein precipitation with tungstic acid. In addition a method has been devised for measuring density and volume of the extirpated lung. Normal values with their biological variations have been established on a series of guinea pigs.

Factors influencing venous pressure in foot of erect human during quiescence J P HEARY,

O H GAUER* AND E E MARTIN * *Aero Med Lab, Air Materiel Command, Wright-Patterson Air Force Base, Dayton, Ohio*

Three subjects were submitted to 6 series of tilt table experiments in an air-conditioned room adjusted to induce vasomotor responses ranging from sweating to shivering states. The rate of increase of venous pressure in the foot after tilting from the horizontal to upright posture depended upon the degree of vasodilation. When hot the pressure attained 80% of the full potential value of approximately 100 mm Hg in less than 20 seconds, when comfortable, this required 90 seconds and, when cool, 3 minutes after tilting the pressure was still less than 50% of the full value. Tests during quiet standing showed that normal postural activity reduced the mean venous pressure when cool and when comfortable to approximately 55 and 65 mm Hg, respectively. When sweating, these movements were insufficient to sustain a low pressure and the mean level became 90 mm Hg. This pressure exceeds by 30 mm Hg any probable counter-balancing combination of osmotic and tissue pressures. The rhythmic inflation of counter-pressure clothing (anti-g suits) can be substituted for the massaging effect of the muscles on the vessels. Thus during standing-walking, when hot, the mean pressure in 9 subjects was 67% and when cool, 41% of the resting value. When seated and using the suit, the corresponding values were 72% and 47%. It is suggested that such clothing might be employed to aid circulation in the lower extremities in certain clinical conditions.

Maximal oxygen intake and renal plasma flow in patients with aortic insufficiency AUSTIN HENSCHEL, THOMAS B GIBBONS* AND CARLETON B CHAPMAN * *Lab of Physiological Hygiene, Univ of Minnesota, Minneapolis*

The maximal oxygen intake and renal plasma flow were measured in 8 normal young men and 7 male patients of similar age, physique and exercise habits, who had mild to moderate aortic insufficiency due to rheumatic fever. Renal plasma flow was measured using a constant injection of para-aminohippurate at rest, during two 16-minute periods of walking at 3 miles per hour on a 10% grade, and at supine rest for 40 minutes following the work. Maximal oxygen intake was determined from 1 minute 45 seconds to 2 minutes 45 seconds of a 3-minute run at 6 miles per hour on a grade adjusted to the performance capacity of each subject. The maximal oxygen intake was 44.03 cc/kg/min and 37.43 cc/kg/min for the normal and cardiac subjects, respectively. The 15% difference in maximal oxygen intake for the 2 groups of subjects is statistically highly significant. The mean resting renal plasma flow cor-

rected to 1.73 sq meters of body surface was 495 and 576 cc/min in the patients and normal subjects, respectively, the difference is statistically significant. During exercise the renal plasma flow was decreased by 25% of the resting value for each group. Forty minutes after the cessation of the exercise the renal plasma flow had returned to the pre-exercise resting level in most of the normal and cardiac subjects.

Dielectric properties of tissues important in microwave diathermy J F HERRICK, D G JELATIS* AND G M LEE * *Mayo Foundation, Rochester, Minn*

Microwave diathermy has become an integral part of medical diathermy since it was approved by the Council of Physical Medicine and Rehabilitation of the American Medical Association. This relatively new type of diathermy has proven to be an efficient method for heating certain localized areas of the body. The ultimate objective in studying the dielectric properties of animal tissues was to understand the heating of these tissues by microwaves. We wished to explain, if possible, the experimentally observed temperature distribution produced in the tissues by microwaves. The amount of heat developed in biological tissues by microwave diathermy is dependent on the dielectric properties of the tissues. Since these properties vary with frequency, it is desirable to measure them at the actual frequencies used for microwave diathermy. Observed values are presented for the dielectric constant and loss of freshly excised samples of such tissues as liver, muscle, fat, bone and bone marrow. The dielectric properties of certain body fluids will also be presented. The design of a 'transformer' for increasing the transfer of microwave power into a particular tissue such as muscle is given as an example of the utility of the dielectric data. The equipment used for measuring dielectric constant and loss was the microwave dielectrometer which was designed and constructed by one of us.

Simultaneous partitional calorimetry and estimation of blood flows in main skin regions of the body ALRICK B HERTZMAN AND WALTER C RANDALL *Dept of Physiology, St Louis Univ School of Medicine, St Louis, Mo*

Cutaneous evaporative, radiative and convective heat losses and blood flows were estimated repeatedly and approximately simultaneously in the forehead, cheek, chest, abdomen, arm, forearm, thigh, calf, hand and foot of nude subjects in the operative temperature range of 27-37°C over an observational period of 4 hours. Although each region exhibited variations in the rate of heat loss during an observational period, comparison of the mean values for the total 4-hour heat loss from each region indicates a tendency towards

constancy in each area with the exception of the forehead (heat loss rises) and arm (heat loss falls) as the operative temperature rises. Scatter in the individual data appear with the onset of sweating, increases with the rise in temperature and correlates with the sudomotor patterns of individual subjects. The simultaneous estimations of the cutaneous blood flows from the skin pulses and also from the thermal data generally agree with respect to the onset and development of the cutaneous dilatations in all areas. Skin pulse estimates of blood flow (calculated from the flow equivalent of the finger pad pulse) were usually about 40% higher than the thermal estimates in all areas other than the forehead and dorsum of the hand and foot. The three latter areas exhibited large discrepancies in flow estimates. Both criteria agree on the existence of high flows in head skin, palm and sole of foot as may be predicted from vessel counts.

Energy cost of flying multi-engined aircraft FRED A HITCHCOCK *Med Research Lab, Civil Aeronautics Admin, Aeronautical Center, Oklahoma City, Okla*

As the first phase of an investigation of the factors involved in pilot fatigue, the respiratory metabolism and energy output of pilots while flying multi-engined aircraft (DC-4) has been measured. The subjects were all experienced pilots who were enrolled in refresher courses at the Aeronautical Center. The procedure followed was as follows. The subject rested for 20 minutes on a bunk located just to the rear of the pilot's compartment. Then expired air was collected for 10 minutes by means of an A-14 mask and Douglas Bag. During this part of the experiment the plane was in the air with another student pilot at the controls. After being in the air for about one hour this student landed the ship and the pilot who was serving as subject for the day took over the controls. After he had had the ship in the air for about 10 minutes expired air was collected for a second period of 10 minutes while he executed various maneuvers with the aircraft. The volume of expired air was measured with a calibrated dry test meter. Samples were collected over mercury and analyzed by means of the Haldane Air Analysis apparatus. The ventilation volume, O_2 consumption, CO_2 production, total calories and calories per sq m of body surface were then calculated, a) while the subject was resting and b) while he was flying the aircraft. The average for 10 such tests show that the caloric output while flying the plane is about twice that while resting.

Facilitating effect of local strychninization on pressor responses evoked from cerebral cortex E C HOFF, H G LANGFORD,* J W VESTER,* W W BECKNER* AND P R THOMAS * *Neuro-*

logical Science Lab of Med College of Virginia, Richmond

Hoff and Green (*Am J Physiol* 117 411, 1936) reported that stimulation of certain areas of the frontal lobe results in transient elevations of blood pressure. From the cat's cortex posterior to this pressor area as far back as the sylvian fissure, blood pressure falls have been elicited. These alterations in pressure are regularly reproducible and under standardized conditions exhibit constant latency, amplitude, and duration. In the present experiments, the influence of local strychninization upon the excitability of the pressor areas has been examined in cats. In control animals, weak faradic stimulation of symmetrical foci on the left or right anterior sigmoid gyrus evoked rises of blood pressure of 20-40 mm Hg, enduring 15-20 seconds. Four to 6 minutes after local application of 3% aqueous solution of strychnine sulfate to the excitable focus on the left or right anterior sigmoid gyrus, stimulation of the strychninized focus or the symmetrical contralateral focus gave greatly enhanced pressor responses. These blood pressure rises after local strychninization were of the order of 80-150 mm Hg above the baseline, the arterial tension subsiding in 4-20 minutes. This facilitation of blood pressure responses from the pressor area of the feline cortex is derived from the localized action of strychnine at the application site, or may be due to more generalized effects upon the excitability of the CNS as a result of absorption into the blood stream. Experiments are in progress to determine this point.

Effect of oxygen on response of living cells to X-rays with special emphasis on bacteria ALEXANDER HOLLAENDER, G E STAPLETON* AND F L MARTIN * *Biology Division, Oak Ridge Natl Lab, Oak Ridge, Tenn*

Suspensions of bacteria saturated with air are considerably more resistant to x rays if nutrient broth is used as a medium than when irradiations are done in physiological salt solution or phosphate buffer. If air is replaced by N_2 , the resistance of the bacteria increases manyfold, there being little difference if they are suspended in broth or salt solution. There is an increased response, with decrease of number, of bacteria in the suspension at low concentration ($<10^5$ bac/cc) as well as a decreased response with an increase of concentration of the broth. Five times as many roentgens are necessary to produce an equivalent killing effect when irradiations are done in the absence of oxygen than when O_2 is present. At 60,000 r the survival is 10,000- to 50,000-fold greater in N_2 than in O_2 . Similar but less pronounced effects were observed with fungi (*Aspergillus terreus*). There is very little, if any, effect at the energy

values used here if bacteria or fungi are added to the suspension after irradiation of the medium. In all experiments great care must be taken that the suspensions are well saturated with the gas under investigation. The moderate decrease of sensitivity of living cells to x rays by reducing the supply of air has been reported in a few scattered publications. Striking reduction in percentage of chromosome deletions in *Tradescantia* (GILES AND RILEY) and percentage of sex-linked lethals in *Drosophila* (BAKER AND SGOURAKIS) if air is replaced by nitrogen during irradiation, has been described from this laboratory.

Appetite studies in a patient with complete esophageal obstruction and jejunostomy FRANKLIN HOLLANDER AND HERBERT A. SOBER * *Gastroenterology Research Lab, Mount Sinai Hospital, New York City*

The subject was an ambulatory male, 18 years old, with cardio esophageal obstruction and jejunostomy for alimentation. He was fed our pre-digested aliment as the sole source of nutrition. Food was ingested orally, as prompted by appetite, but it never passed the esophageal obstruction and was regurgitated shortly after ingestion. Hence, the influence of daily intake of jejunal aliment upon body weight and appetite, measured by calories ingested orally, could be studied. The investigation of this and other phenomena lasted for about 15 years, during which time the subject was employed in the Laboratory specifically for this purpose. Three sets of observations, each lasting about one month, indicated that appetite is inversely—though not precisely—related to nutritional intake. Subsequent repetitions of these experiments, however, failed to confirm this, i.e. oral intake varied during constant jejunal intake, and sometimes the two were correlated directly. The latter was encountered during periods of overt emotional upset, not manifested during the earlier part of the study. In conclusion: 1) there is evidence that appetite diminishes as nutritional intake increases, even when no food can enter the stomach, 2) this relation may be upset and even reversed by emotional disturbances, 3) generalized inferences regarding physiological reactions of man, from observations on a single individual, must derive from extensive repetition of the experiments, and should preferably be held in abeyance until confirmed on other subjects. These precautions are particularly important for work with patients possessing gastrointestinal fistulae, because of the profound emotional disturbances frequently encountered in such cases.

Metabolic response to adrenaline of pre- and post-operated patients J. W. HOLLER, * A. DURY, R. BURTON, * E. H. KEUTMANN * AND C. SMITH *

Dorn Lab Med Research, Bradford Hospital, Bradford, Penna., and Depts. of Medicine and Surgery, Univ. of Rochester School of Medicine, Rochester, N. Y.

The effect of a single intramuscular injection of 0.4 ml. aq. adrenaline (1:1000) upon the plasma potassium, glucose, absolute eosinophiles and other blood constituents was measured in patients before and 3 days post-operative. The surgeries were of the type classed as minor operations. The percentage change in these blood constituents from the overnight-fast level was calculated for the periods of 4, 20, 60, and 120 minutes after an injection of adrenaline. The mean change levels in the surgical patients after adrenaline were compared with those calculated for normal adults and Addisonian patients after a like injection of adrenaline. The eosinophile response in the pre-operated group was similar to the normal adults 60 and 120 minutes after adrenaline. After surgery, the eosinophiles were unaffected by the adrenaline stimulus. The mean change in the blood sugar of the pre-operated and normal adult groups were comparable 20 and 60 minutes after adrenaline. The pattern of change after adrenaline in the blood sugar post-operative was similar to the Addisonians and of much lesser extent than the normals. The mean change in plasma potassium in the pre-operated patients was similar to, but of lesser extent, than in the Addisonians after the adrenaline injection in all the time periods. The mean changes in plasma potassium in the post-operated group after adrenaline were slightly above the preinjection level in all the time periods and suggest very little response to the adrenaline stimulus. These data indicate minor surgery can affect glucose and potassium metabolism, and the responsiveness of the adrenal (eosinophile decrease) to adrenaline stimulus to an extent comparable to that observed in Addisonian patients.

Induction of convulsions in rats STEVEN M. HORVATH AND G. M. AUSTIN * *Univ. of Pennsylvania, Philadelphia, and State Univ. of Iowa, Iowa City*

This paper deals with the effects of continuous radar pulsations at short distances on a group of 47 rats. The dorsal surface of the head was exposed to a wave length of 12.2 cm. for varying periods of time. Sixteen rats were exposed to a 60-watt energy output at 2.5 cm. distance, and 25 rats exposed to a 90-watt output at zero distance from the skull. In 6 rats the director was focused over the spinal cord. Those rats exposed to a 60-watt output showed clonic type convulsions in a mean time of 2.8 minutes. These rats had an average increase in rectal temperature of 4.2°F. Those exposed to 90-watt output convulsed in a mean time of 1.2 minutes. There was no generalized

hyperthermia in these rats. Brain temperature measured in 8 rats showed an average temperature of 110.4°F immediately after onset of convulsions, although in those exposed to 90 watts there was no associated increase in rectal or thigh muscle temperature. No pathological changes were found in any of the brains of sacrificed rats severe enough to explain the convulsions. Mild pyknosis, in the Purkinje cells of the cerebellum, in the Pyramidal layer of Ammon's Horn, and in the third layer of the cerebral cortex were the only findings outside of moderate congestion. It is suggested that a specific rise in the temperature of the brain itself is the most important factor in these convulsive attacks.

Possible renal vascular shunt in dogs during intravenous epinephrine C RILEY HOUCK *Division of Physiology, Univ. of Tennessee, Memphis*

Physiologic evidence for a shunting of blood from the cortex to the medulla of the dog kidney, 'Trueta shunt,' during the injection of epinephrine hydrochloride was sought using the clearance, tubular saturation and extraction techniques (renal vein catheterization via jugular vein), simultaneously wherever possible. In 14 dogs under Nembutal, doses of epinephrine from 21 to 81 γ /kg were given intravenously over a period of from 8 to 20 minutes. Mean blood pressure, renal resistance, renal extraction and consumption of oxygen and the hemoconcentration of renal vein blood were substantially increased to a degree proportional to the dose. Filtration rate, urine flow and renal blood flow were decreased to a degree proportional to the dose. The Tm of glucose was normal. The Tm of PAH at low doses was normal but 70% reduced at high doses. The filtration fraction (creatinine extraction) was elevated at low, depressed moderately at intermediate, and increased at high doses. Extraction of PAH was normal at doses from 21 to 67 but decreased 30% at 81 γ /kg. Since extractions of PAH, creatinine and oxygen, and the Tm's of glucose and PAH were not significantly decreased by moderate doses of epinephrine in the presence of a moderate to marked decrease in glomerular filtration rate, urine flow and renal blood flow, no appreciable shunting of blood from the kidney cortex to the medulla was indicated. Such an interpretation was less certain with larger doses.

A carbonyl reaction differentiating the fetal zona reticularis of the human adrenal cortex from the mouse X zone EVELYN HOWARD AND R. S. BENUA * *Dept. of Physiology, Johns Hopkins Univ. School of Medicine, Baltimore, Md.*
The involution of the X zone of the mouse adrenal cortex is a process which occurs after sexual maturity in females and earlier in males.

It has been suggested that the mouse X zone might be comparable to the human fetal reticularis primarily because its degeneration constitutes a spontaneous regression of a large part of the inner portion of the cortex, occurring apparently as a part of the normal maturation of the gland. The application of the histochemical technique of Ashbel and Seligman for the detection of lipoidal carbonyl groups has now revealed a clear-cut differentiation between these zones in mouse and human adrenals, in that the human fetal reticularis is strongly positive for the presence of lipoidal carbonyl groups, whereas the mouse X zone gives no indication of the presence of appreciable amounts of these compounds. This test substantiates histogenetic indications of differences between these adrenal zones. It is concluded that there can be no simple homology between the mouse X zone and the human fetal reticularis. The facts that the mouse X zone does not reveal free ketosteroid, and that it occurs as an independently proliferating part of the cortex, direct attention to the possibility that reticularis cells without free ketosteroid may have a special function, and are not merely waiting for senescence under all circumstances. The X zone has been further characterized in that it has been found to be markedly suppressed by a low protein diet on which body growth is reduced.

Effect of desoxycorticosterone and pitressin on the water balance of amphibia KUANG-MEI HSIEH (introduced by ROBERT GAUNT) *Dept. of Zoology, Syracuse Univ., Syracuse, N. Y.*

It has been demonstrated (Brunn) that frogs injected with posterior pituitary extracts show a temporary gain in body weight when in suitable environments due to the uptake of water. We have attempted to determine whether this action of posterior pituitary extracts in amphibians can be antagonized by administration of adrenal cortical steroids as can the effect of such extracts on water excretion in mammals. The uptake of water by *Rana pipiens* injected with 0.5 units of posterior pituitary extract (pitressin) has been confirmed. Three hours after injection control frogs had lost 0.17 gm while those receiving pitressin had gained 4.02 gm. Frogs injected with 0.1 or 0.2 mg of DCA showed no significant weight change when compared with untreated controls (untreated = -1.83 gm, DCA-treated = -0.42 gm). One ml of adrenal cortical extract (Upjohn) also was without effect. When frogs were injected with 0.2 mg DCA and 0.5 units of pitressin together there was a questionable potentiation of the action of pitressin by DCA. In order to determine the effect of pitressin and the adrenal steroids on the rate of water excretion, frogs were given distilled water (0.5 ml/10 gm body weight) by stomach tube and the

urine excreted was measured over a period of 9 hours Pitressin (0.5 units) caused a temporary inhibition of urine flow which lasted for less than 3 hours. Both DCA (0.2 mg) and cortical extract (1.0 ml) caused an inhibition of urine excretion which lasted over the 9 hour period of observation. When DCA and pitressin were given together there were no additive effects and the duration of action of DCA appeared to be reduced. It is apparent that the effect of these hormones in frogs differs markedly from that seen in mammals.

Blood volume studies at 5,280 feet altitude DAVID M. HUEY* AND JOSEPH H. HOLMES *Fitzsimmons General Hospital and Dept. of Medicine, Univ. of Colorado, Denver*

In view of the higher hematocrit values at this altitude (5,280 feet) studies of blood and plasma volumes were undertaken in 3 different groups. The first group a) comprised 50 active duty soldiers stationed at Fitzsimmons General Hospital (average age 27), the second group b) 32 male medical students (average age 28), and the third group c) 22 female hospital workers (average age 25). For the soldiers the plasma volume was 41.8 cc/kg, the blood volume was 80.7 cc/kg, and the hematocrit was 50.0%. For the medical students the plasma volume was 39.8 cc/kg, the blood volume was 77.0 cc/kg, and the hematocrit was 49.5%. For the female hospital workers the plasma volume was 41.4 cc/kg, the blood volume was 71.0 cc/kg, and the hematocrit was 44.1%. When compared with average values obtained by Gregersen and co-workers near sea level on 53 normal male medical students the effect of altitude would appear to be an increase in the percentage cell volume and hematocrit values and a decrease in the plasma volume. Values obtained for serum protein concentration and circulating protein per unit body weight were not significantly different from the values obtained by Gregersen near sea level.

Factors influencing active ion uptake by the isolated frog skin ERNST G. HUF *Dept. of Physiology, Medical College of Virginia, Richmond*

The net uptake of Cl^- , Na^+ , H_2O from Ringer's solution (R) by the surviving frog skin has been studied using skin bags. The bags (epithelium inside) were filled with 5 ml of R and immersed for 12 hrs in 2.5 l of R of the same concentration (*Federation Proc.* 8:78, 1949). Cl^- and Na^+ were determined iodometrically and flame photometrically respectively. After 12 hours amount and ion concentration of the fluid in the bag had diminished. The changes were dependent on salt concentration, pH and temperature of the R. The net uptake of ions and H_2O from 0.4 R (48×10^{-3} mEq Cl^- or Na^+ /ml) was only slightly less than the uptake from 1.0 R (120×10^{-3} mEq/ml).

The Cl^- and Na^+ gradients across the skin after 12 hours were higher in experiments with 0.4 R than in experiments with 1.0 R. Acid reaction of the R or lowering its temperature (3) decreased the net uptake of ions and H_2O from 0.4 and 1.0 R. In the majority of experiments more Na^+ than Cl^- was absorbed by the epithelium. The apparent Cl^- excess (Na^+ deficit) in the bag increased with increasing temperature. The solution touching the epithelium became more acid than the solution touching the chorion side of the skin. It can be calculated from QO_2 that enough acid metabolites are produced to combine with the apparently unbalanced amounts of Na^+ taken from and of Cl^- left over in the solution in the bag.

Muscle spindle excitation by efferent fibers in cat CARLTON C. HUNT* AND STEPHEN W. KUFFLER *Wilmer Institute, Johns Hopkins Med. School, Baltimore, Md*

Stimulation of small diameter (3-8 μ) nerve fibers which comprise 25-30% of the lumbosacral ventral root outflow in cat does not cause increased muscle tension. These nerve fibers influence the discharge from stretch receptors in muscle spindles, presumably by excitation of the muscle elements within the spindles, the intrafusal muscle fibers (Leksell, Kuffler and Hunt). The cat's small-nerve system therefore differs from that found in the frog where motor nerves of about 5 μ cause considerable tension by setting up localized contractions around the neuromuscular junctions (Kuffler and Gerard). The small-nerve effect on the afferent discharge from spindles was detected in various ways, particularly by stimulating individual efferent nerve fibers in the ventral roots while recording the discharges from a spindle in an appropriate single afferent nerve fiber in the dorsal root. When an efferent small-nerve fiber is stimulated repetitively (50-120/sec), it can produce in a single afferent fiber a response rate which, after a short period of facilitation, follows the stimulating rate. This 'driving' of the spindle discharge depends to a large extent on the external stretch applied to the muscle and not all units can be driven in such a manner. The efferent spindle response can be maintained by small-nerve excitation for long periods. A similar driving can be obtained by applying mechanical oscillations to the tendon or body of the muscle and certain stretch receptors can 'follow' a tuning fork vibration at 50-200/sec if the muscle is under appropriate external stretch. It is suggested that in both instances of driving the mechanism of excitation is similar. One may assume that the intrafusal muscle fibers, when stimulated through their nerves, can stretch and thus excite the sensory nerve endings within a spindle. This excitation is superimposed on that

already provided by a given external stretch. The most reasonable explanation of the above phenomenon appears to be that the intrafusal fibers in the spindles can respond with distinct tension increments to each small-nerve impulse at relatively high frequencies.

Development and loss of resistance to cell division blocking action of nitrogen mustards JOHN O. HUTCHENS AND BETTY PODOLSKY * *Dept. of Physiology and the Toxicity Lab, Univ. of Chicago, Chicago, Ill.*

Methyl, bis (β -chloroethyl) amine (HN-2) and tris (β -chloroethyl) amine (HN-3) at 10^{-6} – 10^{-4} M produce the following sequence of events when added to cultures of *Chilomonas paramecium* growing (logarithmic phase) in ammonium acetate media: 1) cell division continues until 30–50% of the cells have divided, 2) division then ceases for about two cell generation times (12–24 hours depending on temperature) the cells meanwhile growing larger, 3) the division rate briefly exceeds the control rate to which it finally returns. Resumption of division occurs while the medium contains active mustard as evidenced by the fact that previously unexposed cells exposed to it at this time are inhibited. Addition of freshly prepared mustard solutions to inhibited cultures about to resume division does not prevent or further delay division. This resistance is maintained if the cells are continued in a mustard-containing medium. Sensitivity to mustard action is regained (<3 cell generation times) when the cells are returned to mustard-free solutions. The observed effects are difficult to explain on the basis of genetic mutations. It seems more likely that the cells discover an alternate metabolic pathway for synthesis of a constituent critical for cell division. We have previously shown that HN-2 and HN-3 do not seriously affect respiration or carbohydrate metabolism in this range of concentrations. Protein, pentose nucleic acid and desoxy-pentose nucleic acid synthesis are all affected, however. It seems most likely that the critical constituent lies in one of these categories.

Effect of sustained elevation of renal venous pressure on sodium excretion in the unanesthetized dog W. HWANG,* L. AKMAN,* A. MILLER,* E. SILBER,* J. STANLER* AND L. N. KATZ *Cardiovascular Dept., Medical Research Inst., Michael Reese Hospital, Chicago, Ill.*

Influence of increased renal venous pressure (RVP) on sodium excretion was studied in 5 trained unanesthetized dogs. Renal plasma flow (RPF), glomerular filtration rate (GFR), and sodium excretion (with and without intravenous hypertonic sodium input loads) were studied

serially before and after partial ligation of the inferior vena cava just above the renal veins. Venous pressure distal to the ligation was consistently maintained at elevated levels of 250–350 mm of saline for at least 2 weeks postoperatively. The initial postoperative clearance studies (3rd day postoperatively) revealed the increased RVP to be uniformly associated with a depression of GFR and therefore with the amount of Na presented to the tubules. Under these circumstances, 3 of the dogs exhibited depressed Na excretion, while the other 2 showed normal Na excretion. Thus, depressed GFR was frequently, but not uniformly, associated with depressed Na excretion. Depression of GFR persisted for 5–8 days postoperatively, returning thereafter to control rates despite persistent elevation of RVP. Na excretion also returned to control levels. Thus under our experimental conditions, sustained increases in renal venous pressure did not necessarily cause reduction of sodium excretion. It would appear that no simple cause and effect relationship exists between chronic renal venous pressure elevation and Na excretion in the dog.

Effect of purified growth hormone on the glycogen content of tissues of the rat BARBARA A. ILLINGWORTH* AND JANE A. RUSSELL *Dept. of Physiological Chemistry, Yale Univ., New Haven, Conn.*

A single intraperitoneal injection of purified growth hormone (1–2 mg/100 gm body weight) into fasted normal adult albino rats results in an increased accumulation of glycogen in gastrocnemius, heart and diaphragm muscles at the end of 6 hours. This effect is observed in animals in which the fast is continued and in those fed glucose by stomach tube. The liver also shows an increase in the treated fasted rats. The magnitude of the net increase can be accounted for by assuming a decreased catabolism of carbohydrate. The minimal effective dose in glucose-fed animals was of the order of 100 γ /100 gm for several different preparations of hormone. That the action of growth hormone on carbohydrate metabolism is probably not mediated through an increased secretion of insulin is indicated by the following: a) The minimal effective dose of growth hormone is not hypoglycemic, b) a dose of insulin (0.1 μ protamine zinc insulin/kg) resulting in a hypoglycemic response similar to that due to the higher dose of growth hormone fails to augment the glycogen content of tissues other than the diaphragm, and c) the respiratory quotient is depressed by growth hormone and elevated by insulin administration. Experiments with adrenalectomized rats fed glucose showed that whereas neither growth hormone nor a minimal dose of A.C.E. (0.5 cc of Wilson's aqueous ex-

tract) alone have any effect, simultaneous treatment results in a significant elevation of carbohydrate stores

Comparison of effects of a stress on the glycosuria of force-fed partially depancreatized and of partially depancreatized-adrenalectomized rats

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The activation of the adrenal cortices during stress might be expected to intensify the diabetes of partially depancreatized rats. To the contrary (Ingle, in press), the injection of toxic amounts of formaldehyde caused some decrease in glycosuria. In the present experiments on 40 mildly diabetic rats, 20 were adrenalectomized and treated with amounts of adrenal cortex extracts (ACE) which sustained the pre-adrenalectomy level of glycosuria. Subcutaneous injections of 1.5% formaldehyde were made twice daily in doses of 0.25, 0.5 and 1.0 cc for periods of 7 days and of 0.25 cc for 28 days in both adrenalectomized rats. The animals were force-fed a medium carbohydrate diet. All rats showed a decrease in glycosuria during stress, but the fall was more striking in the adrenalectomized rats. These data support the hypothesis that the increased secretion of adrenal hormones during stress goes to meet an increased 'need' and tends to maintain homeostasis rather than to represent hypercorticalism. The increased cortical activity during the stress did have a positive effect upon carbohydrate metabolism in that it tended to prevent the fall in glycosuria noted in the adrenalectomized rats whose intake of cortical hormones was uniform.

Nature of influence of pH on hemolysis by sodium oleate

M H JACOBS AND CAROLYN STOUT *
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The peculiar nature of the pH dependence of sodium oleate hemolysis illustrates the manner in which the interaction of several factors, each in itself relatively simple, may lead to situations which when not properly analyzed appear to be hopelessly complex, difficult to reproduce, and at times bizarre. Three such factors have so far been separated by the methods here employed. The first is a direct effect of the oleate on the cell surface, leading to a condition of free cation-permeability, within a pH range of approximately 6-8 it decreases with increasing pH. The second is the swelling of the cation-permeable cell through the operation of the Donnan ionic equilibrium, this is greatest at the acid and alkaline ends of the range and least near its middle. The third is a 'protection' of the altered cells, apparently by interfacial layers of oleate, which prevent the escape of hemoglobin under conditions where it would otherwise occur. This effect is favored by

acidity, and is opposed, or may even be reversed, by alkalinity. The interaction of the 3 factors is influenced not only by the pH of the medium but by the concentration of the oleate, the number of cells on which a given amount of it may act and the temperature. Other things being equal, the effect of any given pH change depends on its simultaneous, and in general dissimilar influence on the three factors in question, and possibly on others of an as yet unknown nature.

Structure of gels from ovalbumin and serum albumin

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Gels have been prepared from Armour crystalline ovalbumin and serum albumin. *Ovalbumin* Appropriate combinations within the ranges 0.5 to 7%, pH 4.2 to 2.4, ionic strength less than 0.01, will yield gels when heated for 2 minutes at 100°C. Above 1% protein gels form over a small pH range (at 2% protein pH 4.3-4.7) which decreases as the protein concentration increases. Electron micrographs of viscous solutions prepared by heating 0.01% protein at pH 4.4 reveal fibrous networks whose strand width is approximately 200 Å. Reversion of 2% gels by sodium hydroxide at pH 11.0 and 23°C for 10 minutes produces products resembling native ovalbumin in molecular weight and gel-forming properties. *Serum Albumin* Appropriate combinations within 1-8% protein, pH 4.15-3.9 and ionic strength 0.1 (acetic acid buffer) yield gels when heated for 2 minutes at 100°C. Several gels show strong static double refraction. Electron microscope examinations were made on 4% gels centrifuged at 240,000 g for 120 minutes and dispersed by ultrasonic treatment. The specimens were faintly shadow cast with chromium. Dispersed fibrils with approximate dimensions 200 × 2000 Å were observed. Alkaline reversions of the original gels gave products resembling the native protein. The basic structure of both gels appears to be a cross-linked system of fibrils. Although reversion products have not shown to be identical with initial protein they suggest that individual fibrils may be constructed of corpuscular units.

Maturation of pituitary-adrenal axis in the newborn rat

JOSEPH W JAILER *Depts of Medicine and Obstetrics and Gynecology, Columbia Univ College of Physicians and Surgeons, New York City*

Exposure of adult rats to stress results in an almost immediate fall in adrenal ascorbic acid in intact but not in hypophysectomized animals. The administration of adrenocorticotrophic hormone produces the same effect without regard to the absence or presence of the animals' pituitary (LONG, SAYERS *et al*). The purpose of this in-

vestigation was to determine whether the pituitary-adrenal axis can be similarly stimulated in the newborn rat as it is in the adult. The subcutaneous administration of 0.04-0.05 mg of epinephrine to infant rats, 2, 4 or 6 days of age, fails to cause a decline in adrenal ascorbic acid. At 8-10 days of age, the same procedure results in a fall of approximately 30% in adrenal ascorbic acid. Rats 4, 6, 7, 11 and 14 days of age were refrigerated at 5°C for from 75-100 minutes without producing any alteration in adrenal ascorbic acid. However, 16- and 25 day old rats exposed to the same experimental conditions responded with a 20% decline in adrenal ascorbic acid. When rats 1, 4 and 6 days of age are treated with ACTH they respond with a decrease in adrenal ascorbic acid of about 30%. Consequently, it would appear that when exposed to stress the newborn rat's pituitary cannot respond by liberating adrenocorticotrophic hormone but their adrenals are capable of responding to exogenous ACTH. Further evidence has also been adduced which shows a lack of response to stress in the newborn rat, as compared with older animals.

Relation of urinary pepsinogen excretion to gastric secretory activity in man HENRY D. JANOWITZ* AND FRANKLIN HOLLANDER *Gastroenterology Research Lab, Mount Sinai Hospital, New York City*

The relationship between gastric secretory activity (HCl and pepsin) and urinary pepsinogen excretion in man was studied by simultaneous complete collection of gastric secretion and urine. It is generally held that a parallelism exists, but this is inadequately supported. Sixty-one subjects, with a wide range of gastric secretory levels, were employed for this study. Some of these were ulcer patients. Simultaneous collection of unstimulated gastric juice and of urine was made under resting conditions for periods of 3 hours. Gastric pepsin activity was measured by Bucher's modification of the Anson-Mirsky method, adapted recently in this Laboratory to a lyophilized hemoglobin substrate, and was expressed in terms of mEq of tyrosine liberated. Uropepsin was measured by I. A. Mirsky's modification of this procedure. HCl was measured by colorimetric titration. The production of acid and pepsin, and excretion of uropepsin were calculated in terms of average hourly output. In these subjects, the gastric acid output ranged from 0 to 7.9 mEq/hr, the pepsin output from 0 to 21,219 units/hr, the urinary pepsinogen output from 0 to 373 units/hr. In these ranges the excretion of urinary pepsinogen varied directly with gastric pepsin output, this supports the concept of an exocrine-endocrine partition of gastric pepsinogen and justifies the use of uropepsin determinations

as a measure of gastric enzyme secretory activity under resting conditions. The correlation of acid and pepsin outputs in the interdigestive phase may account for the association of acid production and uropepsin output noted by others. These relations will be evaluated statistically.

Comparison of pressure and flow pulses in the unopened carotid artery of the dog KENNETH E. JOCHIM *Dept. of Physiology, Univ. of Kansas, Lawrence*

Flow pulses were recorded from the unopened carotid artery of anesthetized dogs by means of the electromagnetic flowmeter. Pressure pulses were recorded simultaneously from the same point in the artery with a Lilly capacitance manometer by introducing a fine catheter into the vessel through a small branch. Control records in different animals consistently showed that the systolic rise began simultaneously in both pressure and flow pulses, but that maximum flow was reached earlier than maximum pressure. The incisural drop in flow was relatively greater in magnitude than the corresponding drop in pressure. The diastolic portion of the flow curve was more variable than that of the pressure curve, sometime it presented a very gradual descending slope, and occasionally a prominent positive wave was evident. In some animals a momentary backflow occurred at the end of systole. Epinephrine in doses ranging from 0.5 to 10.0 gamma/kg decreased mean carotid blood flow, the larger decreases (more than 50%) occurring with the larger doses. The ratio mean carotid pressure/mean carotid flow was increased, sometimes by more than 300%, for periods of 8 minutes or more. Histamine intravenously in doses of 100 gamma/kg decreased the carotid flow by as much as 66% and reduced the pressure/flow ratio by almost the same percentage. Both flow and pressure pulses were altered in such a way that they became almost exactly superimposable.

Effect of theophylline, theophylline ethylenediamine and 8-chlorotheophylline on electrocorticogram of rabbits R. J. JOHNS* AND H. E. HIMWICH *Medical Div, Army Chemical Center, Md*

Theophylline, theophylline ethylenediamine, and 8-chlorotheophylline were administered i.v. to locally anesthetized, curarized, artificially respired rabbits and electrocorticograms and electrocardiograms were recorded from monopolar electrodes with a 4-channel Grass electroencephalograph. Preliminary experiments showed that with low rates of injection it required more drug to produce seizures than was required at high injection rates. Furthermore, at very low rates (about 1 mg/kg/min) no amount of drug would invoke seizures. For that reason the rates of in-

jection were based on animal's weight and theophylline content of the drug, being 4 mg/kg/min for theophylline, 5 mg/kg/min for theophylline ethylenediamine, and 4.75 mg/kg/min for 8-chlorotheophylline. With theophylline and theophylline ethylenediamine grand mal-like cortical seizures were obtained at comparable dosages (156 mg/kg of theophylline and 204 mg/kg of theophylline ethylenediamine which is equivalent to 163 mg/kg of theophylline). 8-chlorotheophylline produced no changes in the electrocorticogram at this rate of administration, nor did it produce seizures when given more rapidly and in much larger doses (up to 1333 mg/kg). Further work is being done to investigate the marked difference between 8-chlorotheophylline and the other 2 drugs, whether due to pharmacological inactivation from chlorination in the 8 position, low solubility, or to an inability to permeate the blood-brain barrier.

Inhibition of luminescence by homologous series of carbamates FRANK H. JOHNSON, RICHARD SIMPSON,* ELIZABETH A. FLAGLER* AND KATHERINE MCGEER* *Biological Lab., Princeton Univ., Princeton, N. J.*

The action of a representative series of carbamates from n-methyl to n-octyl, as well as some other derivatives, on bacterial luminescence was studied in relation to drug concentration, temperature and hydrostatic pressure. At low temperatures and normal pressure, each caused a reversible inhibition involving a ratio of between 1 and 2 carbamate molecules/enzyme molecule, and the concentration required for a given inhibition appeared directly related to the activity coefficient of the derivative, from methyl to butyl but not all the way to octyl carbamate. The amount of inhibition at a given concentration was variously affected, among the different derivatives, by a rise in temperature. The data indicated that a given carbamate formed multiple equilibria with the enzyme affected, the predominant equilibrium depending upon conditions of drug concentration, temperature and pressure. At optimum temperature, pressures up to 8,000 lbs/sq. in decreased initially moderate inhibitions by methyl, ethyl or propyl carbamates, had little influence on moderate inhibitions by butyl or amyl carbamates, and increased the initially moderate inhibitions caused by hexyl or octyl carbamates.

Ether concentration in gas and blood samples obtained during anesthesia in man and analyzed by mass spectrometry CECIL S. JONES,* EDWARD J. BALDES, AND ALBERT FAULCONER, JR.* (with the technical assistance of JOHN SAARI)* *Mayo Clinic, Rochester, Minn.*

Various authors reporting on the concentrations of ether in respired gas mixtures, on the concentra-

tion of ether in arterial and venous blood, and on the ratio between the concentrations of ether in a blood and a gas phase at equilibrium (distribution ratio), have presented conflicting values for such measurements during ether anesthesia in animals and in human beings. As a preliminary to further study of these problems, methods of utilizing the mass spectrometer for qualitative and quantitative analysis for di-ethyl ether in samples of gas and blood were evolved. These methods require refinement to increase their accuracy. They were applied to the determination of ether concentrations in samples drawn from 10 surgical patients receiving ether anesthesia and to the determination of the *in vitro* values for the distribution ratio. The results so obtained do not depart too widely from the generally accepted figures given in standard tests. Values for the distribution ratio for ether for the samples of blood from 14 subjects ranged between 12.2 and 16.9. Between the thirtieth and sixtieth minutes of ether anesthesia, the concentrations of ether in arterial blood varied with the depth of anesthesia, between 78 mg and 153 mg ether/100 ml of blood. The mean value of 100 mg ether/100 ml venous blood falls within the extremes reported for man in respect to concentration of ether. Concentrations of ether in the inspired gas mixture varied, the mean value being 8.5 volumes of ether vapor %. Analysis of samples of tracheal air showed that the inspired ether mixtures were diluted in their passage toward the lungs.

Effects of excess potassium followed by Ringer's solution on responses of skeletal muscle ARTHUR J. KAHN* AND ALEXANDER SANDOW *Washington Square College of Arts and Sciences, New York Univ., New York City*

The current studies are a continuation of research previously reported (Federation Proc. 8:177, 1949). Muscles immersed in a 48 mg % K Ringer's solution produce a depth (R) of latency relaxation in isometric twitches which within 4 minutes is 270% of normal and then rapidly decreases to zero in about 50 minutes. Tension output (T) shows a similar time course, although the initial increase is only 25%. We interpret the immediate enhancing effects on R and T as an indirect consequence on the contractile system of the direct action of K on the membranes of the muscle fibers, and the later depressant effects as due to penetration of K and its direct action on the contractile system. When a muscle is immersed in the K-enriched solution for 4 minutes, thus developing the enhanced R and T responses, and is then returned to normal Ringer's, the enhanced effects are nevertheless maintained always above normal, though with some decreases, for about 2 hours. The continuance of the enhanced respon-

ses of the muscle in Ringer's after the initial K-treatment indicates 1) that the initial K-treatment has resulted in some relatively permanent alteration of the system responsible for generation of the latent relaxation and tension output, and 2) that the absence of the depressant effects is evidence that their production, when the K acts continuously beyond the initial 4-minute interval, is due to its penetration and thus direct action on the contractile system

Enzymatic activity of surface denatured catalase

J GORDON KAPLAN (introduced by W W WALCOTT) *Dept of Zoology, Columbia Univ, New York City*

That a 'surface-denatured' enzyme may retain its enzymatic properties has already been demonstrated for the pepsin-albumin system. An intracellular enzyme, catalase, was studied for activity in the surface-denatured state, since this class of enzymes probably exists within the cell at the surface of cellular structures. A suspension of crystalline beef-liver catalase buffered at pH 6.9 was placed drop-wise on the air-water interface of a Langmuir trough buffered at the same pH, allowed to form a monolayer and then compressed into a solid fiber (Devau effect) which was lifted from the trough by a needle. These fibers, after washing, were the site of a vigorous bubble formation when they were covered with a dilute H_2O_2 solution, after heating at $100^\circ C$ for a few minutes all their activity was lost. A comparative study of the effect of temperature on the enzyme in solution vs the enzyme as fiber revealed a striking difference between the two. Heating at $50^\circ C$ up to 4 hours increases the activity (residual) of the fiber, whereas it decreases the activity of the catalase in solution. All measurements were made in the Warburg respirometer. The evidence permits the following conclusions: a) The change in behavior of the fiber is due to its altered physical properties, and b) The difference in behavior suggests that it is the fiber, not contaminating native molecules in solution, which possesses the enzymatic activity.

Testicular and crystalline hormone implants in the rat seminal vesicle

SEYMOUR KATSH (introduced by HARRY A CHARIPPER) *Washington Square College, New York Univ, New York City*

Testis autografts (averaging 2.5 mg) and homografts (averaging 11.6 mg, from 15-day-old rats) serve to maintain the reproductive accessories of castrated adult hosts which receive these transplants to the seminal vesicle. The criteria employed are weights, and histological and cytological evidence of stimulation of the vesicular-prostate complex. The greatest androgenic effects are seen in closest proximity to the grafts. Micro-

scopic examination of the serially sectioned implanted-vesicles reveals the autografts to be more severely disorganized than the homografts. Implants of pancreatic, salivary, thyroid, fetal membrane, and placental tissues and cholesterol, estrone and progesterone crystals have no androgenic effects as measured by the three above-mentioned criteria, but the efficacy of testosterone propionate is well demonstrated since each vesicle receiving these crystals weighs more than twice the normal and shows more intense cytological activity. The contralateral vesicles and ventral prostates of the testosterone-implanted animals are morphologically and histologically normal. Thus the results of the testicular and testosterone series indicate the establishment of an androgenic field with greatest potency at the site of implantation. In the testicular series a tall columnar epithelium is found close by the graft while more distantly a low epithelium is seen, the testosterone-treated vesicles are at least twice as large as their mates. Previous studies with adrenal and ovarian implants support these results.

Effect of intravenous hydrochloric acid-amino acids on plasma chloride

YALE J KATZ (introduced by GEORGE E FAHR) *Clinical and Lab Service, St Barnabas Hospital, Minneapolis, Minn*

Occasions arise when it is desirable to administer intravenously the chloride ion without the use of the sodium ion. Significant amounts of chlorides cannot be given in the form of free hydrochloric acid. A mixture of 0.05 molar HCl and a 5% commercial protein hydrolysate was found to have a pH of 4.0, and 0.10 M HCl in 5% amino acid to have a pH of 3.3, while unbuffered 0.05 N and 0.1 N HCl are pH 1.4 and pH 1.1 respectively. Commercial protein hydrolysate with 3.3 gm HCl added per liter was injected into the ear vein of the rabbit, in an amount of 20 cc/kg. The plasma chloride content was increased an average of 24 mg % (Expressed as NaCl it would be 39 mg %). Proportional rises were found with repeated injections. Since amino acids are metabolizable the chloride remains in excess. A decrease in the alkaline reserve results. This type of solution should provide the clinician a means of simultaneously treating hypochloremia and alkalosis.

Age and serum cholesterol concentration in normal man

ANCEL KEYS, OLAF MICKELSEN, RUSSELL HAYES* AND ERMA V O MILLER* *Lab of Physiological Hygiene and the Students Health Service, Univ of Minnesota, Minneapolis*

Serum total cholesterol concentrations were measured in 2050 urban Minnesota students, business and professional men who were found clinically normal in detailed examinations. Both Liebermann-Burchard Bloor extract and Sperry-

Schoenheimer digitonin methods were used without significant differences between results. For 1114 males from 17 through 45 the relation holds $Y = 126.8 + 2.35X$, $E_b = \pm 0.18$, where Y = cholesterol mg %, X = age, E_b = standard error of the slope. For 287 males 45 through 55 years the equation is $Y = 187.9 + 1.21X$, $E_b = \pm 0.71$. For 52 males aged 55 through 78 the equation is $Y = 476.6 - 3.86X$, $E_b = \pm 0.75$. These slopes are all significantly different. For 564 females aged 17 through 30 years we have $Y = 134.4 + 1.98X$, $E_b = 0.43$, compared with $Y = 126.0 + 2.39X$, $E_b = \pm 0.33$ for 1025 males of the same ages. For age 22.0 years estimated values for males and females are, respectively, mean = 178.7, 177.8, S.D. = ± 33.9 , 31.2, S.E. = ± 1.06 , 1.31. The least-squares equation for 30 normal boys aged 6 through 12 is $Y = 176.1 + 1.19X$, $E_b = \pm 2.91$. For all ages the standard deviations are between 24 and 36 mg %. The data demonstrate a marked curvilinear relation, with minimal values in late adolescence, maximal values in the 50's, and progressive decrements in old age. Preliminary norms and fiducial limits are established for ages 17 through 78 (females 17 through 30) for comparable populations. For ages 20, 30, 40, 50, 60, and 75, the means are, respectively, 173.8, 207.3, 230.8, 248.3, 267.0, and 202.1.

Further studies on prophylaxis and therapeutics of experimental renal hypertension with renal extracts J. P. KIELY,* J. M. KIELY,* BESS G. OSGOOD,* JOHN MARSHALL* AND G. E. WAKERLIN *Univ. of Illinois College of Medicine, Chicago*

During the past 10 years our research has conclusively demonstrated that certain renal extracts are very effective in the treatment and prophylaxis of experimental renal hypertension in dogs. During the past year the following progress was made toward determining the mechanism of the antihypertensive action and toward achieving optimal methods of extraction. Of 5 renal hypertensive dogs treated with crude hog renal cortex extract containing renin 2 showed good, one questionable, and 2 no reductions in blood pressure. Of 4 renal hypertensive dogs treated with semi-purified hog renin (70 DU/mg N) 2 showed good and 2 no reductions in blood pressure. Prophylaxis of 4 dogs with crude hog renal cortex extract containing renin was successful in 3 of the animals. The results suggest that a 48-hour aqueous extract of acetone-ether desiccated and defatted hog renal cortex powder is less effective than a 16-hour extraction, that a previous course of extract of whole hog kidney interferes with the therapeutic effect of crude hog renal cortex extract containing renin and of semi-purified hog renin, and that anti-renin may be involved in

the therapeutic and prophylactic effects seen. Renal clearance studies strongly suggest that the therapeutic and prophylactic effects were not due to changes in glomerular filtration rate or renal blood flow. We are now conducting further therapeutic and prophylactic experiments with semi-purified hog renin (for which a high yield method of preparation has been developed), with crude hog renal cortex extract containing renin, and with hog renal medulla extract.

Psychomotor aspects of the orbitofrontal cortex H. E. KING (introduced by CARNEY LANDIS) *Dept. of Research Psychology, New York State Psychiatric Inst., New York City*

In an investigation of the effects of frontal lobe damage upon higher motor functions in the human it has been found that the surgical procedures of prefrontal venous ligation (the bilateral occlusion of all the cortico-dural veins visible between a point 2 cm. caudal to the tip of the frontal pole and a point 2 cm. caudal to the coronal suture), superior topectomy (the bilateral excision of Brodmann's cortical areas 9, sometimes 10, 8 and 32) or orbital topectomy (bilateral excision of areas 11, sometimes 47, 10 and Walker's 13) all significantly decreased motor performance when measured at a period 10 days after operation, but measures made 3 months after operation revealed the patients to again perform at their preoperative level of ability. Measures have been made of response time, speed of continued movement and movement dexterity upon these operative groups. When all cases (24) were considered together the reduction in performance following operation was approximately 15%, and was statistically significant. When different operative subgroups were considered separately they were seen to be affected differentially, with the superior topectomy cases showing the greatest temporary reduction, prefrontal venous ligation cases showing the next greatest reduction and the orbital topectomy cases showing the least interference with performance. It appeared unlikely that these reductions could be explained in terms of general post-operative sluggishness and inattention since no such alteration was noted in a large number of sensory tests administered at the same testing intervals which required close attention for their adequate performance. It would appear that operations involving the orbitofrontal cortex constitute a temporary interference with a motor integrative system at the higher levels, this interference being greater when the damage is on the superior surface of the brain.

Longevity as a function of diet in the C3H mouse JOSEPH T. KING AND M. B. VISSCHER *Dept. of Physiology, Univ. of Minnesota, Minneapolis*
Several workers have reported increased life

span in rats and mice on caloric restriction In this study C3H males were used in order to avoid the problem of mammary cancer The mice were housed individually, put on the diets at weaning, and kept in an air-conditioned room One group was fed *ad libitum* on commercial chow, the other three groups were given the semi-purified diet described by Visscher *et al* A control group was fed *ad libitum* One restricted group received approximately 66% of the normal caloric intake, with normal protein, vitamin and mineral intake The other restricted group received approximately 50% of normal caloric intake with about 15% restriction of protein, vitamins and minerals The group on 33% restriction had the longest life span (av 755 4 days) Those on 50% restriction followed (av 652 9 days), the controls were next (av 623 5 days) Those fed commercial chow showed the shortest life span (av 585 8 days) Moderate caloric restriction results in the greatest life span

Hemolysis by intracellular crystal formation

BRUNO KISCH *Fordham University and Marine Biological Laboratory, New York City and Woods Hole, Mass*

In eels caught in a deadly sick condition a kind of hemolysis was observed up to now unknown in hematology One or more crystals of prismatic shape developed in red blood cells, there stretching and growing up to twice the length of a normal erythrocyte, finally rupturing the cell membrane These crystals are not hemoglobin but are soluble in water In the polarizing microscope the extinction occurs at 90° It is supposed that an infectious disease, probably a virus infection, is the reason for this type of hemolysis

Ventricular complex of the electrocardiogram of birds BRUNO KISCH *Mount Sinai Hospital and Yeshiva Univ, New York City*

Previous literature on birds states that the electrocardiogram of birds has no *R* but only a deep *S* as main ventricular initial deflection In 20 experiments seagulls and chickens were investigated and the following results were obtained The usual extremity leads, taken with needle electrodes show left axis deviation with a very low voltage in Lead *I* Leads *II* and *III* are very similar to each other A small *R* and a deep *S* is nearly always present in Leads *II* and *III* *T* is upright in *II* and *III*, very low in *I* Chest leads taken in the level of the ventricles with Wilson's Central Terminal, show at the right side of the chest no *R* as a rule but a *QS* Those from the left side of the chest show an *rS* always Direct V-leads show a *QS* on the pectoral surface of the right ventricle, a small *R* and deep *S* on the left ventricle and a *QS* on the dorsal surface of the ventricle *T* is diphasic, often with a quick deep final phase

Inside each of both the ventricles and auricles the ventricular complex starts with a high upstroke (*R*) In most cases there is no, or a very small, *S*, and always an inverted *T* The same type of ventricular cardiogram can be registered from the surface of both auricles and of the aorta The ventricular complex in the unipolar extremity leads is characterized by a high *R* in the *VR* potentials Because these high *R* waves exist nowhere on the surface of the ventricle of the investigated birds but are typical for both cavity potentials, this is a proof that the right arm potentials really reflect the cavity potentials of the ventricle By changing the position of the heart the *R* in the right and left arm potential can be changed too The average of the heart rate of 12 chickens was 380, their rectal temperature was between 107° and 109°, the average heart rate of 8 seagulls was found to be 450 and the temperature between 104 5° and 106° Auricular fibrillation can be produced in birds by local application of acetyl choline on the auricles

Analysis of retrograde conduction to the atria from premature ventricular contractions, a common occurrence in the human heart ALBERT D KISTIN* AND MILTON LANDOWNE *Cardiovascular Research Unit, Veterans Admin, Washington, D C*

Retrograde conduction to the atria from premature ventricular contractions, generally considered rare, was demonstrated in 15 of 33 unselected individuals by means of esophageal electrocardiographic leads, although rarely detectable in a simultaneous lead *II* In esophageal leads *P* waves due to retrograde conduction occur within a limited range of time (0 09-0 46 sec) following the premature *QRS* They differ in contour from sinus *P* waves and/or are premature In the latter case the interval to the next sinus *P* wave is longer than a sinus *P-P* interval Atrial fusion complexes occur *Ventriculoatrial conduction time* is not consistently greater than atrioventricular conduction time Thus there is no evidence for 'normal' unidirectional block in the human In two cases where analysis was possible, ventriculoatrial conduction time varied directly with the degree of prematurity of the ventricular contraction and inversely with the sinus rate This suggests relative refractory period and vagal influence upon retrograde conduction, similar to conditions governing normal antegrade conduction In addition, there were two discontinuous orders of magnitude of ventriculoatrial conduction, differing by 0 11 sec in one case and 0 23 sec in the other, two retrograde pathways were hypothesized The limited degree of prematurity of retrograde atrial activation following an antecedent premature ventricular contraction, favors spontaneous dis-

charge of the sinus node which may explain the 'compensatory pause' frequently observed. In some cases, however, retrograde atrial activation was sufficiently premature to invalidate this explanation, and in these the 'compensatory pause' is unexplained or fortuitous.

Protein content and tryptic activity determined by several methods in canine pancreatic juice under different conditions of stimulation S A KOMAROV, HERMAN SIPLET* AND HARRY SHAY Samuel S Fels Research Inst, Temple Univ School of Medicine, Philadelphia, Penna

Protein content was determined by trichloroacetic acid precipitation (KOMAROV, S A, G O LANGSTROTH AND D R McFAE, *Canad J Research* 17 113, 1939), tryptic activity was determined after activation with various enterokinase preparations by 1) a modification of Mett's method (METT, S G, *Diss St Petersburg*, 1889), 2) by the method of TOMARELLI, R M, J CHARNEY AND M L HARDING *J Lab & Clin Med* 34 428, 1959, and 3) the photoelectric method of Riggs and Stadie as adopted by FRIEDMAN, M H F, *Gastroenterology* 7 526, 1947, for pancreatic juice. In dilution experiments with different types of pancreatic juice obtained in acute experiments 1) after secretin alone, 2) secretin and 'urecholine' stimulation and 3) postprandial secretion from dogs with chronic pancreatic fistula, only Mett's method gave a rectilinear relationship consistently over a wide range of dilutions up to 1:20. Results with the other methods gave a rectilinear relationship for velocity constants only over a narrow range of dilutions and even then not consistently (<50% of the experiments). In experiments with secretin alone and with Urecholine stimulation superimposed Mett's method showed a better correlation between protein content and units of tryptic activity (coefficient of variation from 14.7 to 33.3%) than did the Tomarelli method (C V = 11.8% to 123.2%) or Friedman's adaptation of the Riggs and Stadie method (C V = 14.4% to 54.6%). Urecholine in wide range of dosage when superimposed on secretin stimulation consistently produced a significant increase in the output of enzymes when measured by the output of protein nitrogen or by the Mett method, while the other methods failed to show this increase in some experiments.

Effect of inhalation of oxygen at high partial pressure upon cerebral circulation and cerebral oxygen consumption in man R H KOUGH,* D Y COOPER, JR,* G L ENMEL,* H H LOESCHKE,* C J LAMBERTSEN* AND C F SCHMIDT Lab of Pharmacology, Univ of Pennsylvania, Philadelphia

To measure the effects of high tensions of inspired oxygen on cerebral blood flow (CBF), cere-

bral oxygen consumption ($CMRO_2$) and cerebral vascular resistance (CVR) 3 groups of healthy young men were studied by the nitrous oxide method of Kety and Schmidt. Group 1 (6 subjects), the control group, breathed dry air through a demand regulator for one hour. Measurements A were made at the beginning and measurements B were made at the end of the one-hour period. The conditions of Group 2 (8 subjects) differed from those of Group 1 in that 100% oxygen was substituted for air after measurements A were made. The conditions of Group 3 (6 subjects) differed from those of Group 2 in that the last 15 minutes of oxygen breathing were at an ambient pressure of $3\frac{1}{2}$ atmospheres. Group 3 was considered to be in the preconvulsive stage of acute oxygen toxicity when measurements B were made. Means of measurements are tabulated below in terms of 100 gm of brain/min.

	GROUP 1		GROUP 2		GROUP 3	
	A	B	A	B	A	B
CBF (cc)	56	53	55	47	58	43
$CMRO_2$ (cc O_2)	3.3	3.1	3.4	3.5	3.4	3.5
CVR (mm Hg/cc)	1.3	1.3	1.2	1.5	1.1	1.6

The table indicates that a high tension of inspired oxygen, either directly or indirectly, may cause a decrease in cerebral blood flow, an increase in cerebral vascular resistance, but no appreciable change in cerebral oxygen consumption.

Valves of the hamster caecum HUGO KRUEGER AND RUDY RIESCHEL, JR * Dept of Zoology, Oregon State College, Corvallis

The caecum of the hamster is slightly sacculated and divided into an apical and a basal portion. Internally, this division is marked by a semilunar valve and externally by a groove in the wall of the caecum, the groove corresponding to the fixed border of the semilunar valve. At least 4 valves can be found in the caecum of the hamster: the ileo-caecal valve, an apico-basal semilunar valve, a basal semilunar valve and a chevron valve. The ileo-caecal valve located at the ileo-caecal junction has a semilunar lip which projects into the lumen of the ileum. The apico-basal semilunar valve is located immediately on the apical side of the ileo-caecal junction. This valve projects into the lumen of the caecum and may be considered as a continuation of a portion of the ileal wall into the caecum. Aided by partial constriction of the caecum the apico-basal semilunar valve could presumably prevent caecal contents from reaching the apex of the caecum and as a result the contents coming from the ileum would be shunted into the colon. The basal semilunar valve is parallel with

the apico-basal valve and is located about 5 mm toward the colon from the apico-basal semilunar valve. The chevron valve at the caecal-colonic junction is formed by the terminations of the many ends of muscular chevrons found in the colon. The first of these chevrons projects into the caecal lumen and forms a pocket on the caecal wall.

Preparation and physical properties of crustacean sinus gland hormone. ELOISE KUNTZ (introduced by RUTH E. CONALIN) *Brown University, Providence, R. I., and Vassar College, Poughkeepsie, N. Y.*

A method of isolating crustacean sinus gland hormone from extracts of evertalks by repeated adsorption on Norite A has been developed. Elution is accomplished with 10% NH_4OH in 50% ethanol at 70°C. The preparation causes expansion of the dark chromatophores of *Uca minax*. It is also effective in prolonging the life of evertalkless *U. minax* when given in frequent injections. The preparation exhibits ultraviolet absorption peaks at 280-285 and 335-340 $\text{m}\mu$. Measurements of relative fluorescence intensity at different hydrogen ion concentrations reveal a broad peak extending from pH 2.5 to 6.5 in the presence of phosphate.

Unipolar electromyograms of patients with dermatomyositis. E. H. LAMBERT, SIBYL BECKETT,* C. J. CHEN* and L. M. EATON* *Sections on Physiology and Neurology, Mayo Foundation and Mayo Clinic, Rochester, Minn.*

The electrical activity of muscle in 15 patients with dermatomyositis was detected with unipolar needle electrodes and recorded by a cathode-ray oscilloscope. In resting muscle, numerous potentials indistinguishable from single-fiber potentials occurred spontaneously. There was also increased irritability to needle movement. In voluntary contraction, muscular weakness was associated primarily with a decrease in size (amplitude and duration), rather than in number of motor-unit action potentials; the latter, in some instances, approaching the appearance of single-fiber potentials. Polyphasic motor-unit potentials were increased in number. The motor-unit action potentials in myositis were similar to those found in progressive muscular dystrophy and to those produced by the injection of curare in normal muscle. Average values for motor-unit potentials of muscle biceps brachii in myositis were: amplitude, 198 ± 14 μV ($n = 137$), total duration, 5.7 ± 0.3 msec ($n = 136$), spike duration, 2.1 ± 0.09 msec ($n = 108$), negative phase, 1.4 ± 0.06 msec ($n = 106$). Comparable values in normal biceps were: 507 ± 15 μV , 8.4 ± 0.2 msec, 3.4 ± 0.07 msec and 2.2 ± 0.05 msec, respectively ($n = 180$). In contrast, values in lower motor neuron disease were: 2011 ± 218 μV , 12.9 ± 0.5 msec, 4.6 ± 0.25 msec and 2.8 ± 0.14 msec, respectively ($n = 58$). The electro-

myograms indicate that in myositis disintegration of motor units occurs by successive loss of muscle fibers from the unit. The occurrence of single-fiber potentials at rest, together with biopsy studies, suggests that this occurs by derangement of nerve terminals or motor end-plates. Degeneration of muscle fibers may also occur, but nerve trunks are intact.

Effects of inhalation of oxygen at high partial pressures upon arterial and internal jugular blood gas content, tension and pH. C. J. LAMBERTSEN, G. L. ENMEL, D. Y. COOPER, H. H. LOESCHKE and R. H. KOLGH (introduced by A. N. RICHARDS) *Lab. of Pharmacology, Univ. of Pennsylvania, Philadelphia.*

In studies of the effects upon man of inhalation of oxygen at high partial pressure (OHPP) samples of arterial and internal jugular blood were collected during control periods of air breathing at sea level and from the same subjects during oxygen inhalation at an ambient pressure of 3.5 atmospheres. Several subjects developed convulsive seizures due to toxicity of oxygen at the high arterial pO_2 produced. The table presents average results of measurements on 10 normal men, expressed in conventional units.

BLOOD MEASUREMENTS	AIR, SEA LEVEL		O_2 , 3.5 A°	
	Art.	Ven.	Art.	Ven.
O_2 content	18.6	12.3	25.8	17.7
% Hb sat	96.3	64.0	100	89.7
pO_2		37	2350	74
CO_2 content	50.2	56.1	47.3	55.4
pCO_2	44	56	40	59
pH	7.38	7.32	7.41	7.28

At 3.5 atmospheres the arterial plasma oxygen content of 6.5 volumes % nearly met cerebral metabolic requirements, as indicated by the near-arterial level of jugular hemoglobin saturation. OHPP produced a decrease of arterial and increase of jugular pCO_2 . The former suggests a moderate increase in pulmonary ventilation which was observed but not routinely measured. It is not possible to state whether these findings were related to pulmonary irritation or to central effects of CO_2 retention. Alteration of internal jugular pCO_2 and pH by OHPP are probably not important factors in development of oxygen convulsions.

Fragmentation of biliary calculi by ultrasound. HAROLD LAMPORT, HERBERT F. NEWMAN* and RALPH D. EICHORN* *Lab. of Physiology, Yale Univ. School of Medicine, New Haven Conn., and Beth Israel Hospital, New York City.*

Many types of human biliary calculi, whether

fresh or dried, were fragmented when placed in water in cellophane bags and exposed to a strong 400-kilocycle ultrasonic beam from a Crystal Research Laboratory generator in an oil bath for 5-60 seconds. Because of the change in acoustic impedance at the oil-tissue interface, the ultrasound was attenuated and excessive heat and destruction of tissue resulted when skin or fluid-filled intestine were placed in the oil ultrasonic beam. Even though the beam was attenuated on passing into water, its subsequent tissue-heating effects ceased to be serious, as measured by the appearance of tissue immediately after 15 seconds of irradiation, although longer periods still can damage tissue. Since the gall bladders of the easily available laboratory animals are not large enough, the small intestine and appendix of the rabbit have been studied preliminarily. Approximately 80% of biliary calculi placed in exteriorized loops of intestine filled with saline, but with blood supply intact, were fragmented during exposures of 10 seconds. No immediate change in the gut appeared. The later results of exposure of intestine and gall bladder to calculus fragmenting intensities of ultrasound have not yet been determined. The superior efficiency of the strong ultrasonic oil-propagated beam on the cellophane-encased stones as compared to that of the attenuated beam propagated in water suggests that a short exposure to a more intense ultrasonic beam may fragment biliary and perhaps other calculi without damage to surrounding tissue.

Effects of hypophysectomy on the respiration and cellular components of rat bone marrow DAVID LANDAU* AND ALBERT S. GORDON *Dept of Biology, Washington Square College of Arts and Science, New York Univ., New York City*

Male hypophysectomized rats (24-4 months post-operative) and intact littermate controls of the Sprague-Dawley-Holzman strain were used. The marrow cells were suspended in partially neutralized serum from animals of the same endocrine status and the cu mm O_2 consumed/mg tissue nitrogen/hour determined by the standard Warburg technique. The percentages of mature erythrocytes and nucleated cells as well as the distribution of cell types in the latter group were determined from marrow smears of the suspensions. The QO_2 (N) for the bone marrow of hypophysectomized rats— 39.2 ± 0.6 (S.E.)—was not significantly different from that of the controls— 41.3 ± 1.5 . The mean nucleated cell content of the normal rat marrow suspensions was 58.6% as compared to 69.3% in hypophysectomized rat marrow. Since mature erythrocytes have an extremely low O_2 uptake, recalculation was made of the respiratory metabolism in terms of the percentage of nucleated cellular elements. On this basis, the QO_2 (N) of the

marrow of hypophysectomized rats is found to be 19% lower than that of normal rat marrow. Hypophysectomy did not result in any alteration of the concentration of nucleated erythroid cells but did induce an increase of lymphocytes and a decrease in the numbers of immature neutrophilic myelocytes within the marrow. Preliminary experiments indicate that the bone marrows of hypophysectomized rats injected with purified growth hormone show a significantly higher QO_2 .

Chronic studies of effect of hydroquinone on man S. LANG,* N. R. BREWER AND A. J. CARLSON *Physiology Lab., Univ. of Chicago, Chicago, Ill.*

As was previously reported to the Society in April 1949, hydroquinone added to food fats in the amount of 0.01% decreases the rate of oxidative rancidity from 2 to 50 times. To further study the effects of hydroquinone 19 adult human subjects volunteered to participate in series of experiments. Two males ingested 500 mg of hydroquinone/day for 5 months after an initial control period of one month. Eleven subjects, male and female, ingested 300 mg of hydroquinone/day for 5 months after an initial control period of one month. Five males and one female ingested 300 mg of hydroquinone/day for 3 months. Thus our subjects ingested amounts approximately equivalent to a) a 30-year supply of hydroquinone in 5 months, b) a 16-year supply in 5 months, and c) a 10-year supply in 3 months, assuming that a 70 kilogram man was to ingest 1 gram of fat/kg/day and all of the fat was treated with 0.01% hydroquinone. Periodic blood and urine analysis were made throughout the duration of the experiments. Analysis of blood included percentage hemoglobin, red blood cell count or hematocrit, differential white blood count, sedimentation rate, platelet count, coagulation time and icteric index. Analysis of urine included albumen, reducing sugars, white blood cells, red blood cells, casts and urobilinogen. None of the subjects showed any symptomatology of toxicity during the course of experiments and in no case were the analyzed data significantly deviant from accepted human norms.

Bladder function following acute denervation and in spinal shock L. L. LANGLEY AND J. A. WHITESIDE (introduced by JOHN M. BRUHN) *Dept of Physiology, Med. College of Alabama, Birmingham*

The urinary bladder adapts to varying volumes maintaining a low, approximately constant pressure. Whether this is an inherent ability of the bladder musculature or is due to reflex action has been questioned. In experiments on more than 20 dogs we find that the bladder retains its capacity to accommodate to changing internal volume following complete denervation, during spinal shock at which time the micturition reflex is ab-

sent, as well as following Etamon (tetraethylammonium chloride) administration in sufficient doses to block micturition. We conclude that this phenomenon is an inherent property of the bladder musculature. Ten cc of saline introduced rapidly into the normal bladder causes an increase in pressure of approximately 3 cm H₂O. If the pelvic nerves are cut the average increase upon the introduction of 10 cc saline is 6-7 cm. This sharp rise is due to a marked increase in the tonus of the bladder wall. Severing the hypogastric nerves results in no marked or consistent change when they are cut either before or after the pelvic nerves are cut. Following complete section of the spinal cord at T₄ the micturition reflex cannot be elicited. During this period of spinal shock 10 cc saline increases the pressure only 1-2 cm, that is, the bladder is atonic. However, if the pelvic nerves are now cut, or if Etamon is given the bladder becomes hypertonic. Loss of the bladder's ability to accommodate to varying volumes is seen in spinal shock only if the bladder has previously been over-distended.

Do anesthetics depress nerve cells by depressing oxygen consumption? MARTIN G. LARRABEE, JUAN GARCIA RAMOS* AND EDITH BULBRING*
Dept of Biophysics, Johns Hopkins Univ Baltimore, Md

Effects of anesthetics or neuronal metabolism and synaptic transmission were compared by simultaneous measurements of oxygen consumption and transmission in excised superior cervical sympathetic ganglia of rabbits. A ganglion was placed in flowing solution in a small chamber in which oxygen concentration was measured with a polarized platinum electrode. Rate of oxygen consumption was determined by measuring the rate of fall of oxygen concentration when the flow of solution was stopped. Transmission was measured by height of postganglionic action potential evoked by preganglionic nerve stimulation. All anesthetics tested except urethane (chlorotone, Nembutal, ether, chloroform, also alcohols from methanol to octanol) depressed synaptic transmission reversibly without slowing the resting oxygen consumption. Urethane reversibly slowed oxygen consumption by a small amount which did not seem to increase significantly as the concentration rose from levels sparing to those blocking transmission. All anesthetics slowed resting oxygen consumption markedly at sufficient concentrations. When oxygen consumption was depressed by Nembutal or chlorotone, it could be restored by methylene blue, but depressed transmission could not. The extra oxygen consumption caused by repetitive activity was readily depressed by anesthetics, but only in direct proportion to depression of activity. In contrast with anesthetics, azide and cyanide reduced the resting

rate of oxygen consumption considerably, before depressing transmission. Cyanide depressed the extra oxygen consumption of activity more than the activity itself. These facts suggest that anesthetics depress neurones by some mechanism which does not interfere with oxygen consumption. Further interpretations are assisted by observations on sympathetic nerve trunks.

Constancy of responses to intra-arterial injection of mecholyl in isolated blood-perfused extremity. perfusion with pump-oxygenator system. BEN J. LAWRENCE, JR,* ADAM B. DENISON, JR,* J. MAXWELL LITTLE AND HAROLD D. GREEN
Dept of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N C

Fluctuations in blood flow in an isolated extremity perfused by an animal are due in part to a constrictor produced by the animal (GREEN, *et al Federation Proc* 1950). To eliminate such humoral factors we substituted a constant pressure pump and an oxygenator composed of glass beads for the animal. In this preparation flow was initially 10-20 cc/min/100 gm, it dropped to 2-4 cc during the first 2 hours, rose almost to the initial level at 3-4 hours then decreased practically to zero at 6-8 hours. The percentile responses to intra-arterial mecholyl varied inversely with the flow except during the terminal drop when they decreased progressively. Edema, as evidenced by leg weight gain, appeared early and increased rapidly during the terminal decline in flow but the resulting increase in viscosity was insufficient to account for the reduction in flow. Addition of chloromycetin, aureomycin or streptomycin, which prevented bacterial contamination, prolonged the experiments to 18-24 hours without materially affecting the sequence of flow changes or edema formation. Tetraethylammonium administered to leg and blood donor dogs, addition of the B complex vitamins to the perfusion system, regulation of blood pH, and, preliminary deplatelization of the blood by centrifugation at 3200 rpm for 15 minutes, to one hour were all without significant effect. Storage of pooled blood for 3-5 days at 9°C with daily addition of heparin and streptomycin minimized the fluctuations in flow.

Influence of dietary protein or thyroglobulin on liver and plasma protein levels of thiouracil-fed rats. J. H. LEATHEM
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Adult male rats were fed either 78% or 18% casein diets with or without thiouracil (0.5%) for 20 days. Liver protein was greatest following thiouracil and 78% casein but was significantly increased and to the same extent by 78% casein alone and by 18% casein plus thiouracil. Liver

weight, however, was only markedly increased by thiouracil. Liver water content was unchanged. Thiouracil feeding increases total plasma protein levels due to an increase in plasma globulin in addition to its effect on the liver. When adult male rats were fed thiouracil (0.25 per cent), thyroglobulin (0.05 per cent) (Prolid) or both for 20 days the plasma and liver protein effects of thiouracil were abolished. Thyroid, pituitary and liver weight increases induced by the goiterogen were counteracted by thyroglobulin whereas the cardiac hypertrophy induced by thyroid was antagonized by thiouracil.

Conversion of acetate and lactate to succinate in the intact mammal JUI SHUAN LEE* AND NATHAN LIFSON Dept of Physiology, Univ of Minnesota Med School, Minneapolis

After the administration of $\text{CH}_3\text{C}^{13}\text{OOH}$ plus normal malonate to fasted rats, $\text{C}^{13}\text{OOHCH}_2\text{CH}_2\text{C}^{13}\text{OOH}$ was recovered from the urine (LEE AND LIFSON, *Proc Soc Exper Biol & Med* 70:728, 1949). Similar studies have been made of the conversion of the following labeled compounds to succinate: $\text{NaHC}^{13}\text{O}_3$, $\text{C}^{13}\text{H}_3\text{C}^{13}\text{OOH}$, $\text{CH}_3\text{CHOHC}^{13}\text{OOH}$, $\text{C}^{13}\text{H}_3\text{C}^{13}\text{HOHCOOH}$. The results may be summarized as follows: 1) $\text{NaHC}^{13}\text{O}_3 \rightarrow \text{C}^{13}\text{OOHCH}_2\text{CH}_2\text{C}^{13}\text{OOH}$, 2) $\text{CH}_3\text{C}^{13}\text{OOH} \rightarrow \text{C}^{13}\text{OOHCH}_2\text{CH}_2\text{C}^{13}\text{OOH}$, 3) $\text{C}^{13}\text{H}_3\text{C}^{13}\text{OOH} \rightarrow \text{C}^{13}\text{OOHC}^{13}\text{H}_2\text{C}^{13}\text{H}_2\text{C}^{13}\text{OOH}$, 4) $\text{CH}_3\text{CHOHC}^{13}\text{OOH} \rightarrow \text{COOHCH}_2\text{CH}_2\text{COOH}$, 5) $\text{C}^{13}\text{H}_3\text{C}^{13}\text{HOHCOOH} \rightarrow \text{C}^{13}\text{OOHC}^{13}\text{H}_2\text{C}^{13}\text{H}_2\text{C}^{13}\text{OOH}$. In the case of result (1), the concentration of labeled carbon was of borderline significance only. These isotope distribution patterns in the urinary succinate are consistent with the *in vivo* operation of the tricarboxylic acid cycle as influenced by malonate inhibition of the conversion of fumarate to succinate.

Nutritional and metabolic factors in peripheral vasomotor reactions: vasocompensatory impairment induced by avitaminosis C RICHARD E LEE (introduced by B W ZWEIFACH) Dept of Medicine, Cornell Univ Med College and The New York Hospital, New York City

As compared to 12 pair-fed controls and an adequately supplemented synthetic diet, 11 guinea pigs deficient in vitamin C for 21-23 days show no demonstrable differences in blood pressure or in their ability to respond in pressor manner to intravenous and intra-arterial injections of epinephrine. However, when subjected to standardized graded hemorrhage, in contrast to the animals on a fully supplemented diet, the vitamin-C-deficient animals were consistently incapable of elaborating the renal humoral vasoconstrictor material, VEM. Tests were carried out on the unfractionated plasma using the rat mesoappendix method. In addition, these animals were much less resistant

to hemorrhage than their pair-fed controls. The amount of blood loss necessary to reduce the blood pressure to $\frac{1}{3}$ its initial value was approximately 50% of that of the controls. The removal of an additional cc of blood, which was well tolerated by the supplemented animals, was followed by a gradual fall of blood pressure to zero in 8 of the ascorbic-acid-deficient animals.

Effects of the shear bacterial polysaccharides upon cardio-vascular response CHESTER E LEESE, WILLIAM E POEL* AND HAROLD BERMAN* Dept of Physiology, George Washington University Med School, Washington, D C

Rabbits were given doses of polysaccharide from 100 to 1200 gamma/kg. This substance has been shown to be effective in destroying cancer tissue, but possesses strong toxic properties. Electrocardiograms showed tachycardia as the most persistent finding with inversion of the P and T waves, and abnormally high S-T takeoffs. All irregularities were reversible and disappeared within 6 hours after intravenous administration. Blood pressure dropped within 10 to 40 seconds after polysaccharide administration, and was maintained at $\frac{1}{3}$ to $\frac{1}{2}$ of its original level for 5 to 10 minutes. A gradual return to normal limits was then observed, which required 15 to 40 minutes. Following recovery of the pressure level, injections of the agent had no appreciable effect. It appears that the depressor action may be tachyphylactic in nature. Histamine was suggested as the depressor agent because of the destructive power of the polysaccharide upon cancer cells. Antihistamine drugs did not prevent the depressor response. Circulation time was increased in response to polysaccharides, measured by the cyanide method. The recommended dose of cyanide proved fatal in several instances following polysaccharide, but was never fatal to the controls.

Degradation of fatty acids to 'acetate' by neurospora JOSEPH LEIN AND PATRICIA S LEIN (introduced by W W SWINGLE) Dept of Zoology, Syracuse Univ, Syracuse, N Y

A mutant of *Neurospora crassa*, S210, which grew in minimal medium supplemented with oleic acid was isolated. Further study showed that the mutant did not grow when supplemented with amino acids or water-soluble vitamins but did grow well in acetic acid. Growth of the mutant in acetic acid could be very markedly inhibited by arginine or lysine but not by other amino acids. Experiments were carried out in which oleic acid or acetic acid was used as the sole carbon source, and it was found that the mutant could derive its carbon as well as its necessary supplement from both acids. This result was thought to indicate that the mutant was actually an acetate mutant and that its acetate could be derived from

oleic acid The possibility also exists that acetate itself is not the fragment formed from the fatty acid but rather that a compound closely related to it may be the one produced *in vivo* With this reservation in mind, naturally occurring fatty acids were tested to see if they could serve as 'acetate' donors for the mutant The compounds were tested for inhibition effects using wild type so that inhibition phenomena would not mask their ability to enable the mutant to grow From the growth studies it appeared that *Neurospora crassa* can degrade butyric, caproic, caprylic, capric, lauric, myristic, oleic, linoleic and linolenic acids into 'acetate' enabling the acetate mutant to grow Stearic and palmitic acids were not degraded to usable 'acetate' compounds

Positivity in ventral horn during bulbar reticular inhibition of motoneurons JEROME Y LETTVIN AND PAUL C DELL (introduced by WARREN S McCULLOCH) *Manteno State Hospital and Dept of Psychiatry, Univ of Illinois College of Medicine, Chicago*

We have previously shown that the suppressor bulbar reticular fibres in the ventrolateral fasciculus terminate in the internuncial nucleus of the Lateral Column of Ramon y Cajal (the Tractus Cellularum Intercornualis Lateralis of Jacobsohn) and that stimulation here inhibits and only inhibits the motoneurons even when the current is strong enough to excite initially the motoneurons themselves We now show that this inhibition is accompanied by a long positive potential in the motoneuron pool resembling the positive 'synaptic potential' recorded by Eccles with intersegmental reflex inhibition

Effect of insulin on transport of certain substances across cell barriers R LEVINE, M S GOLDSTEIN* AND B HUDDLESTON* *Dept of Metabolic and Endocrine Research, Med Research Inst, Michael Reese Hospital, Chicago, Ill*

The intimate mechanism by which insulin acts has been looked for in a 'biocatalytic' effect on one or several of the enzymatic steps of the intermediary metabolism of glucose However, insulin may not exert any direct action whatever upon the known enzyme systems of the accepted carbohydrate scheme, but it may act by promoting the rate of transfer of glucose (and perhaps other substances) across certain cell membranes Accordingly we are investigating the effect of insulin upon the rate of entry into tissues of substances other than glucose, preferably non-utilizable, organic, naturally occurring compounds We have recently reported that insulin increases the rate and extent to which galactose is distributed in the tissues of eviscerated nephrectomized dogs (*J Biol Chem* 179: 985, 1949) On the basis of existing evidence it was then assumed that the administered galactose was not 'utilized' (transformed in any way) This

has now been experimentally demonstrated The above experiments were repeated using eviscerated nephrectomized rats and analyzing the whole carcass Our data show that at the end of the experimental period none of the administered galactose has disappeared as a result of insulin administration It can be concluded, therefore, that insulin is affecting some 'permeability' process by which galactose gains entrance into cells Preliminary experiments indicate that this type of insulin effect can also be obtained by using fructose and lactate but that insulin does not affect the distribution of urea, creatinine or sucrose

Quantitative determination of fluorescein passage through capillaries following ultraviolet irradiation of transparent rabbit ear chambers EDWARD A LICHTER* AND ALFRED A SCHILLER *Dept of Physiology, Univ of Illinois College of Medicine, Chicago*

Modified transparent chambers, similar to those described by Ebert, have been inserted in rabbit ears to study the manner in which fluorescein dye injected *iv* enters and leaves the tissue spaces under various dosage levels of ultraviolet irradiation calculated to affect changes in capillary permeability The radiation was directed through the polished quartz table of the chamber in dosage levels approximating rabbit belly threshold erythral responses Alterations in the size of the vascular bed were determined from serial photomicrographs taken during the control and experimental periods Approximately 4-5 hours after ultraviolet irradiation of the chamber, 50 mg/kg of Na fluorescein in 5 % solution was injected intravenously followed by measurements of the intensity of fluorescein fluorescence in a given microscopic field in the chamber at 1- to 2-minute intervals for a period of 60-90 minutes Fluorescence was determined photometrically by a sensitive, suitably filtered, multiplier photocell adapted to the microscope ocular The photocell was standardized against a piece of stable fluorescent glass placed on the microscope stage above a constant intensity lamp The arithmetically integrated areas delimited by the time-intensity (fluorescence) curves provide indices of comparison between control and irradiation conditions In several animals under comparable experimental conditions, it was observed that the rate and peak intensity of dye transudation and resorption was greater following irradiation, each chamber serving as its own control Characteristic deviations of the experimental chamber from the control, during 3 successive 20-minute intervals, were a) 103 % increase in dye, b) 11 % decrease, and c) 27 % decrease, respectively

Non-chronotropic T-wave changes produced by acetylcholine IRVING M LIEBOW AND HERMAN

K HELLERSTEIN (introduced by VICTOR LORBER) *Dept of Medicine, School of Medicine, Western Reserve Univ, Univ Hospitals, Cleveland, Ohio*

The effects of acetylcholine upon the blood pressure and upon intracavitary and extracavitary leads of the electrocardiogram of the intact nembutalized dog were studied. Acetylcholine in amounts from 0.01 microgram to milligrams was injected into the right atrium and mid abdominal aorta via indwelling catheters. 1) Intra-atrial and intra-aortic doses as small as 0.01 μ g produced a significant fall in blood pressure. The former route produced changes in the electrocardiogram. Acetylcholine therefore is effective in minute doses in the intact anesthetized animal. 2) PTA depression and increase in the amplitude of the T wave (extracavitary leads) were found to be early and independent of the blood pressure changes. PTA depression occurred in 31 of 34 experiments before blood pressure fall and in the absence of chronotropic effect. The same was true of T wave changes in 9 of 31 experiments. The changes are considered to be a direct effect of acetylcholine. 3) Acetylcholine produced greater negativity of the intracavitary T wave and greater positivity of the extracavitary T wave. On the basis of current concepts, acetylcholine either hastens repolarization of the ventricular sub-epicardial lamina or retards it in the sub-endocardial lamina.

Electro-icongo-grams from the cerebral cortex (cats) at the pial surface 'spontaneous' activity and responses to endorgan stimuli under anesthesia JOHN C LILLY AND WILLIAM W CHAMBERS (introduced by H C BAZETT) *E R Johnson Fdn for Med Physics, Univ of Pennsylvania, Philadelphia*

A new technique and some of the results of its use are described briefly. The Bevatron (A I E E - I R E Symposium Volume on Instrumentation in Medicine and Nucleonics, to be published by the American Institute of Electrical Engineers in 1950) records some 2 dimensional cross sections of the 3-dimensional electrical activity of the brain (electro-icongo-grams). The input field is picked up by 25 electrodes in a 5 x 5 square array (8 mm by 8 mm) on the pia, is amplified by 25 amplifiers, and used to intensity-modulate 25 light sources in a 5 x 5 square array at the output. The moving and fixed figures and forms of the electrical input field are thus transformed into corresponding patterns of variation of luminosity of the output field. The output field is photographed with a motion picture camera at 128 frames/sec to obtain the records called electro-icongo-grams or E I G.s. By projecting the E I G.s at 16 frames/sec on a screen subtending a visual angle of 0.1° to 1.0°, the following results can be seen by the observer. 1) In deep barbiturate anesthesia, the cortical responses to nor-

mal endorgan stimuli can be seen to spread in the primary afferent areas in a stereotyped fashion from the zone first activated. 2) 'Barbiturate waves' are seen to be simple forms which travel or which 'grow' around a focus and fade away in place. 3) Spontaneous activity in the conscious animal has traveling, shifting figures of various velocities, sizes, and forms, depending, grossly, on previous stimuli.

Behavioral and EEG changes following chronic brain stem lesions D B LINDSLEY, L H SCHREINER,* W B KNOWLES* AND H W MAGOUN *Depts of Anatomy and Psychology, Northwestern Univ, Evanston, Ill*

The possibility that wakefulness and EEG desynchronization, in the arousal reaction to afferent stimuli, might depend upon an ascending influence of the brain stem reticular formation, rather than upon the influence of afferent volleys arriving at the cortex over classical sensory paths, was explored by observing the behavior and EEG's of cats with large, chronic lesions of the midbrain or diencephalon. After destruction of the anterior midbrain tegmentum or hypothalamus, sparing sensory connections to thalamus and cortex, the animals usually appeared asleep. Their EEG's exhibited slow waves and spindle bursts. Ordinary stimulation was ineffective, but stronger auditory or nociceptive stimuli evoked motor responses and EEG desynchronization. Arousal was brief, usually not outlasting stimulation. After mesencephalic interruption of the medial and lateral lemnisci and spinothalamic tracts, the animals were not abnormally sleepy and showed waking EEG's. When relaxation or sleep occurred, arousal and EEG activation was readily induced by afferent stimuli and usually persisted for long periods after stimulation. Of these two systems influencing the cortex, that through the tegmentum and hypothalamus thus appears of greater importance in maintaining wakefulness and inducing EEG arousal upon afferent stimulation. However, after large rostral tegmental or hypothalamic lesions, afferent stimuli are still able both to arouse the animal and activate its EEG. These effects seem attributable either to the direct influence of afferent volleys upon the cortex, or to collateral excitation of diffuse projection nuclei at the thalamic level. The consequences of chronic thalamic lesions are under study.

Nature of muscle membrane potential GILBERT LING (introduced by R W GERARD) *Dept of Physiology, Univ of Chicago, Chicago, Ill*

Earlier work led to the view that the membrane potential (MP) of skeletal muscle fibers (single unpaired frog sartorius fibers) is of the Donnan type and varies with metabolic and other conditions as the balance of permeable and imperme-

able ions is altered. Thus the A potential (55-80 mV) was shown to depend on impermeable CrP and to fall in proportion as this splits to permeable PO_4 and Cr. The B potential (0-55 mV), less metabolism sensitive, has been related to other ions, especially carnosine and hexose phosphates. If hexose 6 P is increased by soaking in adrenaline, the B potential is proportionally increased. The late rise of B potential, previously found under IAA action, has similarly been shown to parallel a rise in hexose phosphates. It is also associated with a rise of internal cations, not of K at this stage (though some bound K may become free) but of NH_4 , released from split ATP, and probably others. Such appearance of extra ions inside reflects itself in an increase in osmotic pressure, and in fact muscle weight increases in strict parallelism with the B potential. The normal MP should equal $\frac{RT}{F} \ln \frac{K_i}{K_o}$ and also equal $\frac{RT}{F} \ln \frac{(R_i)}{(K_o)}$

+ $\frac{(\text{Na}_o)}{(R_i)}$, where (R_i) is the total inside concentration of impermeable anions. From the known or directly measured values in these equations, the MP calculates, respectively, to 105 and 100 mV. The directly measured MP (corrected for junction potential) is 97.6 ± 5.7 mV, maximum 110. Under non-equilibrium conditions, as in IAA poisoned muscle, when NH_4 is being produced at the same time as K^+ is leaking out, the more complete equation of Hodgkin and Katz (*J Physiol* 108: 37, 1949) is preferable, as the inside-outside ratios of different permeable ions are temporarily unequal.

Role of changes in metabolism in acclimatization of albino rats to reduced barometric pressure

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Re-investigation of possible reduction in metabolism in acclimatization to oxygen lack was suggested by reports of such decrease in congenital heart disease. Twenty-four 170 gm male albino rats were acclimatized to 18,000 feet simulated altitude by exposure for 30 days, the final pressure being attained gradually over a one-week period. Maintenance of growth and increase in hemoglobin indicated true acclimatization. O_2 consumption was measured by an open circuit method with specially designed mask and valve system. Metabolism of experimental animals declined 32.7, 38.5 and 40.5% from control values after 0, 2, and 4 weeks at reduced pressure, respectively, and remained at -38.5 and -36.8% for 2 weeks following return to ground level pressures. Values for littermate controls at ground level pressures declined 23.1, 23.1, 32.7, 38.5 and 34.6% on corresponding dates. Decreases from control levels are statistically significant in both experimental and control

groups but not between these groups, and are probably due to factors of training and increasing age of animals. These data on growing rats thus support the concept that reduction in total O_2 consumption plays no role in acclimatization to anoxia and raise a question as to the presence of true acclimatization in congenital heart disease.

Effect of egg white injections upon excretion of hemoglobin in the rat

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We have previously reported that when hemoglobin is administered intravenously, after the production of proteinuria by intraperitoneally administered bovine albumin, there is a sharp increase in the rate of hemoglobin excretion and a pronounced depression of the threshold serum hemoglobin concentration, when compared with control animals that have received intraperitoneal saline solution. In current experiments we have investigated the effect upon hemoglobin excretion of intraperitoneally administered crude egg white, with concomitant proteinuria. Experiments were made according to the plan of previous work. Rats received 3 injections of saline solution intraperitoneally, the second injection containing 1 gm crude egg white. Hemoglobin was administered intravenously after subsidence of the edema and vascular disturbance that follows egg white administration, but during maximal proteinuria. It was found that, under these conditions, the threshold serum hemoglobin concentration is slightly increased, but the rate of excretion at higher concentrations is slightly diminished. Creatinine clearances were determined simultaneously and, when corrections for the change in glomerular filtration rate are introduced, it appears that egg white reduced the tubular reabsorption of hemoglobin but did not significantly affect glomerular permeability to hemoglobin. This is in contrast to the effect of bovine albumin, which reduced tubular reabsorption of hemoglobin but which sharply increased glomerular permeability to hemoglobin.

Effect of renin upon excretion of hemoglobin in the rat

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By the technique previously reported, the effect of intravenous renin administration upon the excretion of intravenously administered hemoglobin was studied in the rat. Animals were given 3 intraperitoneal injections of saline solution, succeeded by intravenous administration of hemoglobin solution containing 4 Goldblatt dog units of renin. Control animals received similar injections, except for omission of the renin. Simultaneous cre-

atimine clearances were performed. When correction was made for the slight fall in glomerular filtration rate which accompanied renin administration, it was seen that there had been a sharp increase in the rate of hemoglobin excretion, when compared with control animals at comparable serum concentrations. This is considered to indicate a pronounced increase in the glomerular permeability to hemoglobin under the influence of renin. Preliminary results indicate that the tubular reabsorption of hemoglobin, after renin administration, is significantly increased.

Serial determinations of morning gastric secretion in patients with peptic ulcer A. LITTMAN,* C. E. ROSIERE* AND A. C. IVY, *Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago*

At intervals, usually of 2 to 4 months, we aspirated the fasting unstimulated morning gastric secretion for 90 minutes in 16 ambulatory patients with duodenal ulcer. These studies were carried out for 1- to 3-year periods to determine the possible relationships of the acid and volume output to the patients' clinical courses, treatment and other variables. In 8 cases there were sharp drops in hourly acid output after the first tests, these patients all had typical ulcer symptoms at the time of the first test. However 5 of the 8 patients failing to manifest such a drop with remission also had active symptoms. Of 9 cases in which tests were done during subsequent recurrences, in 8 there were no accompanying rises in acid output, indicating that morning gastric secretion was not influenced by 'activity' of the ulcer. However, all recurrences under observation were of brief duration prior to testing, while the episodes present at the time of the first tests were sometimes protracted and severe, suggesting that sharp falls from initially high levels in the first tests occurred most often in association with remission from prolonged and severe clinical episodes. In only a few instances was there a possibility that antacid therapy was related to fluctuations in acid output. Also, treatment with atropine in 3 of 7 cases resulted in pronounced diminution of acid secretion although medications were always withheld for 36 hours before each test. Stopping atropine in these three cases was followed by sharp rises in acid output.

Gastric secretory inhibitor factor in canine gastric juice GEORGE R. LIVERMORE, JR.* AND CHARLES F. CODE, *Section on Physiology, Mayo Clinic and Mayo Fdn., Rochester, Minn.*

Gastric juice was collected from Heidenhain and Pavlov types of gastric pouches while these were secreting in response to subcutaneous injections of mecholyl or histamine. After neutralization, a

fraction of the juice was precipitated by the addition of ethanol until the concentration of ethanol in the sample was 80%. The gastric secretory inhibitor activity of this precipitate after it had been dissolved or suspended in physiologic saline solution was determined by the assay procedure of Code, Blackburn, Livermore and Ratke (*Gastroenterology* Dec., 1949). In most instances the intravenous injection of the precipitates inhibited gastric secretion. The inhibitor factor was most concentrated in juice from Pavlov pouches after stimulation with mecholyl and least in juice from Heidenhain pouches after stimulation with histamine. A higher concentration of the active material was present in juice from a Heidenhain pouch after stimulation with mecholyl than in juice from a Pavlov pouch after stimulation with histamine but the inhibition produced by equal weights of the 2 types of precipitates was slightly greater in the case of the juice from a Pavlov pouch after injection of histamine. Changes in body temperatures during the assays were recorded and the correlation between these and the amount of inhibition has been studied. It was found that in many cases a correlation did exist between body temperature and inhibition but in addition it was noted that a high degree of inhibition sometimes occurred without fever and conversely a high fever was sometimes present without significant gastric secretory inhibition.

Establishment and relief of asphyxial blocks in spinal motoneurons DAVID P. C. LLOYD, *Labs. of Rockefeller Inst. for Med. Research, New York City*

An exploring electrode may be so placed on, or in, the cord that antidromic responses of intramedullary myelinated axons (M-segment), initial axonal segments (I-segment), bodies, and dendrites of motoneurons are distinguishable. Interpretation of asphyxial changes in these responses is facilitated by observations on cocaine block of nerves in volume conductors. Below a block normally triphasic, responses become monophasic positive deflections. Above a developing block, the 2nd (negative) phase increases *pari passu* with loss of the 3rd phase, demonstrating that intensity of the sink at a point increases when that point does not subsequently become a source for sinks developing beyond that point. Thus, in volume, blocks are located between points at which negative phases increase and points at which negative phases disappear. On asphyxia M-segment responses, normal for some 3 minutes, increase rapidly to a maintained maximum, while all other responses disappear. Promptly, on restoring ventilation, M-segment responses decrease precipitately, but incompletely, I-segment responses re-

cover and overshoot, while dendritic responses remain absent. Then, slowly, as dendritic responses recover, M segment responses further decrease, and I segment responses decline from overshoot level toward normal. Therefore, asphyxial block, established between axon segments, on reventilation shifts toward the dendrites before resolving. After repeated asphyxial insult, blocking time increases and, coincidentally, the influence of asphyxial hyperexcitability becomes apparent as a transient augmentation, before block is reestablished, of response in depressed dendrites. Asphyxial changes in dendritic response now resemble somewhat those, described by Brooks and Eccles, in the 'antidromic focal potential' of spinal cords depressed by sodium pentobarbital (Nembutal).

Sodium as inhibitor of potassium effect upon frog nerve fibers ANDERS LUNDBERG (introduced by R. LORENTE DE NÓ) *Rochefeller Inst for Med Research, New York City*

Treatment of frog nerve (spinal roots or sciatic nerve trunk) with sodium chloride at 2-3 times the concentration present in Ringer's solution (0.1N) results only in a slight depolarization of the nerve fibers, nevertheless, the presence of an excess of sodium ions in the external fluid of the nerve fibers markedly inhibits the depolarizing action of a given excess of potassium ions, insofar as both the rate at which the depolarization is established and the maximal depolarization itself are considerably less than when the excess of potassium ions is present in an isotonic (0.1N) solution. On the other hand, decreasing the tonicity of the solution by withdrawal of sodium chloride results in an increase in the rate of depolarization by potassium chloride (0.05N). An oscillographic analysis has been carried out of the effect of an excess of sodium ions upon the nerve fibers. Even after several hours 0.33N sodium chloride does not block conduction of impulses. The most remarkable change observed consists in a reduction in the fast electrotonus.

Circulation in membranes of the frog and hamster after stasis, trauma and under pathological conditions BRENTON R. LUTZ, GEORGE P. FULTON* AND ROBERT P. AKERS* *Dept of Biology, Boston Univ, Boston, Mass*

Blood flow in small vessels of the frog retrolingual membrane, vocal sac, bladder and mesentery, and of the hamster cheek pouch was studied for sludged blood, under normal, pathological and surgical conditions, and after stasis. Reflected and transmitted light were used with magnifications from $\times 54$ to $\times 520$. True sludged blood was not found in hamsters with advanced tumors, staphylococcus infection or surgical trauma, nor in frogs

traumatized or with 'red leg'. Apparent sludged blood occurred in some hamsters in vessels examined by reflected light at low magnifications ($\times 54$, $\times 90$). The same vessels seen by transmitted light at higher magnification ($\times 200$) showed erythrocyte groups separated by plasma, white cells and platelets. When these erythrocyte groups were compressed by a microprobe, the individual erythrocytes separated freely, and passed separately into capillaries. After stasis for days, erythrocytes packed in vessels remained unagglutinated and flowed individually when the vessel was rubbed with a microprobe or cut with a microknife. A significant result of venous occlusion, for 20-45 minutes by clamping cheek pouch vessels, was mass platelet accumulation on some vessel walls at release. Individual platelets or small groups broke off into the stream, then additional platelets collected at the same place. Frequently channeling of blood flow through complex routes in adherent platelet masses occurred, but sludged blood was not observed under these conditions. These platelet masses may be the basis of local or remote thrombus formation following injury and stasis.

Determination of amount of ice formed in blood at various freezing temperatures BASILE LUYET AND PAUL SCHMIDT* *Dept of Biology, St Louis Univ, St Louis, Mo*

Though freezing is a classical method for producing hemolysis, it has been reported that blood can be frozen solid, above -3°C , without appreciable hemolysis (STRUMIA *Conference on Blood Preservation* Harvard 1949, p. 160). It was suggested (LUYET, *ibid*, p. 144) that the erythrocytes escape congelation and injury because they become gradually dehydrated by exosmosis in the concentrated plasma left when water crystallizes out of blood in the process of freezing itself. To check this possibility we determined calorimetrically the amount of ice formed in blood exposed to temperatures from -1° to -25°C . The blood to be frozen—oxygenated ox blood—was sealed in glass ampules, and these were cooled in a mercury bath maintained at the desired temperature. The following values, taken from a curve obtained with the blood of one animal, represent the essential findings.

Temperature ($^{\circ}\text{C}$)	-2°	-4°	-6°	-8°
% of water-content frozen	75	84	84	89

The amount of ice formed did not increase further when the temperature was lowered from -8° to -25° . Thus at -2° only some $\frac{1}{4}$ of the water is frozen (59% of the entire blood, the water-content being 77.9%), this is enough to make the blood appear 'frozen hard,' but sufficient room is left in

the interstices for the partially dehydrated erythrocytes to be preserved in 'cold storage'

Resumption of rhythmic contractions by amniotic muscle after its solidification in liquid nitrogen

BASILE LUYET AND FEDERICO GONZALES * *Dept of Biology, St Louis University, St Louis, Mo*

Excised fibers of skeletal muscles were reported previously to respond to electric stimulation after they had been solidified in liquid nitrogen if the rate of cooling and rewarming was of the order of several hundred degrees/sec. The present report is concerned with the resumption of rhythmic contraction after the same treatment in a system which is spontaneously contractile, the nerve-free smooth muscle of the amnion. Pieces of amnion of the chick were partially dehydrated by immersion in a solution of ethylene glycol, then, after the excess solution was blotted off, they were dipped in liquid nitrogen, finally they were rewarmed rapidly by immersion in Tyrode solution at 40°. Rhythmic contractions were usually resumed upon contact with a glass rod and they continued spontaneously for 15-30 minutes. Some injury resulted from the low temperature treatment: contractions were often less pronounced than in the controls, the percentage of responsive strips was smaller and the survival time was shorter. The nature of the conditions permitting recovery—ultra-rapid cooling, ultra-rapid rewarming and dehydration—and dependence of the degree of injury on the non-fulfillment of these conditions, support the view that life is preserved to the extent to which crystallization is avoided.

Substitutes for procaine in ventricular tachycardia

E L McCawley, J E Stanwood* and N A David *Dept of Pharmacology, Univ of Oregon Med School, Portland, Ore*

Surgical manipulation during operations involving the great vessels (Blalock, Smithwick) and coarctation predisposes to ventricular arrhythmias especially when the most preferred anesthesia, cyclopropane, is used. While procaine hydrochloride has been found effective in preventing these arrhythmias, a large dose is required and its action is brief. We have undertaken an investigation of the possibility of using other compounds for this purpose. Using dogs, ventricular arrhythmias have been produced by cyclopropane-epinephrine and chloroform-barbiturate-epinephrine combinations and by traumatic manipulations in the thoracic and abdominal cavities during cyclopropane anesthesia. The mesenteric Pacinian corpuscles appear to be particularly involved as a source of stimulus initiating irregularities in the sensitized heart. Diphenhydramine (J M White and E L McCawley, *Abstracts, Pharmacology Soc Meeting, Indianapolis, 1949*) has proven superior to procaine in preventing the ventricular arrhythmias initiated by these various procedures. Diphenhy-

dramine has a lower minimally effective dose and a greater duration of action. If diphenhydramine solution is administered slowly, no significant hypotension occurs. While there appears to be no change in the level of anesthesia, muscular twitchings may occur. We found 'Pronestyl' (N'-(2-diethylamino ethyl)-p-aminobenzamide) also effective and to have a longer duration of action than procaine. Ventricular fibrillation occurs in one-fifth of the animals developing arrhythmias if no protective drug is administered. Procaine, diphenhydramine and Pronestyl markedly reduces this incidence. In no case, however, could ventricular fibrillation, once established, be reversed.

Location of receptors for tonic neck reflexes

G P McCouch, T H Ling,* I D Deering* and D Scott, Jr * *Depts of Physiology and Anatomy, Univ of Pennsylvania School of Medicine, Philadelphia*

In 8 decerebrate, labyrinthectomized cats, the tonic neck reflex to head turning was retained after either section of all muscles connecting head with neck and atlas with axis (4 cats), or bilateral section of muscular branches of the first 3 cervical nerves (2 cats), or section of both muscles and nerves (2 cats). In 6 decerebrate, labyrinthectomized cats the first 2 or 3 cervical nerves were exposed with as little dissection of muscle as possible. The tonic neck reflex to turning the head was clearly present. An attempt was then made to denervate the upper neck joints by circumsection of the first 2 or 3 cervical nerves at their emergence through the intervertebral ligaments without injuring muscular branches. When this was done unilaterally, the neck reflex was abolished in the ipsilateral extremities, when bilaterally, it was absent in all 4 limbs. In a few preliminary experiments designed to study the discharge of joint receptors, discharges have been recorded from atlanto-occipital and atlanto-axial ligaments and from the region of the 2nd cervical root between its emergence from the ligament and the ganglion after section of branches traversing muscle. These discharges have been elicited by flexion and extension and by rotation of the head. It is concluded that receptors for the tonic neck reflex to turning the head are located in the atlanto-axial and occiputo-atlantal joints. If there be muscular receptors which facilitate this reflex, their effect in isolation is subliminal under the conditions of these experiments.

Physiological and pharmacodynamic studies of blood from x-ray personnel

DAVID I MACHT AND STANLEY H MACHT * *Depts of Pharmacology and Radiology, Sinai Hospital, and Dept of Radiology, Baltimore City Hospitals, Baltimore, Md*

The senior author has called attention in many publications to the fact that certain seedlings grown in hydroponic plant-physiological solutions

under strictly controlled ecological conditions, are able to detect abnormal or toxic constituents in 1% solutions of blood serum, even though these are not detectable by ordinary pharmacological or biochemical methods. Thus it was shown that blood sera of various animals, including men, exposed to x-rays are definitely toxic for the growth of *Lupinus Albus* roots. In the present investigation, studies were made in two Baltimore hospitals of blood specimens taken from physicians, students, technicians, clerks and other personnel employed in their radiological departments. Phytopharmacological tests were made on the one hand and morphological examinations on the other hand. It was discovered that almost all of the blood sera from x-ray personnel possessed phytotoxic properties, even before morphological changes could be discovered in the blood smears. A considerable number of the subjects also revealed changes in the blood cells, such as leucocytosis, etc. The findings point to the fact that stray radiations do occur in x-ray laboratories in spite of all the ordinary precautions taken to keep them out. This was corroborated by tests with supersensitive films. The hygienic implications of the above findings are obvious.

Phytotoxic properties of normal and some pathological spinal fluids DAVID I. MACHT, *Dept. of Pharmacology, Sinai Hospital, Baltimore, Md.*

The author has been studying for 25 years the phytotoxic properties of normal and pathological blood sera. As a result of these studies, it was definitely established that the blood sera of a few pathological conditions produced definite phytotoxic effects, mainly inhibition of root growth of *Lupinus Albus* seedlings grown under standardized, ecological conditions in plant physiological solution (*J. Lab. & Clin. Med.* 26:597, 1941 and *Bull. Torrey Botanical Club*, 76:235, 1949). Thus, it was shown that the following conditions produced marked inhibition of root growth: cataplexy, pernicious anemia, leprosy, trachoma, pemphigus and various psychoses. More recently studies were begun on spinal fluids. It was found that a 2% solution of normal spinal fluid yields an average index of growth of 92%. Experiments with spinal fluids from menstruating women, and from patients afflicted with pernicious anemia, pemphigus, and both functional or organic psychoses all yield characteristic phytotoxic indices of growth.

Changes in vital capacity following release of tourniquets obstructing venous return I. F. S. MACKAY, *Dept. of Physiology and Pharmacology, Univ. College of the West Indies, Jamaica, B. W. I.*

It is well known that the vital capacity diminishes when the recumbent position is assumed. The mechanism responsible has been examined by Hamilton and Morgan (*Am. J. Physiol.* 99:526,

1932). These authors suggest that when a subject lies down, blood is poured from the dependent veins into the lungs. In support of this they showed that tourniquets applied to the four limbs at diastolic pressure caused an increase in vital capacity. The changes in vital capacity following the release of the tourniquets in the recumbent position form an interesting pattern. There is firstly an immediate and sharp decline in vital capacity followed by a rise and then a fall to a plateau. The technique used was that of Mackay (*J. Physiol.* 107:89, 1943).

	SUBJ. A	SUBJ. B
Recumbent vital capacity, liters		
With tourniquets	4.77	4.90
After tourniquets are released (at ½ min. intervals)		
0.5	4.55	4.62
1.0	4.66	4.62
1.5	4.62	4.72
2.0	4.58	4.68
2.5	4.51	
3.0	4.47	4.66
4.0		4.77

Cardiac acceleration to atropine cannot be conditioned THAYER M. MACKENZIE* AND W. HORSLEY GANTT, *Pavlovian Laboratory, Phipps Clinic, Johns Hopkins Univ., Baltimore, Md.*

This laboratory has been concerned with the laws governing conditional reflex (cr) formation. The criterion as to whether a reaction can be conditioned depends not upon the reaction but upon the method of its production. Previous studies from this laboratory have shown that a response involving central nervous system excitation can be conditioned, but the same response produced by peripheral stimulation can hardly be conditioned. Thus a) salivation evoked by pilocarpin, gastric secretion from histamine injection, and hyperglycemia from adrenaline injection cannot be conditioned, but b) salivation and gastric secretion as components of food reaction, and the emotional hyperglycemia are readily conditioned. In cases under a) effect is produced by action on afferent nerve endings or directly on tissues, while in b) the action is result of central nervous excitation. Similarly we have formed cardiac crs where acceleration occurs as a component of central excitation, e.g. food, pain. Atropine was injected from another room by special technique to eliminate presence of experimenter, a marked unconditional increase to atropine could not be conditioned. Using saline as a conditional stimulus and 0.1 mg/kg atropine as unconditional stimulus the results were (average of 10 experiments) Rhett atropine 93 control to 141 and saline control

116 to 106, 13th minute after injection Ehza atropine 94 control to 181 and saline 103 control to 93, 11th minute after injection The cardiac like other reactions, to be conditioned, must result from central rather than peripheral excitation

Effect of lithium on the electrocardiogram of animals VICTOR A. MCKUSICK (introduced by JAMES A. SHANNON) *Cardiovascular Clinic, U S Marine Hospital, Baltimore, Md*

Elevation of serum potassium produces a pathognomonic sequence of electrocardiographic changes in animals and in the human. This study had as its objective a definition of the electrocardiographic effects of the lithium ion. In experiments roughly parallel with those of Winkler *et al* (*Am J Physiol* 124 478, 1948) for the potassium ion, isotonic (0.65%) lithium chloride solutions were infused intravenously in 6 dogs, 1 cat and 2 rabbits. Intermittent electrocardiographic observations were made and the blood level of lithium correlated therewith. In 6 guinea pigs the lithium chloride solution was administered intraperitoneally in intermittent doses and only the lithium blood level at cardiac arrest was determined, although the full sequence of EKG changes was recorded. In all these animals a consistent series of EKG events was observed: 1) amplification of the T waves, 2) auricular standstill or fibrillation, 3) widening of the QRS complex, and 4) appearance of a bizarre biphasic QRS-T complex at a low rate—omen of cardiac arrest. This is the same sequence as observed with potassium. However, roughly 2–3 times as high a lithium level was necessary to produce a given change as is required of potassium. With the hyperlithemia, it was observed that there was progressive increase in the concentration of serum potassium from about 4.0 mEq/l to about 10 mEq/l. The electrocardiographic effects of lithium may be secondary to its effects on the concentration of potassium in the heart muscle and in the extracellular water or in the ratio of these two.

A critical study of the Shay rat ROBERT J. MADSEN AND HELEN H. RAMSBURG (introduced by M. O. LEE) *Experimental Biology and Medicine Inst., Natl. Insts. of Health, Bethesda, Md*

Large numbers of male Sprague-Dawley rats were preoperatively starved for 24 hours, the pylorus ligated and starvation continued postoperatively for 18 hours, water being withheld. This standard method gave a weight (gm)/volume (cc) of gastric secretion ratio of 12 ± 5 , the pH varied from 1.1 to 1.8 and 80% of such rats developed forestomach ulcerations. The stomachs of these rats were greatly distended but not necessarily to an unphysiological degree as shown by three lines of evidence. The vital dye benzo sky

blue (C.I. 520) was injected intravenously into rats whose stomachs were distended and ulcerated, and simultaneously into control rats whose stomachs were equally distended but because of therapy with aluminum hydroxide had no ulcers. In both cases the dye uniformly stained the tissues proving that capillary circulation was functioning in the ulcerating tissue. Intragastric pressure in these rats averaged 6–7 cm of water. Finally, direct observation showed that capillary flow did exist in the ulcerating tissue. The volume of gastric secretion could be increased by postoperative hydration with saline or decreased by additional preoperative starvation and dehydration. Rats placed on an adequate purified diet 10 days prior to operation and compared to those on a stock diet showed no significant differences postoperatively. Ligation of both vagi, ureters or common bile duct produced a profound reduction in gastric juice volume and forestomachs free of ulceration.

Normal blood sugar in sheep and lambs DAVID E. MANN* AND M. X. ZARROW *Purdue Univ., Lafayette, Ind*

It has been reported that the normal blood sugar of sheep is consistently low compared to that of man. Dukes in *'Physiology of Domestic Animals'* (1935) gave a range of 40–65 mg % and Hitchcock and Phillipson (*J Physiol* 105 42, 1946) reported that 72% of their values were between 38–50 mg % and 16% were lower. A series of blood sugar values were obtained in 4 normal sheep of the Dorset and Shropshire strain throughout the months of November and December. Blood was obtained either from the jugular vein or an ear vein and determinations made by the Folin-Wu micromethod. The average blood sugar for this group was 67 mg % with a range of 56 to 75 mg %. Approximately half of the values were between 70 and 75 mg %. In 3 nursing lambs an average blood sugar of 126 mg % was obtained with a range of 63 to 225 mg %. It is apparent that though there is a tendency towards low blood sugar level in sheep a large percentage of the values approach the lower limits observed for other species. The wide variations observed in the lambs was probably due to the fact that the blood was obtained at different intervals after nursing which would also account for the high concentration of the blood sugar.

Effect of neuro- and adeno-hypophysectomy on water metabolism and on hypothalamic nuclei in the rat THOMAS H. MAREN AND DAVID BODIAN (introduced by E. K. MARSHALL, JR.) *Dept. of Pharmacology and Exper. Therapeutics, and Dept. of Epidemiology, Johns Hopkins Univ., Baltimore, Md*

Hypophysectomies of several types were as-

essed quantitatively by study of serial sections of the head. Cell counts were made of supraoptic and paraventricular nuclei. Hypothalamus and median eminence were uninjured. Sixteen animals were observed for 3-6 months post-operatively. The animals fall into 3 groups on the basis of anatomical and physiological findings. In *Group I* (7 rats) anterior lobe function appeared normal and 55-100% of that gland remained. Water intake was markedly elevated in all cases, and ranged from 100-170 ml/24 hr. In every case, the neurohypophysis was only partially ablated, the amount of normal appearing remnant being 33-71% (av. 50%). Response to water diuresis was normal. The typical anti-diuretic effect of nicotine was abolished in 6 of the 7 rats. In *Group II* (6 rats) the neurohypophysis was also partially ablated, remnant being 12-76% of normal (av. 57%), but the anterior lobe was totally removed with corresponding loss of function. There was no polydipsia. Water diuresis was sharply diminished, but could be restored by administration of desoxycorticosterone. In *Group III* (3 rats), there was subtotal removal of both lobes, anterior remnant was 7-18% and posterior 19-43%. There was mild polydipsia, and moderate water diuresis. Nicotine effect was abolished. In the hypothalamus a striking difference, as yet unexplained, was found between *Groups I* and *II*, where neurohypophysial remnants had similar volumes (50, 57%), but in *Group II* there had also been total adenohypophysectomy. These latter animals showed greater loss in the magnocellular hypothalamic nuclei, this was most notable in the paraventricular, where *Group I* averaged 35% remaining cells, and *Group II* only 16%.

Relation of length to tension in double-function muscles J. E. MARKEE AND M. F. WILLIAMS *

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We have previously reported that those thigh muscles such as the gracilis, semitendinosus, and biceps femoris which act as double muscles possess the physiological characteristics of flexors in one part of the muscle, and of extensors in the other part. That is, the flexor portion fatigues more rapidly, develops less tension per unit of weight, and develops effective tension through a larger proportion of the range of shortening of the fibers than does the extensor portion of the same muscle. The present study deals with further details of the ability of the flexor portion of these muscles and some of the flexor muscles which act over the ankle of the dog to develop effective tension through most of the range of movement, whereas the tension developed by the extensor portion of these muscles, or by adjacent extensors is markedly influenced by the length of fibers at the time of the contraction. In the case of the flexor muscles

or flexor portions of the double muscles, increasing the length of the fibers results more rapidly in an increased resting tension and less rapidly in an increased total tension which can be developed during contraction. Consequently, the active tension is influenced less by the length of the fiber than is true of the extensor unit. Flexor muscles or the flexor portion of double muscles are able to exert almost maximum active tension throughout more than three fourths of that range of shortening which the fibers could accomplish *in situ*.

Cholinergic sensitivity in an afferent cerebral system AMEDEO S. MARRAZZI AND C. ROSS HART *

Toxicology Section, Med. Div., Army Chemical Center, Md.

In the past, efferent cortical cells have been activated by large doses of acetylcholine protected by eserine. The size of the doses required has made physiological interpretation difficult. In the cat it has been possible, by submaximally activating the optic tract electrically and recording the evoked post synaptic potentials from the optic cortex, to study the effects of drugs on the 2 synapses between the tract and cortex ((1) MARRAZZI, A. S., *Federation Proc.* 2: 33, 1943). In these experiments, the drugs reached the tissues in a natural fashion through the blood supply and in reasonably small quantities. With intracarotid injection in cats lightly anesthetized with sodium pentobarbital, the following consistent group of findings was obtained: a) acetylcholine, 2.5 gammas, produced a marked, quickly reversible augmentation in the potentials evoked from both optic cortices on stimulating one optic nerve; b) DFP produced a prolonged augmentation; c) atropine sulfate decreased the augmented response. The blocking action of atropine indicates that the anticholinesterase action of DFP was involved. Experiments in progress will test whether the actions may not be localized exclusively to the synapses. The effects of acetylcholine, of an anticholinesterase and of the blocking agent, atropine, illustrate an unequivocal cholinergic sensitivity in the cerebral portion of the optic system and therefore a mechanism potentially capable of participating in synaptic transmission. The mechanisms suggested by the above results and the previously described adrenergic inhibitory effects on the same system 1) could constitute a humoral, perhaps accessory, system transmitting excitation and inhibition in an afferent pathway in the central nervous system.

Quantitative micro-analysis of nucleic acid purines and pyrimidines without prior isolation of the nucleic acids A. MARSHAK AND H. J. VOGEL *

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Thirty mg lipid-free, trichloroacetic acid-extracted material are digested in an open tube with 0.16 ml 12 N perchloric acid at 100°C for 1 hour, diluted with water to 1 ml and centrifuged. Aliquots of the supernatant are subjected to paper chromatography. Base recovery (as N) is 97% from calf thymus desoxypentose nucleic acid and 95% from yeast pentose nucleic acid. Lipid- and acid-soluble-free Arbacia sperm and Arbacia eggs contain, respectively, 0.98 and 0.91 gram-moles bases/gram-atom phosphorus. Pentose- and desoxypentose-linked pyrimidines may be estimated since differential splitting is obtained under appropriate conditions. Analyses, as molar ratios relative to adenine

	ADE NINE	GUA NINE	CYTO SINE	URA- CIL	THA- MINE
Arbacia sperm	1.00	0.70	0.64	0.00	1.01
Asterias sperm	1.00	0.69	0.62	0.00	0.91
Arbacia eggs	1.00	1.37	1.06	0.46	0.00
<i>M. tuberculosis</i> (H37Rv)	1.00	1.86	1.92	0.28	0.51
<i>M. tuberculosis</i> (H37Ra)	1.00	2.01	1.86	0.30	0.56
<i>M. sp</i> (M 2)	1.00	1.60	1.21	0.58	0.12
<i>M. sp</i> (B5-1)	1.00	1.64	1.25	0.56	0.12
PNA, yeast	1.00	1.13	0.71	0.73	0.00
DNA, calf thymus	1.00	0.70	0.69	0.00	0.97

Nucleic acids from diverse sources may have similar or very different base ratios. The tetranucleotide theory is not supported. *M. tuberculosis* contains uracil. Since B5-1 contains Feulgen-stainable nuclei while M 2 does not, cytochemical procedures for determining DNA depending upon microscopically visible amounts of Feulgen-positive material are not reliable.

Some liver function tests on dogs in adrenal cortical insufficiency CONSTANCE R. MARTIN* AND W. D. COLLINGS. *Dept. of Physiology, State Univ. of Iowa, Iowa City, and Michigan State College, East Lansing*

Control values for several liver function tests were obtained on 5 dogs. After complete 2-stage adrenalectomy, the tests were repeated with the dogs in varying stages of adrenal insufficiency resulting from treatment with different dosage levels of adrenal cortical extract (ACE) (Eschatin) or desoxycorticosterone acetate (DCA) (Percorten). Bromsulphalein (BSP) excretion was maintained at control levels in the operated animals on maintenance doses of ACE or DCA. BSP retention occurred during periods of inadequate therapy. The retention roughly paralleled rises in serum non-protein nitrogen, but often preceded changes in hematocrit and blood pressure. Changes in serum alkaline phosphatase were inconsistent; 3 animals had increased phosphatase activity during mild insufficiency providing food intake

was adequate. During severe adrenal deficiency, phosphatase fell sharply, probably because of inanition. Albumin/globulin ratios were better maintained with ACE than with DCA, but albumin fell and globulin rose, prior to serum sodium changes, in all mildly adrenal-deficient dogs. Once the ratio fell below control levels, it was not restored by any therapy. The galactose tolerance test was performed on 4 of the dogs. Maintenance of preoperative values was best with ACE, although control values were never achieved in 3 dogs following adrenalectomy. Galactose tolerance always fell as the therapy dose was reduced, but this was not dependent upon hemodynamic changes. Average values follow:

	CON- TROL	MILD INSUFF	SEVERE INSUFF
BSP % ret. 30 min	2.7	5.5	12.6
Serum A/G	2.17	1.63	1.01
B1 Galactose 120 min	0	16	40

Central nervous stimulation by implanted high frequency receiver ALEXANDER MAURO, P. D. WALL, L. M. DAVEY AND A. M. SCHER (introduced by D. H. BARRON). *Lab. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.*

The receiver consists of a parallel resonant circuit of 4 turns of No. 30 enameled wire $\frac{1}{4}$ " sq., tuned by a 50 mmfd condenser. It employs a germanium diode rectifier, and is sealed in an envelope of pure polyethylene. One output lead is connected to a silver plate which projects from the envelope and the 2nd to a monopolar stimulating electrode. Implantation on the motor cortex involves placing the envelope in the sub-apneurotic space and fastening it to the pericranium. The stimulating electrode is inserted through the dura via a bone button and is immobilized by stitching to the dura. The pulser employs a low-power oscillator, driving a power amplifier tuned at 30 megacycles. Repetition rate and pulse width can be widely varied. The pulser can be coupled to the receiver either by a single 5' loop or by 3 loops at right angles to each other around the animal's cage. In the monkey, the receiver has been maintained in use for long periods without complications. The entire system has been operated intermittently during the past 2 years and has proved to be practical. Present uses include investigations on the motor cortex, antiepileptic drugs, control of gastric secretion, and stimulation of the hippocampus and rhinencephalon.

Instantaneous electrocardiographic equipotential lines for right and left bundle branch block and left ventricular hypertrophy ALEXANDER

MAURO,* LOUIS H. NAHUM, HYMAN M. CHIRNOVICH* AND RAJINDER S. SIKAND* *Lab. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.*

The electrocardiographic equipotential lines on the body surface for a given instant have already been charted by the authors for normal humans and anesthetized dogs. In the present study the observations have been extended to patients with intraventricular block of the right and left bundle branch type, as well as to patients with left ventricular hypertrophy. While the plots vary from one individual to another, they all exhibit a) unusual complexity of the individual equipotential lines, b) marked asymmetry of their distribution c) complete absence of any central potential line about which the entire distribution 'rotates' from instant to instant. These observations support the conclusions already derived from the study of normal individuals that at no time in the cardiac cycle does a surface doublet distribution exist.

Exchange of albumin between plasma and lymph

H. S. MAYERSON AND KARLMAN WASSERMAN* *Dept. of Physiology, School of Medicine, Tulane Univ., New Orleans, La.*

Human serum albumin, tagged with radioactive I (I^{131}), has been injected into the jugular veins of dogs and its appearance followed in thoracic duct lymph for periods of from 2-36 hours. Curves drawn from specific activity determinations show that the radioactive iodo-albumin leaves the plasma exponentially at a very slow rate, the average k value being 0.0009. Thus, at the end of 5 hours, only approximately 20% of the radioactive iodo-albumin has left the circulation. A second injection, given approximately 2 hours after the first, yields a disappearance curve the slope of which is similar to that derived from a single injection. Radioactive iodo-albumin first appears in the lymph in measurable count in from 10-20 minutes after the injection depending on the rate of lymph flow. Analysis of curves drawn from specific activity determinations indicate the consistent presence of 3 slopes: 1) a relatively steep slope, lasting about 40 minutes in most experiments, 2) a more gradual slope lasting approximately 70 minutes, 3) a still more gradual slope persisting until equilibrium is reached with respect to the specific activity of albumin in plasma and lymph. This equilibrium, as determined by extrapolation, is reached in approximately 14 hours after the initial injection. Thereafter, the albumin disappears from both tissues at approximately the same rate. Experiments in progress are designed to elucidate the significance of these slopes. Tentatively, it would appear that the initial slope represents the appearance of lymph from the liver and that the second

and more gradual slope indicates the mixture of liver lymph with lymph from the intestines and other more remote areas. The final slope may be interpreted as representing that of radioactive iodo-albumin after the lymph from all parts has become homogeneous with respect to the radioactive iodo-albumin.

Decerebrate rigidity produced by discrete lesions

MARIE J. MAYO (introduced by W. S. WILDE) *Dept. of Physiology, Tulane Univ. School of Medicine, New Orleans, La.*

Attempts were made to imitate the gross transection of the brain-stem necessary to produce decerebrate rigidity by producing multiple discrete lesions with stereotactically directed electrodes in an effort to determine which structures were significantly involved. Since rigidity is best obtained when the brain is transected just above the lateral vestibular nuclei, this level was selected for the placement of the lesions. Cats were placed under ether anesthesia, the lesions placed, and the animal allowed to recover from the anesthesia. Following this the reflexes and muscle tone were observed and the animal photographed. The criterion of totality of rigidity was based on the determination of increase of rigidity by subsequent ischemia produced above the level of the lesion following ligation of the carotid and basilar arteries. It was found that the lesions which consistently produced the best decerebrate rigidity were those which involved both the superior vestibular nuclei and the corticospinal tracts. Lesions in either structure alone produced no effect or flaccidity. Lesions of the superior vestibular nucleus and the medial inhibitory reticular formation produced decerebrate rigidity but to a much less extent than when both the superior vestibular nuclei and the corticospinal tracts were destroyed. The latter rigidity was never increased by subsequent ischemic decerebration.

Effects of vitamin B₁₂ and thyroprotein on growth-inhibiting action of diethylstilbestrol in rats.

JOSEPH MEITES AND H. W. NEWLAND* *Dept. of Physiology and Pharmacology, Michigan State College, East Lansing*

Growth depression induced by diethylstilbestrol in rats has been accounted for on the basis of reduced appetite (MEITES *Am. J. Physiol.* 159:281, 1949). It was hypothesized that these inhibitory effects were induced by increasing certain vitamin needs and/or decreasing thyroid secretion. This hypothesis was tested by treating 5 evenly divided groups of 50 male rats for 20 days. Diethylstilbestrol and thyroprotein were fed in a commercial ration, while B₁₂ was injected subcutaneously at a level of 0.2 μ g daily. During the first 10 days, B₁₂ failed to counteract the growth depression induced by diethylstilbestrol but pro-

tially overcame the effects of the latter during the last 10 days. Thyroprotein plus diethylstilbestrol induced greater growth inhibition than the latter alone. Addition of B_{12} to the diethylstilbestrol-thyroprotein combination completely counteracted the growth-inhibiting action of the latter and only slightly that of the former. Food intake was increased both by B_{12} and thyroprotein.

Measurement of resistance in the human digital vascular circulation MILTON MENDLOWITZ
Mount Sinai Hospital, New York City

The relationship between pressure and flow in the human digital circulation was studied by graded compression of the brachial artery with a special clamp, sympathetic nerve tone having been released by indirect heating. Pressure was measured with a Gaertner capsule and flow with the digital calorimeter. When plotted on logarithmic paper using the formula $Q = aP^k$, a and k being constants, k was found to vary from 0.8 to 1.1. This contrasts with the pressure-flow relationship observed in animal perfusion experiments in which k is 1.4–1.8. This difference is believed to be attributable to the fact that in the vasodilated finger tip the preponderance of flow is through digital arterio-venous anastomoses rather than through capillaries. Poiseuille's law is hence more applicable to the digital circulation than to the circulation as a whole. Application of the law enables measurement of 1) vascular configuration volume index, 2) digital vascular caliber, 3) resistance in dyne seconds per cm^4 , and 4) resistance in dynes. The normal variations and the changes in hypertension and polycythemia are discussed.

Urolipase in normal and depancreatized dogs

H. C. MENG (introduced by CHARLES E. KING)
Dept. of Physiology, Vanderbilt Univ. Med. School, Nashville, Tenn.

The pathological and physiological significance of urinary lipase (urolipase) is poorly understood, and some investigators even question its existence. The purpose of this investigation was to attempt to find the origin of urolipase and determine its significance in relation to fat metabolism. Catheterized urine specimens were obtained from normal healthy dogs, and it can be stated definitely that urolipase appeared in the urine, but there was considerable variation in concentration between different dogs and from time to time in the same dog. Ten of these dogs were given 30 gm. of olive oil/kg. by stomach tube, and 8 of them showed a significant increase of urolipase. However, the increase was not correlated with the increase in serum lipase. Dogs receiving 3 gm. of olive oil/kg. intravenously as a 10% emulsion showed a still more marked elevation of urolipase. Depancreatized dogs showed a somewhat smaller increase of urolipase following intravenous injection

of fat emulsion. It thus seems likely that part of the urolipase originates in the pancreas.

Immediate increase in adrenaline 'sensitivity' of the sympathectomized nictating membrane of the cat following SC-1950 GEORGE W. MEYER*
AND KEITH S. GRIMSON *Dept. of Surgery, Duke Univ. School of Medicine, Durham, N. C.*

Increased contraction of sympathectomized nictating membranes with epinephrine is a phenomenon commonly attributed to degeneration of post-ganglionic fibers. A new quaternary amine, 2,6-dimethyl, diethyl piperidinium bromide, SC-1950, blocks autonomic ganglia and has been used to test this hypothesis. Dose of SC-1950 used was 4 mg/kg. and of epinephrine or nor-epinephrine 5 μ g. Left ganglionectomy and right preganglionic sympathectomy was performed in 9 cats 7–9 days before tests. Then epinephrine in 7 and nor-epinephrine in 2 caused contraction of the left nictating membrane greater than the right. Immediately following first or occasionally second injection of SC-1950 in 8 of the 9 cats, contraction of the left preganglionic side increased. With epinephrine in 6 increase was partial in 4 from an average of 2.5 cm. excursion of the writing lever (Rt.) and 6.1 (L.) before SC-1950 to 4.3 (Rt.) and 6.0 (L.). Increase was complete in 2, average 1.5 (Rt.) and 4.9 (L.) before to 4.8 (Rt.) and 4.9 (L.) after SC-1950. With nor-epinephrine increase was partial in one and complete in one. Epinephrine or nor-epinephrine was given to 3 cats a week after left ganglionectomy, leaving the right normal with no increase on either side following SC-1950. Also, ganglionectomy was performed on each side in 2 cats and a week later contractions were equal and did not change after SC-1950. The isolated superior cervical sympathetic ganglion of the cat a week after decentralization evidently exerts a buffering influence against nictating membrane contraction with epinephrine.

Parathyroid extract hyperphosphaturia in hypoparathyroidism ALEXANDER J. MICHIE AND JEANNETTE MCCONNELL SHOREY (introduced by HOMER W. SMITH) *Harrison Dept. of Surgical Research, School of Medicine, and Hospital, Univ. of Pennsylvania, Philadelphia*

As parathyroid extract causes little or no increase in the minute excretion of phosphate in voided urine or normal subjects we wished to determine the renal mechanism by which parathyroid extract causes hyperphosphaturia in patients suffering from hypoparathyroidism. The subjects had had a previous resection of their thyroid following which they developed symptoms of hypoparathyroidism, i.e. tetany, etc. Their hypocalcemia was treated with either vitamin D or AT-10 supplemented with oral calcium. The vitamin D or AT-10 was stopped a month prior

to the performance of the renal clearances. Renal plasma flow and glomerular filtration rate were determined in the usual manner. Bloods and urines were analyzed so that the minute excretion of phosphate in the bladder urine and the minute tubular reabsorption of phosphate were measured. Then 200 μ of parathyroid extract were given i.v. and these examinations repeated. During the subsequent 3 hour period there was no significant change in the tubular reabsorption of phosphate. However, the amount of phosphate appearing in the bladder urine per minute increased 4-fold. The increase in phosphaturia was secondary to a slight increase in glomerular filtration rate. This caused an increased amount of phosphate to be presented to the tubule which did not respond either by increased or decreasing its phosphate reabsorption rate.

Glycogen deposition in the liver and the interscapular fat body of the mouse LOUISE MICKIEWRIGHT (introduced by J. WAITER WILSON)
Brown Univ., Providence, R. I.

A diurnal cycle of glycogen deposition in the interscapular fat body, similar to that in the liver has been observed in the mouse, this similarity is particularly evident in animals whose feeding is restricted to certain hours. Two groups of mice, aged 7 weeks and 35 weeks, respectively, were allowed to feed between 5 P.M. and 9 P.M. only. Animals were killed at 7 A.M., 3 P.M., 9 P.M., and 1 A.M., the glycogen content of the liver and fat body was studied cytochemically. The amount of glycogen in both liver and fat body was maximal at 1 A.M., at 7 A.M. there was a slight decrease and the minimal level in both was observed at 3 P.M. The glycogen cycle is less clear-cut in animals whose feeding is not restricted. In 35-week-old mice the cycles in the fat body and the liver were similar. The amount of glycogen was highest at 1 A.M. and for the remainder of the 24 hours was much lower and rather constant. In 5-week-old mice the fat body contained the maximal amount at 1 A.M. but the glycogen content of the liver was highest at 9 A.M. In these young mice the lowest content in the liver occurred at 9 P.M. but the lowest amount in the fat was observed either at 9 A.M., 1 P.M. or 5 P.M. Within the interscapular fat body the distribution of glycogen among the cells is not uniform even when the total amount of glycogen is maximal.

Certain effects in dogs of inspiring 15-30% carbon dioxide FLETCHER A. MILLER, E. B. BROWN, AND RICHARD L. VARCO (introduced by OWEN H. WANGENSTEEN) *Dept. of Physiology and Surgery, Univ. of Minnesota, Minneapolis*

Normal mongrel dogs were anesthetized with sufficient sodium pentothal to permit tracheotomy or tracheal intubation. Control respiratory mi-

nute volumes and blood pressures were recorded with the animal anesthetized below the excitation plane. Carbon dioxide oxygen mixtures containing 15, 20, 25, and 30% CO_2 were administered via an inspiratory demand valve regulator. After 30-45 minutes breathing each of these mixtures, the respiratory minute volume was measured and an arterial blood sample drawn for pH and CO_2 content. From these data pCO_2 and BHCO_3 content were calculated and compared with preanesthetic values. High pCO_2 apparently exerts an anesthetic effect as indicated by the lack of need for additional pentothal following administration of 15% or higher CO_2 mixtures. Respiratory depression did not regularly begin until the dogs breathed a mixture containing CO_2 in concentrations greater than 15%. *In vivo* plasma CO_2 absorption curves have been constructed with pCO_2 as great as 300 mm Hg. Maximum BHCO_3 concentration is usually reached at a pCO_2 of 140-160 mm Hg and from this point the increase in total plasma CO_2 is accounted for solely by dissolved CO_2 . Arterial blood pH values of 6.6 to 6.7 have been obtained regularly from the blood of dogs breathing 30% CO_2 for 30-60 minutes.

Unidirectional permeability of the vaginal wall of the rabbit N. MILLMAN,* C. G. HARTMAN, J. STAVORSKI* AND J. BOTTI* *Ortho Research Foundation, Raritan, N. J.*

As has long been known, many classes of chemicals are readily absorbed from the lumen of the vaginal tract, as indicated by their appearance in appreciable quantities in the blood. This is true of such diverse substances as salts, sugars, proteins, dyes, alkaloids, chemotherapeutic agents and antibiotics. However, the experiments here recorded indicate that the reverse process occurs only to a minimal extent, if at all. Thus one may build up tremendous concentrations of substances in the blood for long periods and yet be unable to detect any of them in the vaginal tract. Even when high local concentrations are built up, as by injections of the perineal fasciae, there is no release into the lumen. These findings were confirmed by *in vitro* tests in which the vagina was excised and used as a dialysis membrane, with the mucosal surface on the inside. Chemical and fluid shifts on both sides were noted. When the solution of a test substance was placed inside the 'bag', some of the solute was soon found to have penetrated the wall. On the contrary, when the reverse passage was tested, none of the solute was found on the inside. Occasionally, a water exchange was observed. Substances tried *in vitro* and *in vivo* experiments included NaCl, KI, glucose, antipyrine, sulfanilamide, methylene blue, neutral red, light green, safranin, human blood antigen and antibody.

Effects of pituitary growth hormone in normal and depancreatized cats A E MILMAN AND P DE MOOR (introduced by F D W LUKENS) *George S Cox Medical Research Inst, Univ of Pennsylvania, Philadelphia*

The effect of purified anterior pituitary growth hormone on glucose and nitrogen excretion has been studied in normal and depancreatized cats. Growth hormone produced nitrogen retention in depancreatized cats maintained on constant insulin dosage and food intake. Nitrogen retention occurred in spite of increased glycosuria, but the amount was less than that obtained in normal cats treated for the same 4-day periods at the same dose levels (3 and 10 mg/day). The failure of growth hormone to induce maximal nitrogen retention in animals receiving a fixed insulin dosage and lacking the capacity to augment their own secretion of insulin, supports the hypothesis that in normal animals growth hormone induces the secretion of additional insulin. After withdrawal of the growth hormone, there was an unexpectedly slow return of the glycosuria to pre-injection levels, a phenomenon which is unexplained. Glycosuria developed in one normal cat given 10 mg of growth hormone daily for 3 days. In acute experiments in fasted normal cats, and in diabetic cats deprived of insulin, the intraperitoneal injection of growth hormone produced a depression of the blood amino-nitrogen level in 2-6 hours. This observation, similar to that reported in normal and partially diabetic rats (alloxan), provides the first evidence of an effect of growth hormone on nitrogen metabolism completely independent of insulin.

Recording two-channel photofluorometer for in vivo studies with fluorescein DAVID MINARD AND MAYNARD EICHER *Naval Med Research Inst, Bethesda, Md*

To measure time of appearance of fluorescein after intravenous injection and to record rate of change in fluorescence in 2 tissue areas simultaneously, a recording 2-channel photofluorometer has been constructed, each unit utilizing a photomultiplier tube (RCA 931A) as the photosensitive element and a GE F-5000 mercury vapor lamp as the ultraviolet source. The paired elements are fixed in either arm of a V-shaped holder with a one-inch window at the apex. For deeply situated organs, polished quartz rod extensions serve as 2-way conductors of activating ultraviolet and emitted fluorescence. Dark purple ultraviolet transmitting filters (Corning No 5970) over the U-V sources and yellow filters (Corning No 3486) over the photomultipliers, insure selective sensitivity to wave-lengths emitted from activated fluorescein. An electronically regulated power supply provides stable D C voltages up to 1200 volts to each photomultiplier. The outputs from

the photomultipliers are read directly from panel microammeters or recorded by oscillographic galvanometers (Consolidated type 7-115) with a slit camera. Additional channels permit simultaneous recording of other physiologic variables, such as blood pressure and urine flow. Galvanometer and microammeter sensitivities can be reduced in fixed steps. Bucking current circuits permit zero adjustment at high outputs. The units are standardized against films of graded fluorescence. The instrument is useful in studies on circulation time to brain, skin, kidneys and other areas and in detecting vasomotor reactions in skin and kidneys resulting from the action of drugs, asphyxia, anoxia and nerve stimulation.

Studies on renal cortical ischemia using the recording photofluorometer DAVID MINARD AND ELLIOTT F OSSERMAN (introduced by ALBERT R BEHNKE) *Naval Med Research Inst, Bethesda, Md*

To record repeated observations on both kidneys simultaneously the 2-channel photofluorometer described elsewhere by Minard and Eicher was used in studies of renal cortical ischemia resulting from epinephrine injection, splanchnic nerve stimulation, and asphyxia in anesthetized monkeys, dogs, and rabbits. Arrival of sodium fluorescein at each kidney surface following rapid controlled intravenous injection of small dye doses (2 mg/kg) is recorded as a rising and falling curve of typical form. Failure of injected dye to produce an appreciable increase in fluorescence is the criterion for ischemia. Blood pressure, injection time, and in some experiments urine flow from each kidney are also recorded. Asphyxia and epinephrine in rabbits regularly blocks dye appearance. Epinephrine and splanchnic nerve stimulation in monkeys and dogs produce ischemia but asphyxia is relatively ineffective. In the rabbit denervating one renal pedicle abolishes ischemic responses to asphyxia but not to epinephrine. Preliminary studies indicate anoxia rather than CO₂ accumulation to be responsible for asphyxial ischemia in the innervated rabbit kidney. Active and passive changes in effective area of surface vessels of the intact stained kidney are manifested as changes in fluorescence. Thus in the monkey epinephrine and splanchnic nerve stimulation cause pronounced increases in kidney fluorescence. Bradycardia from peripheral vagal stimulation is associated with a slow rise in fluorescence during periods of asystole and a rapid fall during systole. However no significant changes in kidney fluorescence or in appearance of the typical curve following dye injection result from central sciatic stimulation or bilateral carotid occlusion.

Proteolytic systems in plasma active at acid reactions I ARTHUR MIRSKY, PERRY FUTTERMAN*

AND ROBERT H. BROTH-KAHN *May Inst for Med Research, Jewish Hospital, Cincinnati, Ohio*

Previous studies have demonstrated that pepsinogen is secreted, in part, directly from the gastric peptic cells into the blood stream. Efforts have been made to detect and measure the quantity of circulating pepsinogen. During these studies, it was noted that human and animal plasma contained at least two proteolytic enzymes active at acid reactions. One of these systems exhibits maximum activity at pH 3.5 and does not originate in the gastric mucosa. Another system is active at pH 2 to 2.5. The concentration of this latter system in the blood is apparently related to the activity of the gastric mucosa. It has been demonstrated that much of this activity cannot be attributed to the activation of pepsinogen but is related to the existence in the blood of another proteinase which, like pepsin, is also active at acid reactions. The distribution and some properties of these systems will be described.

Factors influencing intrarenal pressure. A. V. MONTGOMERY (introduced by E. L. PORTER) *Dept of Physiology, Univ of Texas School of Medicine, Galveston*

Intrarenal pressure (IRP) can be dramatically affected by various experimental procedures. Complete renal arterial occlusion or renal decapsulation caused a decrease in IRP. Complete renal venous occlusion or an increase in ureteral pressure causes an increase in IRP, but the effect of either manipulation is diminished by decapsulation. When the renal vein is totally occluded, the IRP is about the same as the pressure on the renal side of the occlusion. That arterial pressure *per se* is not an important component of IRP is indicated by the lack of correlation between systemic arterial pressure and IRP. From these results it is concluded that IRP is similar to the pressure of a fluid contained in an elastic bag. Inside the bag, as well, there are 2 discrete and elastic compartments also filled with fluid. These may be designated as the vascular compartment and the tubular (nephron?) compartment. Any condition which causes dilation of either of these compartments or which causes contraction of the elastic wall of the bag will produce an increase in the pressure of the fluid (IRP) in the bag. It seems likely that the tubular compartment is dilated by increasing ureteral pressure and that an increase in renal venous pressure causes a dilation of the thin-walled venous side of the vascular compartment. But because the walls of the arterial side of the vascular compartment are relatively rigid, changes of pressure within them do not influence IRP.

Critique of the use of sulfanilamide and D₂O for determination of total body water in dogs. P. O. 'B. MONTGOMERY AND M. J. FOGELMAN

(introduced by ARTHUR GROLLMAN) *Dept of Experimental Surgery, Southwestern Med School, Univ of Texas, Dallas*

In the course of studies designed to measure rates of movement of water between various solvent compartments within the body, simultaneous total body water determinations, using D₂O and sulfanilamide were performed on dogs. A known amount of D₂O (0.5 cc/kg/bw) and sulfanilamide (20 mg/kg/bw) were injected intravenously into unanesthetized dogs. Venous blood samples were drawn during intervals up to periods of 2 hours. D₂O was determined by the falling-drop method and sulfanilamide by the method of Bratten and Marshall. Results indicate that equilibration of D₂O or sulfanilamide in whole blood is not attained after 2 hours. Values for total body water calculated from the disappearance curves of D₂O from whole blood vary from 32% to 90% of body weight with an average of 54% body weight. Values for total body water calculated from the disappearance curves of sulfanilamide from whole blood vary from 52% to 97% body weight with an average of 74%. Figures for total body water obtained simultaneously the 2 methods for individual animals cannot be correlated. These discrepancies may be explained by variations in internal diffusion area, and changes in internal convection forces.

Physiological effects of warming, cooling, and exercising the feet during prolonged radial acceleration. LAURENCE E. MOREHOUSE, RICHARD E. HUGHES,* ELIZABETH M. PRANGE* AND JANET WESSEL* *Depts of Physical Education and Aviation Medicine, Univ of Southern California, Los Angeles*

The ability to withstand footward acceleration in aircraft is related to shifts of body fluids. Heat, cold, and exercise in sufficient quantities to alter circulation were applied to the feet before and during 3 minute runs at 3.5 g on the human centrifuge. Ear opacity and pulse, thermistor temperature measurements, and foot size determinations were recorded in 9 male subjects. When the feet were first immersed in hot water before centrifugation, the circulation to the head was diminished. No further circulatory embarrassment was observed during acceleration and the recovery was more rapid when the feet were edematous due to heat. Immersing the feet in ice water raised the pulse rate but did not affect the head circulation either during acceleration or recovery. Exercise also increased the pulse rate but slightly reduced head circulation during acceleration and recovery. It is felt that foot temperature and exercise probably do not affect physiological responses to acceleration enough to influence g endurance.

Investigation of polarometric method for oxygen tension in blood with a rotating platinum elec-

trode EDWARD H MORGAN AND GABRIEL G NAHAS (introduced by HOWARD B BURCHELL) *Mayo Foundation, Rochester, Minn*

In accordance with principles outlined by Kolt-hoff and by Laitenen, a rotating platinum electrode and external circuit were constructed and adapted for measuring oxygen tension in 1.5 ml samples of whole human blood. Coating of the platinum electrode with a film of silicone (dri-film 9987) was found to delay the loss of reactivity of the platinum surface when it was in contact with blood. In samples of blood equilibrated with mixtures of O_2 and CO_2 , in tonometers at $25^\circ C$, the diffusion currents obtained at an applied EMF of $-0.8 V$ varied not only with pO_2 but also with pCO_2 . When the pCO_2 was maintained within ± 3 mm Hg, current values were linearly proportional to pO_2 in the range from 50 to 250 mm pO_2 . Increase in pCO_2 yielded higher current for a given pO_2 . The mean difference of duplicate measurements of the diffusion current obtained from 28 blood specimens at various oxygen tensions was 0.16 microampere (range 0.068 microampere) which was equivalent to 3.3 mm Hg pO_2 (range 0-14.3 mm Hg). Thus far, however, sensitivity and reproducibility of results adequate for routine use have not been attained because of uncontrolled and not completely understood vagaries of the platinum electrode.

Failure of cold pressor response to correlate with personality tests CAMPBELL MOSES, DOROTHY W HULMER* AND DORA CAPWELL* *Addison H Gibson Lab, Univ of Pittsburgh, and the Allegheny Vocational Counseling Center, Pittsburgh, Penna*

The cold pressor response of Hines and Brown (*Am Heart J* 11 1, 1936) and several personality tests were studied in 167 selected individuals. Of this group 60 gave a normal pressor response and 60 were classified as hyperreactors. Forty-seven subjects with essential hypertension were similarly tested. No significant correlation between the cold pressor response and the Bernreuter Personality Inventory, the Rosensweig Picture Frustration Test, the Guilford-Martin Personnel Inventory or the Kuder Preference Record was obtained in either the normotensive, the hypertensive or the hyperreactor subjects. It is suggested that the cold pressor response is of such complex origin that it cannot be correlated with a single personality test.

Comparison of step-up and treadmill exercise using arterial blood and respiratory gas exchange measurements HURLEY L MOTLEY AND JOSEPH F TOMASHEFSKI* *Cardio-Respiratory Lab, Barton Memorial Division of Jefferson Hospital, and Dept of Medicine, Jefferson Medical College Philadelphia, Penna*

A comparison of the variations in respiratory gas exchange measurements during rest and exercise is valuable for appraisal of pulmonary function status in man. The step-up exercise test (30 step-ups 20 cm high in one minute) was compared with treadmill exercise (rate and tilt adjusted so that the patient can continue for 15 minutes). Gas and arterial blood samples and pressures were taken after 10 minutes of treadmill exercise (allowed to attain a steady state). Studies on 54 patients with fibrosis and various degrees of pulmonary emphysema are reported.

	ART O_2 SAT	O_2 CONS	CO_2 OUTPUT	VENTIL. VOL.
	%	cc/sq m/min		l/sq m/min
Rest	92.9	145	118	4.7
Step-up	91.6	474	355	12.2
Treadmill	93.4	484	400	12.6

In 17 cases the treadmill exercise produced a slight decrease in arterial oxygen saturation, in 24 there was a slight increase and in 13 there was a significant increase (3% +). Step-up exercise is a more severe test for the emphysematous patient than a treadmill exercise which can be maintained for 15 minutes. Respiration rate, blood pressure, pulse rate and percentage of oxygen removed did not vary significantly between the two methods of exercise, but in the 36 cases with a significant degree of emphysema (residual air over 35% of total lung volume) the arterial oxygen saturation was decreased more with step-up exercise. The step-up test is as satisfactory as the treadmill in providing information essential for evaluation of pulmonary function impairment.

Polarographic analysis of spinal fluid as an aid in the diagnosis of poliomyelitis OTTO H MÜLLER *Dept of Physiology, Syracuse Univ College of Medicine, Syracuse, N Y*

In a polarographic study of spinal fluid in poliomyelitis and other diseases it was found that the procedure used for determination of the 'protein index' (MÜLLER AND DAVIS, *Arch Biochem* 15 39, 1947) was not applicable because of the small amounts of protein involved. The following technique, similar to that of Homolka and Krupička (*Čas lékařu čes* 87 1326, 1948) was more suitable. To 1.0 ml of spinal fluid is added 1.0 ml of 0.1 N KOH. Of this mixture, a first sample of 0.8 ml is added immediately to 2.0 ml of a buffer consisting of 0.001 M $Co(NH_3)_6Cl_2$, 0.1 N NH_4Cl and 0.1 N NH_4OH and then analyzed polarographically. A second sample of 0.8 ml, taken after 45 minutes is similarly analyzed. The results of the last analysis are expressed in percent of the starting values. In an investigation of over 120 spinal fluids it was

found that in 'normal' samples not only were the starting values small but also the changes produced by digestion. In contrast to this the starting values in poliomyelitis are usually very much higher and increase by 100% or more during digestion. Observations made so far indicate that this change from normal can be correlated with intensity of the disease. Return to normal during convalescence appears to be slower for the polarographic values than for other clinical findings. These results suggest that the proteins of spinal fluid not only change in quantity but also in quality in poliomyelitis.

Uptake of phosphate by frog axons L. J. MUIRHEAD
(introduced by FRANK BRINK) *Dept of Biophysics, Johns Hopkins Univ., Baltimore, Md*

A method has been devised for continuously recording the variation in P^{32} content of axons, while allowing electrical stimulation, changes in gas tensions, or other chemical variations in the surrounding fluid. Over a period of 10 hours, a nerve at 20°C takes up P^{32} to a concentration 5 to 10 times that of the external solution. Phosphate uptake at 0°C in O_2 or at 20°C in N_2 , or at 20°C in O_2 with 0.1 mM azide, is zero. The temperature coefficient of uptake (Q_{10}) ca. 2.5 over the range 10-30°C. At temperatures higher than 30°C the axon deteriorates rapidly with respect to conduction but P_3 uptake is maintained for several hours after conduction has failed. Removal of the connective tissue sheath has been shown to increase the P^{32} uptake to slightly more than twice the normal rate. Stimulation at frequencies of from 60-120/sec completely blocks the uptake of P^{32} .

Effect of repeated injections of epinephrine on self-selection of diet in rats, with special reference to sodium and potassium LOUIS P. MUNAN* AND ERRETT C. ALBRITTON *Dept of Physiology, George Washington Univ. School of Medicine, Washington D C*

Subcutaneously injected epinephrine altered, in opposing fashion, the self-selection of salt solutions offered continuously to two strains of rats. Female rats of the Sprague-Dawley and Long-Evans strains were divided into four groups of ten each and maintained on a synthetic diet free of sodium, potassium, chloride, and inorganic phosphate. They were offered separately water and 3% solutions of the missing salts, namely, NaCl, NaH_2PO_4 , KCl, and KH_2PO_4 . Epinephrine injections were given once and later twice daily in isotonic saline in dosage of .02-.04 mg epinephrine/100 gm of body weight. The control groups received an equal volume of isotonic saline at the same time intervals. In the Long-Evans rats, the Na/K intake ratio was smaller in the experimental than in the control group due largely to an increased potassium intake by the experimental animals. In the Sprague-Dawley rats, the Na/K

intake ratio was greater in the experimental than in the control group, due largely to an increased sodium intake by the experimental animals. These findings point to a species difference in the salt function of the adrenal cortex under epinephrine stress.

Net splanchnic glucose production in normal man and in various disease states JACK D. MYERS
(introduced by WALTER KEMPNER) *Dept of Medicine, Duke Univ. School of Medicine, Durham, N C*

The hepatic blood flow, by the bromsulphalein technique, and the glucose difference between hepatic venous and arterial bloods has been measured in a group of 43 control human subjects and in a series of patients with various diseases. In the fasting state, the hepatic venous glucose concentration always exceeds the arterial concentration. Thus the product of the hepatic blood flow (HBF) and the hepatic venous-arterial glucose difference (GD) provides an estimation of the net splanchnic glucose output (SGO). This quantity is less than the actual hepatic glucose production by that amount of glucose which is utilized by the various viscera of the portal venous circulation. The mean HBF in 43 control subjects was 812 ± 24 (S.E.) ml/minute/square meter of body surface. The mean GD was 8.7 ± 0.4 mg%. This provides a mean SGO of 68 ± 4 mg/min/m². In 9 patients with diabetes controlled by insulin, neither the GD nor the SGO varied significantly from the control values. Twelve patients with Laennec's cirrhosis of the liver had a mean GD of 7.7 ± 0.7 mg% (not significantly subnormal) but a reduction in SGO to 52 ± 5 mg/min/m² because of moderate decreases in HBF. In 8 subjects with congestive heart failure in whom the HBF was strikingly reduced, the GD was only moderately increased to 11.5 ± 1.0 mg%, providing a SGO which was subnormal at a value of 50 ± 4 mg/min/m². Ten patients with hyperthyroidism had mild but not significantly elevated GD and SGO.

Application of buffered paper strips to the chromatography of polymyxins HAROLD A. NASH AND A. R. SMASHER (introduced by CARL A. BUNDE)
Research Dept., Pitman-Moore Co., Indianapolis, Ind

To achieve resolution of the polymyxins, the use of strips treated with buffer solutions previous to descending development with n-butanol was investigated. In the acid range, R_f values were found to be less affected by the pH of the buffer than by the concentration of salts in the buffer system. In a given system and at a fixed pH, the R_f values could be partially correlated with distribution coefficients and with hydration of the paper strips as affected by the buffer system. At low buffer concentrations, where hydration effects were relatively constant, different salts were

found to have different effects on Rf values even when the effects on distribution coefficients were the same. In general, di- and trivalent anions were much less effective in increasing Rf values than the univalent anions tried. By proper choice of buffer system, the Rf values of the polymyxins could be manipulated from less than 0.1 to 1.0. For routine work the buffer system, 0.2 M glycine to pH 2.5 with concentrated HCl was adopted. With this system Rf values of the polymyxins were A, 0.18, D, 0.38, E, 0.54, and B, 0.56.

Electrical activity of single muscle fibers at the neuro-muscular junction

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Utilizing the technique previously reported (NASTUK, W. L. AND A. L. HODGKIN *J. Cell & Comp. Physiol.* In press) microelectrodes have been inserted into single muscle fibers of frog sartorius at the neuro-muscular junction. The muscle was placed in Ringer's solution (22°C), single fibers were impaled at a point averaging 42 μ from the center of the junction, and a stimulus was applied to the nerve. The recordings show an end plate potential which rises sharply from the baseline to 42 mv (av) in 0.3 msec. At this level a propagated action potential originates and the membrane is depolarized more rapidly. Repolarization begins at the usual rate but is apparently hindered by the endurance of the original end plate disturbance as evidenced by an abrupt diminution in rate. Average data obtained at the junction are: resting potential 90 mv, action potential 117 mv, peak end plate potential 101 mv. Average data obtained at a point distant from the end plate are: resting potential 91 mv, action potential 123 mv. After 30 min equilibration in Ringer's solution (23.8°C) containing d-tubocurarine chloride (1.4 μ M/l) blocking of some junctions occurs. At such junctions the end plate potential is greatly diminished or absent. For functions still functioning, the end plate potential rises more slowly requiring 0.5 msec to reach 46 mv (av), at which level a propagated action potential originates. Typical data obtained at a point averaging 55 μ from the center of the junction are: resting potential 90 mv, action potential 116 mv, peak end plate potential (when measureable) 106 mv.

Influence of muscular exercise on uric acid excretion in man

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Strenuous exercise (running on a motor-driven treadmill for 1 minute at 10 miles/hour, 17.5% grade) during a state of water diuresis depressed urine flow, glomerular filtration rate (allantoin clearance) and uric acid clearance to 30, 40 and

30% respectively of the pre-exercise values. Urine flow and glomerular filtration rate returned to normal within 10 minutes after cessation of exercise, but uric acid clearance remained depressed for about one hour. Following the suggestion of Quick (*J. Biol. Chem.* 110: 107, 1935) that the excretion of uric acid is influenced by the blood lactate concentration, a second series of experiments was performed. A 10-minute period of moderate exercise (treadmill running at 6 miles/hour, zero grade) resulted in a very small rise in blood lactate and a slight depression of uric acid clearance. When, however, the subject breathed 8% oxygen in nitrogen during the same type of experiment, the rise in blood lactate was considerably greater and there was a marked depression of uric acid clearance. It is concluded that the diminished clearance of uric acid during exercise and recovery is related to the elevated blood lactate level. The basis of this effect is being investigated.

Simultaneous blood volume determinations in dogs with dye (T-1824), carbon monoxide and radioactive iron Fe^{55}

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Measurements of the blood volume in dogs were made using simultaneously the three following techniques: 1) the T-1824 dye method as described by Gregersen, 2) the carbon monoxide method according to the technique developed by Roughton and Root, and 3) the radioactive iron (Fe^{55}) method using tagged erythrocytes from a donor dog. Beaker experiments on known volumes (50 cc) of blood were run concurrently with the *in vivo* tests in order to check the accuracy of the radioactive iron determinations. In 16 beaker experiments the mean volume determined by radioactive iron differed by only one-half of one % from the true volume; the standard deviation being 3%. In 7 technically satisfactory experiments, the mean value of the blood volume measured by the carbon monoxide method was 2% above the value obtained with T-1824, whereas the mean value obtained with the iron method was 11% below that given by the dye. The latter difference is beyond the limit of experimental error. It is therefore considered to be of physiological significance and not explainable by lack of precision in any of the methods.

Responses of blood vessels of the bat's wing to epinephrine and nor-epinephrine

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Direct microscopic observations were made of

wing vessels in unanesthetized animals with their wing nerve supply either normal or acutely sectioned, or partially degenerated. The substances tested were administered in various ways of which the most efficient was direct topical application on a small area from which the epithelium had been removed on one side. This procedure produced minimal injury, as evidenced by the normal flow, vasomotion and responses of the exposed vessels. Effective concentrations of either epinephrine or nor-epinephrine always constricted the arteries and arterioles. Dilatation was never observed. In structures that normally exhibit rhythmical active vasomotion, such as terminal arterioles, pre-capillary sphincters and venous vessels, both substances increased the magnitude and duration of the constrictor phase. Minimal effective concentrations of applied solutions of epinephrine were between 1×10^{-9} and 5×10^{-8} gm/ml. Nor-epinephrine in comparable tests usually appeared slightly less effective. However, the pH of the applied solutions, the time elapsing between the final dilution and application to the test site, and several other factors so modified the apparent minimal effective concentrations thus determined as to raise serious doubts of the significance of comparing functional responses in terms of limited quantitative differences.

Effects of azide and ATP on retinal potentials

WERNER K. NOELL (introduced by HARRY F. ADLER). *USAF School of Aviation Medicine, Randolph Field, Tex*

In rabbits, rapid intravenous injection of azide is immediately followed by a rise of the resting potential of the eye which within 3 seconds reaches values more than 5 mv above the control level. The decay of this potential depends on the dosage, being a matter of seconds with 1 mg sodium azide/kg body weight. With some delay the rise of the resting potential is followed by an increase in the rate of the spontaneous discharges of the optic fibers reaching values comparable to those obtained by strong illumination. There are also marked changes in the electroretinogram. Reduced and enhanced responsiveness of all components of the electroretinogram are related to the momentary size of the potential change induced. The effect of azide on the electroretinogram is qualitatively similar in frog, cat and rabbit, while the effects of anoxia and iodoacetate vary with the species. The action of azide is interpreted as an inhibition of adenosinetriphosphatase. Essentially the same effects can be obtained by intracarotid injection of adenosinetriphosphate (ATP). Late effects, however, of azide and ATP differ significantly. It appears that ATP and ATPase are essential links in the mechanisms which lead to retinal excitation after illumination.

Slow intravenous injection of large amounts of azide causes inexcitability of optic nerve fibers in rabbits. This may result from its action on other enzyme systems.

Metabolism of tetraethylthiuramdisulfide (Antabuse) WIKTOR W. NOWINSKI, THOMAS P. EDWARDS* AND JAMES P. ELLIS* *Dept of Neuropsychiatry and Tissue Culture Lab, Univ of Texas, Med Branch, Galveston*

It was shown in this laboratory (EDWARDS, *Texas Repts Biol Med* 7: 684, 1949) that Antabuse inhibits 85% of cellular respiration as measured by the oxygen uptake of rat liver homogenates (Warburg technique). In an attempt to elucidate the mechanism of the breakdown of Antabuse in the body, the possible reduction of the molecule was investigated with the Thunberg method. It was found that the reaction time of the digests containing rat liver homogenate and Antabuse (with alcohol as substrate) was 2 to 3 times longer than that without Antabuse. From these results, it was supposed that Antabuse may act in the body as a competitive hydrogen acceptor (at least 4 hydrogens seem to be necessary in order to reduce one molecule of tetraethylthiuramdisulfide) and that inhibition of cellular respiration, as demonstrated by Edwards, could be attributed to this process. To prove this hypothesis, experiments in Warburg manometers were carried out, in which ascorbic acid acted as an additional hydrogen donor. It was found that the inhibition caused by Antabuse could be completely overcome by addition of 20 mg ascorbic acid/Warburg vessel, the actual figures of averages being -125.16 cmm oxygen uptake in one hour, for homogenate plus ascorbic acid and -137.34 for homogenate with Antabuse and ascorbic acid, whereas Antabuse alone gave an inhibition of oxygen uptake of practically 100%. In experiments where smaller amounts of ascorbic acid were present in the system, the reversal of inhibition was accordingly diminished, the averages were -117.21 for control (homogenate and ascorbic acid), -83.18 for Antabuse and ascorbic acid, and -32.87 for homogenate with Antabuse only. In these experiments, 6 mg ascorbic acid vessel restored over 50% respiration. From these experiments, we conclude that tetraethylthiuramdisulfide acts as a competitive hydrogen acceptor and is the cause of inhibition of cellular respiration.

Further proof of the aspiratory effect of inspiration on right atrial inflow DAVID F. OPDYKE AND WILLIAM H. VAN NOATE* *Dept of Physiology, Western Reserve Univ Med School, Cleveland, Ohio*

Although the preponderance of evidence supports the view that blood is aspirated into the

thorax by the act of inspiration, there are opinions to the contrary. It is established that effective right atrial pressure increases during inspiration. However, such changes can be explained either by an increased pulmonary resistance or by an increased right atrial inflow. Indirect evidence that the inspiratory increase of effective right atrial pressure is due to an increased atrial inflow is provided by careful measurements of phasic effective right atrial pressure. The slope of the effective right atrial pressure gradient during the interval from the beginning of ventricular isometric contraction (Z point) to just before the opening of the tricuspid valve (V point) is dependent only upon the volume of atrial inflow since during this time there is no ventricular filling. In every case studied the slope of the Z to V gradient increased during inspiration.

Blood levels of cyclopropane and ether in relation to epinephrine-induced cardiac arrhythmias

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Kety (personal communication) has developed a method for determining cyclopropane and ether in blood when both are present. Epinephrine (0.2–0.4 cc of 1:10,000/kg, rapidly) by vein caused ventricular premature systoles and tachycardia in 18 dogs at blood levels of 4.2 to 9.97 vol % cyclopropane. Three dogs developed ventricular fibrillation at 7.69 to 8.33 vol % when the dose of epinephrine was increased. Ether in blood concentrations of 0.30 to 1.70 cc/liter in 8 dogs decreased or entirely prevented the premature systoles, ventricular tachycardia and attendant falls in blood pressure. Analysis of the site of origin of these arrhythmias by simultaneous recording of three unipolar leads (NAHUM, CHERNOFF AND KAUFMAN *Am J Physiology*, 157:248, 1949) has indicated that most of these originate in the right ventricle (13 out of 20) equally distributed between anterior and posterior surfaces.

Relation between normal human electroencephalogram and Rorschach test factors

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An attempt was made to correlate the EEG alpha rate and index with Rorschach test factors on 99 medical students and school personnel. Cortical potentials were recorded from prefrontal and occipital electrodes under standard conditions. Alpha index was determined for a 30 second tracing for both cortical areas, alpha frequency, by the time required for 5 alpha waves measured in 5 different parts of each record. The Rorschach test was given by the group method of the Har-

rower-Erickson and Steiner (Springfield C C Thomas, 1945) with written responses to a 3-minute review of each blot, followed by a 2-minute review to allow orientation of responses and classification as to F, M, C, CF, FC and Sh. The latter period constituted a modified associational period. The written responses were scored according to Rorschach and Beck, (*Rorschach's Test, I and II* New York Grune and Stratton, 1946). The Rorschach test factors A%, Z, TR, M/sum C, C + CF/FC, and P were plotted against the alpha index, and A% and TR against alpha rate. Although the plot of the composite factor C + CF/FC (here used as a stability indicator) against alpha index has a linear relationship, the statistical evaluation revealed no positive correlation coefficient value. The lack of a positive quantitative correlation between the alpha activity and personality type under these test conditions when compared with the general relationships suggested by other workers (Trans Am Neurol 63:167, 1937, *Brain* 59:366, 1936), may be attributed to inherent difficulties in making accurate measurements of personality factors and alpha indices.

Studies on acclimatization of mice to high carbon dioxide and to low oxygen

ARTHUR B OTIS *Dept of Physiology and Vital Economics, Univ of Rochester School of Medicine and Dentistry, Rochester, N Y*

Mice will survive for at least a week when placed in a mixture of 20% CO₂, 21% O₂ and 59% N₂. Such an exposure increases the tolerance of the animals to 35% CO₂ but sudden removal to room air may result in convulsions which are often fatal. If the CO₂ concentration is reduced more gradually no difficulty is encountered. Normal mice when exposed to 35% CO₂-67% O₂ retain the righting reflex for only about 3 minutes, but mice which have been exposed for one week to 15% CO₂ retain the righting reflex for at least 30 minutes when exposed to the higher concentration. If mice which have been living in 15% CO₂ are removed to air, the tolerance to 35% CO₂ becomes, after 3 days, the same as that of normal mice. The principal changes observed to occur in mice chronically exposed to 15% or 20% are an apparent increased ventilation, a drop in body temperature, loss of weight and an increase in the alkaline reserve of the blood which is of the magnitude necessary to return the plasma toward the normal pH. Mice which have been exposed to 10% oxygen for 7 days show the same tolerance (as judged by time of maintenance of the righting reflex) to 35% CO₂ as do normal mice.

Ionic alterations in chickens infected with *P. gallinaceum* RICHARD R OVERMAN, ANNE C BASS* AND T H TOMLINSON, JR* *Dept of*

Physiology, Univ of Tennessee College of Medicine and Lab of Tropical Diseases, USPHS, Memphis, Tenn

Flame photometric analyses for Na and K and chemical analysis for Cl were carried out on both plasma and packed erythrocytes of 1) normal chickens, 2) chickens infected with *P. gallinaceum* and showing a) 1-100 parasitized cells per 10,000 RBC, b) 100-3000, c) 3000-5000, d) 5000-7500 and e) over 7500, 3) chickens which had spontaneously recovered from a malaria infection, 4) a series of 'refractory' chickens, and 5) chickens inoculated with EE forms of *P. gallinaceum*. Normal values (average of 9 determinations) were as follows: Plasma Na, 153.7 mEq/l, Plasma K, 6.0 mEq/l, Plasma Cl, 120.7 mEq/l, Erythrocyte Na, 18.0 mEq/l, erythrocyte K, 118.8 mEq/l, Erythrocyte Cl, 127.9 mEq/l. Generally speaking, progressive alterations in ion distribution in infected chickens of group 2 occurred as follows: 1) a reduction in plasma Na concentration, 2) a rise in plasma K, 3) a reduction in plasma Cl, 4) an increase in cellular (erythrocyte) Na and, 5) a reduction in cell K. In chickens showing the higher parasitemias the increase in plasma K averaged 48%, the increase in erythrocyte Na 298%, and the reduction in cell K averaged 36%. Chickens which had 'recovered' showed normal average ionic concentrations in both plasma and cells as did the 'refractory' animals and those inoculated with EE bodies. These changes are entirely similar to those reported by Overman for *P. knowlesi* infected monkeys and human with *P. vivax* or *P. falciparum* infections.

Water and electrolyte changes in rat intestine after total body x-radiation E. E. PAINTER AND E. W. PULLMAN * *Univ of Illinois, College of Medicine, Chicago, Ill*

Preliminary experiments carried out on the small intestine of 220-gm male rats before and after a single dose of total body x-radiation (LD₇₀) indicate that increases in sodium content can be correlated with decreases in potassium and with increases in chloride and water content in the first few days after radiation. These increases in sodium content of the intestine have been confirmed by radioactive sodium studies reported earlier by us (*Quart Report, Biol Div, Argonne National Laboratory*, p 88, Nov, 1948). That increase in sodium is in part related to an increase in extracellular fluid content of x-irradiated animals and in part related to the exchange of sodium for potassium in gut cells appears to be a valid conclusion from our results.

Molecular diffusion and filtration from the capillary circulation of mammalian muscle J. R. PAPPELHEIMER, E. M. RENKIN* AND L. M. BOR-

RERO * *Dept of Physiology, Harvard Med School, Boston, Mass*

Inulin, sucrose, glucose, urea or NaCl were introduced suddenly into arterial blood supplying the isolated perfused hindlimb muscles of the cat. The osmotic activity of each molecular species tending to draw fluid from tissue spaces to capillary blood was counterbalanced by continual adjustment of mean capillary pressure by known amounts just sufficient to prevent net fluid exchange. Under these conditions the changes of mean capillary pressure with time are considered to reflect the diffusion rates of the test molecules across the capillary walls and into the tissue spaces. The half-time of the diffusion process is approximately inversely proportional to the free diffusion constant of the molecular species and to the ease with which fluid passes through the capillary membranes under the influence of a known hydrostatic head of pressure (the filtration coefficient). The half-times are independent of blood flow and of tissue fluid volume in the ranges so far investigated. The data indicate that diffusion of substances across the capillary walls occurs many times more slowly than through a watery solution of equivalent surface area (free diffusion). If the capillary exchange occurs through cylindrical pores or rectangular slits in the capillary walls then the collective area of such openings is less than 2% of the total capillary surface. Combination of filtration data with diffusion data is theoretically capable of yielding information concerning the number and dimensions of ultramicroscopic openings in the capillary walls which would account for the experimental results.

Glucose, pH and potassium retention in red cells

A. K. PARPART AND JAMES W. GREEN * *Dept of Biology, Princeton Univ, Princeton, N. J*

Defibrinated rabbit blood was incubated at 35° C for periods up to 50 hours and a study made of the potassium-sodium exchanges which occurred in the red cells at intervals during this time under a variety of pH and glucose changes. Experiments were made to compare the influence of pH and of glucose utilization on potassium retention. Potassium retention has been studied at several pH's (6.2-7.4) with and without the addition of glucose. Phosphate buffers were used. Results indicate that while glucose utilization is an important factor, there is a very marked, and even over-shadowing, effect of pH, within a certain range, on potassium retention irrespective of glucose utilization. In addition, effects of inhibitors of glycolytic activity have been examined.

Absorption of vibratory energy by human body surface H. O. PARRACK, HENNING VON GIERKE, *

HANS OESTREICHER* AND WOLF W. VON WITTERN* *Aero Med Lab, Air Materiel Command, Wright-Patterson Air Force Base, Dayton, Ohio*

Measurements were made of the response of the body surface to mechanical vibrations. Three methods were used in overlapping frequency ranges so as to span a total frequency range of 20-20,000 cps. The results are consistent and describe the mechanical impedance of the body surface and the elastic properties of the tissue. The impedance consists of a frictional resistance and reactance. The resistance is proportional to the square root of the frequency. The reactance is an elastance varying inversely with frequency up to about 50 cps where it becomes zero. Above this frequency the reactance is an inertance that is proportional to frequency throughout the measured range. The vibratory energy absorbed at the body surface and converted to heat in the tissues may be calculated from the impedance. From these results was developed a theory on the mechanical behavior of the vibrating body tissue that considered the tissue as an elastic medium with viscosity. For such a model, and for the frequency range 20-20,000 cps, equivoluminal shear waves with velocities less than sound are predominant. It is only at still higher frequencies that compression waves play an important role. The theory also explains the surface waves observed experimentally.

Changes of electrical potential of the ventral roots of the spinal frog resulting from reflex stimulation LLOYD D. PARTRIDGE* AND ROBERT GESELL *Physiology Laboratory, Univ of Michigan, Ann Arbor*

Single and repetitive electrical stimuli were delivered by a square wave generator. Changes of electrical potential were recorded with the aid of a D C amplifier and a cathode ray oscillograph. One leading off electrode was placed on the ventral root near its point of exit from the cord, the other electrode about one cm. more distal. Each stimulus was found to produce a sudden increase of negativity of the proximal electrode with respect to the distal electrode, possibly the result of a sudden liberation of acetylcholine at the synapse. The rate of subsequent decay of this negativity was in approximate agreement with the law of mass action as applied to enzymatic destruction of acetylcholine. Repetitive stimulation showed, in agreement with Barron and Matthews, a step-like increase of initial negativity. Such increased negativity was followed by a correspondingly increased rate of initial decay at cessation of stimulation. Action potentials, when they occurred, varied in number directly with intensity of the slow potentials. These findings are thought to support the theory of temporal summation

reported by Frev and Gesell at the 1949 meeting of the Physiological Society. They do not, however, preclude the participation of several factors contributory to the decay of electrotonic potentials.

Studies on in vitro inactivation of alpha-estradiol by infantile, malignant and cirrhotic rat liver KARL E. PASCHKIS AND R. H. DEMEIO* *Div of Endocrine and Cancer Research, Jefferson Med College, Philadelphia, Penna*

Inactivation of alpha-estradiol by liver slices and liver homogenates has been extensively studied. Experiments reported here show that the liver of rats, 6 days of age, have a good inactivating power. Studies were also performed on rats treated with the carcinogen 2-acetaminofluorene, livers from animals killed at a stage where no cancer had yet developed showed good inactivating capacity. Homogenates of malignant hepatomas induced both by 2-acetaminofluorene and p-dimethylaminoazobenzene also showed good inactivating power. We have previously reported good inactivation to be present in starved rats (*Endocrinology* 43: 97, 1948) and in rats treated with carbontetrachloride (*Endocrinology* 33: 309, 1943). It is concluded that the *in vitro* action of liver upon estradiol is present early in life, and over a wide range of abnormal conditions, the system or systems involved appear to be very resistant to damage. This is in marked contradistinction to the overall handling of estrogens *in vivo*, in which the excretory function of the liver plays a large role in addition to the metabolizing action, this overall handling and disposition of estrogens *in vivo* is known to be extremely sensitive, and impaired even by minor, 'subclinical' liver damage.

Elimination of bromide from serum and cerebrospinal fluid S. I. PATRICK* AND G. S. EADIE *Dept of Physiology and Pharmacology, Duke Univ School of Medicine, Durham, N. C.*

Serial cerebrospinal fluid and serum bromide determinations were made simultaneously in 3 patients and 6 dogs with high bromide levels by a new photoelectric method described elsewhere. Bromide concentrations and percentage replacement of chloride were invariably less in the spinal fluid than in serum, this was true even during excretion, i. e. when bromide was passing out of the spinal fluid into blood and so to the kidney. This is taken as evidence for secretion of bromide into the blood stream, presumably by the Pacchionian bodies. Other evidence also indicates that spinal fluid is not in equilibrium with serum, and the ratio spinal fluid bromide/serum bromide is thought to be a physiological rather than a physical ratio. No evidence was found for increased permeability of the choroid plexus at

high bromide levels, evidence for this previously adduced by others is explained by inaccuracies in the chemical methods employed. Addition of chloride to the diet increases the rate of bromide elimination only slightly, the increase is of the order of magnitude of differences in individual rates of excretion.

Cortical receptive zone of the chorda tympani

HARRY D. PATTON AND V. E. AMASSIAN * *Dept of Physiology and Biophysics, Univ of Washington School of Medicine, Seattle*

Previous studies, combining cortical ablation and determination of taste thresholds, suggested a cortical localization of taste near the somatosensory face area. This topographical relation has been further tested by mapping the cortical area electrically responsive to stimulation of the chorda tympani. This nerve, although conveying some tactile impulses from the tongue, primarily subserves taste. In cats under deep pentobarbital anesthesia, the lingual nerve was exposed from the tongue to the base of the skull, and divided central to its junction with the chorda tympani, which was left intact. The distal trunk containing the chorda tympani fibers was stimulated with single shocks, while the cortex of the same or opposite hemisphere was explored oscillographically for evoked electrical activity. Characteristic early response of the contralateral cortex was a sharp surface-positive wave (50–200 μV) of 10–12 msec latency. Ipsilateral responses were also obtained. Responses were irreversibly abolished by transtympanic destruction of the chorda tympani in the middle ear. The responsive zone, 4–5 mm² in area, was on the rostral orbital surface, superior to the rhinal fissure and anterior to the anterior ectosylvian fissure. It lay rostral and inferior to the tactile responsive zone for the face, and in its dorsocaudal extent, overlapped the latter. The remainder of the lateral and mesial surface of the cortex was unresponsive to chorda tympani stimulation. Insular cortex around the sylvian fissure, suggested by others as the cortical taste area, was consistently unresponsive.

Contribution to oximeter design using modulated light

W. PAUL (introduced by J. K. W. FERGUSON) *Dept of Pharmacology, Univ of Toronto, Toronto, Ont., Canada*

Using a flickering lamp and A-C amplification, the linear voltage-density characteristics of selenium photocells permit a new method of electrical compensation for varying opacity in the pinna of the ear. Our instrument has the usual two color channels, with an earpiece containing a pressure capsule and a lamp modulated at 125 c/s. The amplified alternating voltage for each channel is rectified and applied to a vacuum tube voltmeter. When the pinna is squeezed, a

positive bias is applied to the rectifier to adjust each channel to a 'zero' point without affecting the curve of output current against optical density. When blood is allowed to flow, compensation for varying Hb-density ('ear thickness') is applied by adjustment of a simple shunt control set according to the 'Infra Red' indication of Hb density. The calibration of this adjustment affords a convenient means of testing an earpiece design without recourse to arterial puncture. The earpiece is applied to the pinna of the ear of a fully oxygenated normal subject. After a preliminary warming period, pressure is applied to the pressure capsule and the bias controls adjusted to a 'zero' reading on both channels. Pressure is released and after Hb-density has become stable, the shunt control is turned to give a 100% reading on the saturation scale. Shunt control readings plotted against 'Infra Red' readings for a number of subjects provide an indication of the potential accuracy of the absolute saturation. Recent earpieces give a precision indicated by a range of $\pm 3\%$ saturation.

Oxygen partial pressures during hypothermia in the dog

K. E. PENROD *Dept of Physiology, Boston Univ School of Medicine, Boston, Mass*

The extent to which hypoxia occasioned by the considerable shift to the left of the hemoglobin dissociation curve is a contributory factor to death during hypothermia has been a subject of speculation since this concept was advanced by von Werz (*Arch f exper Path u Pharmacol* 202: 561, 1943). At 20° C rectal temperature the partial pressure of oxygen in mixed venous blood has been found to be of the order of 10 mm Hg, with a per cent saturation of 50 to 60. The fundamental question becomes: Is this low pressure sufficient to furnish adequate oxygen to the tissues when the metabolism is reduced by the cold? Answers to this question have been sought in several different ways centering about comparisons between dogs breathing air and those breathing 100% oxygen, inasmuch as in the latter case the physically dissolved oxygen is probably sufficient for the oxygen consumption at low body temperatures thus making hemoglobin dissociation largely unnecessary. Comparative oxygen consumption values, oxygen content of coronary sinus blood and of internal jugular blood are presented.

Method for analyzing the mechanical properties of smooth muscles stimulated by cooling JOHN F. PERKINS, JR. AND WILLIAM H. JOHNSON (introduced by A. B. LUCKHARDT) *Dept of Physiology, Univ of Chicago, Chicago, Ill*
Evidence was presented in a previous paper (PERKINS AND NICHOLAS, *Federation Proc* 8: 1949) that cooling causes contraction of smooth muscles

apparently by acting on a different excitatory system than epinephrine, or else acts directly on the contractile system. For this reason, studies of viscous-elastic properties of smooth muscles contracting *a)* under the influence of cooling, as contrasted with *b)* epinephrine or other drugs are being undertaken using the following method. A mechanically produced sinusoidal displacement is applied to a muscle through a spring. The displacements of the oscillator and of the muscle are detected electrically by the use of 2 transducer tubes, one applied through a glass stylus to the oscillator, and the other by the same method to the junction of the muscle and the spring. Each transducer tube gives a voltage output which is proportional to the mechanical displacement. This voltage is in each case amplified and converted to a current by D C amplifiers and cathode follower impedance changers, and the resulting currents are passed through 2 simple oscillographs which record directly on a kymograph drum. By this method, the amplitude and the phase relations between the 2 displacements can be obtained, and from this the viscosity and elasticity terms can be calculated before, during and at the height of contraction, and their frequency dependence also determined. Data obtained by this method will be presented.

A strain gauge amplifier for special biological application CLARENCE V PESTEL* AND JOHN P MARBARGER *Aeromedical and Physical Environment Lab, Univ of Illinois, Chicago*

A versatile instrument for amplifying the output of a strain gauge, such as the Statham, Model P23A or B, has been fabricated and tested. It is designed primarily for use in biological research where it is desired to record such pressures as arterial, venous, respiratory, etc. The instrument is of the carrier-frequency type, complete with a direct current output stage, capable of driving a rugged, high-speed, direct writing oscillograph. It is provided with a 5-position step-gain switch, together with a fine gain control, so that any pressure from a low value to maximum gauge pressure can be made to produce full scale deflection. Another 5-position switch on the instrument permits one to select *a)* zero position, so that any amplifier changes may be detected and corrected (by controls provided) without removing pressure from the strain gauge, *b)* calibration signal, so that a constant degree of amplification can be observed and reproduced, thus allowing one to establish comparable records from day to day, *c)* absolute pressure, *d)* mean pressure and *e)* pulse wave. The maximum sensitivity of the instrument is such that a pressure of 16 mm Hg applied to a Statham gauge, Model P23A, (0-750 mm Hg) will produce an output of 105 v across

3300 ohms. With the Statham gauge, P23B (0-50 mm Hg) much greater sensitivity can be attained. The output is linear with respect to the input until the output voltage exceeds 105 v.

Observations on reflex regulation of circulation

LYSLE H PETERSON (introduced by M E MAXFIELD) *Dept of Physiology, Univ of Pennsylvania Medical School, Philadelphia*

A number of surprising responses of the circulation following stimulation of various reflex systems have been observed in over 600 normal subjects and hospital patients during physiological experiments and surgery. Certain reflex responses appear to differ in man as compared with other species, to differ also with and without anesthesia. Following carotid sinus stimulation there appears to be a primary weakening of ventricular contraction concurrent with bradycardia. The effects are in contrast to those following prolonged diastole following premature or missed contractions. Following stimulation of the mesenteric receptors there also appears to be a primary weakening of contraction independent of pulse rate. Evidence is also presented to show that under various physiological, pathological and anesthetic conditions there is a marked alteration in the manner which the cardiovascular system reacts to changes within itself. This data is presented not only to demonstrate reflex circulatory phenomena in man (some for the first time) but also to stress the need for re-evaluation of present concepts of circulatory reflexes. Such generalizations which are commonly stated as Starling's and Marey's laws and the belief that the ventricles are not directly affected by the vagi must be thought of with reservation. An understanding of reflex balance in man seems necessary for an understanding of diseases of the cardiovascular system of man.

Regional distribution of blood flow after hemorrhage with and without Dibenamine RAYMOND W PICKERING (introduced by LANE H ALLEN) *Dept of Physiology, Univ of Georgia School of Medicine, Augusta*

Using flow meters of various types, the flows through the femoral artery, portal vein, carotid artery, inferior vena cava and hepatic veins have been measured during the course of a stepwise hemorrhage. Leg flow fell abruptly in mid hemorrhage, the resistance being markedly elevated. The high resistance was largely maintained until death. Venous O₂ content was reduced to a low value, and the O₂ consumption of the leg severely reduced. The vasoconstriction was largely prevented by pre-treatment of the dog with 5 mg/kg of Dibenamine. The visceral and carotid beds did not show this extreme resistance rise. As hemorrhage progressed, resistance showed minor eleva-

tion or held steady, and then declined progressively to low levels. Flow was reduced as cardiac output and arterial pressure fell. Hypoxia was less severe than in the legs. No significant differences between control and Dibenamine treated animals were found for either bed. The evidence indicates that vascular beds fall into two broad categories. In one, exemplified by the leg, centrally imposed vasoconstriction was not negated by tissue hypoxia. In the other, viz the viscera, brain, and presumably the heart, vasoconstriction is either absent, less active or is easily reversible by tissue hypoxia. Dibenamine lessens the difference between the two categories.

Extraction and fractionation of urinary corticosteroids GREGORY PINCUS AND LOUISE P. ROMANOFF * *Worcester Foundation for Exptl Biology, Shrewsbury, Mass and Tufts College Medical School, Dept of Physiology, Boston, Mass*

Normal human urine (24-hour sample) brought to pH 1 and extracted in a Hershberg type continuous extractor with CH_2Cl_2 for varying periods of time yields increasing amounts of neutral reducing lipid (NRL) and neutral formaldehydogenic steroid (FS). A maximal yield is had by 18 to 24 hours of extraction, with approximately 35% extractable at 4 hr and 55% at 8 hr. With extraction beyond 24 hr there is a falling off in the total amount. The absolute amounts recovered represent roughly a 4 to 5-fold increase over that extractable by hand extraction in the Heard-Sobel procedure. The neutral lipid extract may routinely be further fractionated by transfer to a silica gel (1-1.5 gm) column with 10-15 cc of benzene eluted by 10 to 30 cc amounts of organic solvents in the following order: a) benzene, b) benzene-ether mixtures, 2:1, 1:1 and 1:2, c) ethyl ether, d) ethyl ether-ethyl acetate mixtures 9:1, 4:1, 3:1, 2:1, 1:1, 1:2, e) ethyl acetate, f) acetone, g) methyl alcohol. b) tends to segregate the less polar steroids (e.g. 17-ketosteroids, 11-desoxycorticosterone-like substance) and c more polar steroids (e.g. corticosterone, 17-hydroxycorticosterone). Partition of the total neutral lipid between benzene and water results in a ratio of approximately 7:1 between fractions b) and c) in the benzene-soluble material whereas the ratio is 1:2 for the NRL and 1:5 for the FS of the aqueous phase. The chromatographed total neutral extract itself gives ratios of 2:1 for NRL and 1:2 for FS. Separation into ketonic and non-ketonic fractions does not alter the total neutral extract ratios markedly, but the presence of considerable non-ketonic corticosteroid is indicated.

Studies of epinephrine effect on portal circulation ELMER B. PRATT AND FRANCIS D. BURDICK (introduced by RICHARD WHITEHEAD) *Dept of Medicine, Univ of Colorado, Denver*

In unanesthetized dogs prepared either with London cannulae on portal and left hepatic veins or with duodenal cannulae for collection of bile, measurements were made of liver blood flow (constant bromsulfalein infusion method), plasma specific gravity, hematocrit, venous pressure and bile volume and specific gravity. The excellent condition of these animals has permitted their use in repeated experiments for more than 2 years. Injection of 0.1 mg of epinephrine hydrochloride into the femoral vein produced a typical cardiovascular response and elevation of the peripheral venous hematocrit. In simultaneous samples the hematocrit showed decreasing values from portal to hepatic to peripheral veins. There was a temporary elevation in plasma bromsulfalein concentration (30-40%) and a decrease (average 66%) in the dye concentration difference between portal and hepatic vein samples. The effect on bile flow and dye concentration was somewhat variable but the bile specific gravity was consistently decreased. The pressure was elevated in both portal and hepatic veins. The same dose of adrenaline injected directly into the portal vein produced no hematocrit, venous pressure, or general cardiovascular effects but gave the same type of bromsulfalein concentration changes. These results suggest that epinephrine acts directly on liver circulation to shunt blood flow away from the parenchymal cells.

New type of gradient calorimeter for rapid, simultaneous determination of heat loss and heat production in laboratory animals LAWRENCE R. PROUTY AND RICHARD W. LAWTON (introduced by McKEEN CATTELL) *Dept of Physiology, Cornell Univ Med College, New York City*

A gradient calorimeter of the Benzinger type, suitable for laboratory animals, has been built and tested. Its size is 32 x 24 x 18 inches. The half-inch thick Dural walls are lined with 2,152 copper-constantan strip thermal junctions wired in series. With an input of 21.6 Calories, 1,700 μv total output is obtained. This represents a drop of $1.85 \times 10^{-4}^\circ\text{C}$ across the gradient layer of 0.05 inch woven glass tape. Water vapor given off by the animal is condensed within a similar but smaller calorimeter unit (plate meter). Ingoing and outgoing air thermocouples measure heat loss to the circulating air. The calorimeter walls give a 100% response to direct radiation in 50 seconds, but the response time is slower with calorimeter contents in place. The thermal constant of the calorimeter is obtainable within ± 0.1 . The large mass of the calorimeter walls affords stability against minor rapid fluctuations in laboratory temperature. Heat production is determined from oxygen consumption, as measured by a Pauling

oxygen meter, and carbon dioxide production, as measured by an infrared gas analyzer. Both direct and indirect calorimetry, rectal and skin temperatures are simultaneously recorded by a Leeds and Northrup eight-point recorder. The calorimeter and associated equipment have the following advantages, a) rapid response to changes in heat production and heat loss, b) furnishing either a stable or rapidly changing thermal environment for the animal subject, c) a simple measurement of vaporization, and d) continuous recording of all measurements. The calorimeter should make possible critical experiments on thermoregulatory mechanisms of animals.

Spread of excitation process in ventricular myocardium of dogs RAYMOND D. FRUITT (introduced by NORMAN M. KEITH) *Mayo Clinic, Rochester, Minn.*

Two series of experiments were carried out. In one, direct-lead electrocardiograms were recorded from the ventricular cavities and epicardial surfaces of isolated, perfused canine hearts, into the cavities of which caustic solutions were introduced. In the second, electrocardiograms of a similar type were recorded from strips of the myocardium which retained attachment to the ventricle at only one end. The following conclusions have been reached. First, if rapid transmission of the excitation process through the endocardial tissues is a function of a specialized tissue (the Purkinje system), destruction of that tissue has not been accomplished. Second, in view of the severity of trauma effected by the agents introduced into the ventricular cavities, escape of such specialized tissue from injury seems unlikely. Third, muscle-strip experiments lend support to the concept that variation in speed of transmission of the excitation process may be related not to differences in the nature of the tissues, but rather to peculiarities in their arrangement. Excitation moves rapidly along a strip composed of longitudinally disposed fibers, and slowly down a strip made up of fibers arranged in a transverse manner.

Accurate registration of intralumen pressures of the digestive tract by two new methods J. P. QUIGLEY, DANIEL A. BRODY,* BILLY MCKAY,* W. C. LANDOLINA* AND J. H. MCALISTER* *Dept. of Physiology, Univ. of Tennessee, Memphis*

Pressures within the lumen of the canine digestive tract have recently been accurately recorded by two new methods. 1) An improved form of the pressure-conduction method of Brody and Quigley (*Gastroenterology* 9: 570, 1949) transmits pressure from a moderately soft plastic tip (3 x 8 mm) within the gut through 0.4 mm bore plastic tubing to a Sanborn electromanometer. The pos-

sibility of tube plugging is minimized by the slow flow (5 cc/hr) of clean, dry gas through the tubing. Lengths of tubing up to 100 cm. are feasible when helium is substituted for air as the conducting medium. The electromanometer permits registration by either optical or direct-writing methods and the sensitivity can be varied at will. 2) A newly developed electromagnetic transducer consists of a 3 x 20 mm transducer placed in the cavity from which pressure is to be measured. This transducer has a self-supporting, center-tipped coil which forms two arms of a balanced induction bridge. Changes in intralumen pressures of the gut cause proportional displacements of an elastically mounted iron core. The apparatus is so calibrated that the resulting electrical imbalance is recorded in terms of pressure. The volume/pressure coefficient of this device is exceedingly small and plugging cannot occur. Pressure observations made by these two methods closely resemble in magnitude and in general form the results we previously obtained by our less refined methods and appear to confirm the validity of these earlier observations.

Simultaneous determination of bloodflow through each lung H. RAHN AND H. T. BAHNSON* *Dept. of Physiology and Vital Economics, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

If the gas content of the arterial blood, the R.Q. and metabolic exchange rates can be determined for each lung separately, it is possible to calculate the mixed venous point and consequently the blood flow through each lung by the Fick principle. This is true only if the arterial gas contents of the two lungs differ. By means of a differential bronchial catheter, alveolar air was recorded continuously and simultaneously from each lung. The gas content of the arterial blood was determined from the alveolar air tensions and the blood gas dissociation curves. The R.Q. can be ascertained from either the alveolar air or the expired air determinations. In order to calculate the gas content of the mixed venous blood, the arterial point for each lung is plotted on a diagram having O_2 and CO_2 blood gas contents as ordinates. Through each of these arterial points a straight line is drawn which has a slope that depends on the R.Q. for that lung. The point of intersection of these lines represents the composition of mixed venous blood which is the same for each lung. In nembutalized dogs breathing air, approximately 45% of the total circulation perfuses the left lung. This fraction is reduced to approximately 25% within 10 minutes after the administration of nitrogen to the left lung only. No further significant changes were observed at the end of one hour of nitrogen exposure. This

vasomotor reflex actually occurs within 1-3 minutes as shown by qualitative measurements employing the rapid infra-red gas analyzer

Species variability in the inactivation of penicillins

G and K NATHAN RAKIETEN, GEORGE VALLEY* AND EDNA W LYON * *Bristol Labs Inc, Syracuse, N Y*

It is necessary to administer per unit body weight a larger total amount of penicillin (repository forms) to a rat than to a rabbit or dog to obtain a measurable blood level for 24 hours. Further, penicillin G, when administered i m to mice in aqueous solution, disappears from the blood more rapidly than it does in rabbits (EAGLE *et al J Bact* 57 122, 1949). In studying the factors which might play a role in the inactivation of pure penicillins G and K *in vivo* and *in vitro* a species difference was noted between the rat and rabbit. In the intact rabbit K disappears from the blood more rapidly than G following i v administration. This is also true in the renal-ligated (R L) rabbit. In contrast in the eviscerated renal-ligated (E R L) rabbit the blood levels obtained after i v administration of K approach those obtained with G after equilibration has occurred. The rat differs from the rabbit in that in the R L animals, and apparently in the intact also, G and K disappear from the blood within the same time intervals. However in the E R L rat both G and K remain in the blood stream at constant levels for at least 3 hours. *In vitro* studies using the Warburg apparatus failed to show any marked differences between rabbit and rat liver slices. Neither inactivated G and both inactivated K. Apparently some mechanism is present in the liver of the intact and R L rat which allows for the inactivation of G as well as K.

Estrogen content of blood of ovarian vein of the dog and spermatic vein of the stallion

A E RAKOFF* AND A CANTAROW *Division of Endocrine and Cancer Research, Jefferson Med College, Philadelphia, Penna*

As part of an investigation of the rate of secretion of hormones by certain endocrine glands, the quantity of estrogen was determined, by bioassay, in blood collected from 1) the ovarian vein of dogs after gonadotrophic stimulation, 2) the spermatic vein of stallions and 3) a peripheral vein. After ovarian stimulation with pregnant mare serum gonadotrophin for 8 to 10 days very little estrogen (10 mouse units/100 cc of serum or less) could be found in blood collected at operation from the ovarian veins of 4 dogs, thus suggesting a relatively slow secretion of small amounts of estrogens. Blood collected from the spermatic vein of 2 stallions showed very high serum estrogen values (264 and 100 MU/100 cc respectively), which were many times greater than those for

peripheral venous blood (20 MU/100 cc) determined simultaneously, indicating a relatively rapid secretion of large amounts of hormone from the testis. The estrogens in the spermatic vein blood were entirely in 'free' form, in the peripheral blood, approximately half of the estrogen was 'protein-bound' in one instance and entirely in the free form in the other.

Functional analysis of the vasomotor innervation of the dog's footpad

W C RANDALL, W F ALEXANDER,* J W COX* AND A B HERTZMAN *Depts of Physiology and Anatomy, St Louis Univ School of Medicine, St Louis, Mo*

During faradic stimulation of the sympathetic chain, vasomotor responses were recorded by the photoelectric plethysmograph from the hind feet of the anesthetized dog. Extensive variations in the position of ganglion swellings required specific reference to precise vertebral levels in localizing ganglia on the sympathetic chain. Although apparent fusion of right and left chains caudally was commonly observed, little evidence of contralateral response to stimulation was noted. Post-ganglionic vasomotor outflow from the ganglion chain to the footpad was determined by stimulation of individual ganglia after section of the chain both superior and inferior to the point of stimulation. Although some variation was observed, the largest postganglionic supply to the footpad was shown to be derived from ganglia at vertebral levels L₆ to S₁. The inflow of preganglionic vasomotor fibers innervating the footpad was determined by recording vascular responses during direct stimulation at successively lower levels on the chain. As additional preganglionic fibers enter caudally, vasoconstriction in the foot became more intense. Individual oblique rami were stimulated wherever possible. Very few vasomotor preganglionic fibers to the footpad were found to enter the chain above the vertebral level L₄, and the maximum inflow appears to be between L₄ and L₆.

Effects of adenine ingestion in the dog

SIGWIN B RASKA *Dept of Physiology and Pharmacology, Univ of North Dakota School of Medicine, Grand Forks*

Oral administration of adenine to dogs in daily doses of 50 to 100 mg/kg produces, within one or two weeks, hemorrhage and/or ulceration of buccal mucosa, gums and ventral surface of tongue. These lesions are usually bilateral and symmetrical. These lesions can be cured or prevented by intramuscular injection of penicillin. The tongue of these dogs often becomes atrophic and necrotic. Mucosal and submucosal hemorrhages are always present. Applied pressure to mucosal membrane results in profuse bleeding. The hemorrhagic tendency resembles that of vitamin K deficiency.

The hemorrhages of the gums especially around the teeth simulate those seen in experimental vitamin C deficiency. Indications for CNS disturbances were a waddling gait with muscular asthenia of the hind parts and clonic convulsions, especially in the pelvic and shoulder girdles. In the late stages there was peripheral anesthesia. The cornea became opaque and the sclera and conjunctiva very inflamed. Vision appeared decreased. In addition to previously described autopsy findings (RASKA, S. B. *Science* 105, 2718, 1947, *Federation Proc* 8 130, 1949) the following was noted: diffuse patchy hemorrhages in both lungs; hemorrhages of myocardium, especially of the auricle and of the ventricle at the auriculo-ventricular junction; section of the heart muscle showed multiple small hemorrhagic foci and in one of the heart muscle sections accumulation of cells suggestive of Aschoff Bodies, congestion of gastric mucosa with extensive hemorrhages in mucosa and submucosa. Scattered patchy hemorrhages and congestion in lower jejunum, ileum, and in the mucosal folds of the sigmoid, pancreas and adrenals showed hemorrhagic foci. Peri-adrenal hemorrhages also were found. Much of the syndrome produced by adenine simulates clinically as well as microscopically the syndrome exhibited by animals suffering from multiple avitaminosis.

Nitrogen retention produced by adenine SIGWIN B. RASKA *Dept of Physiology and Pharmacology, Univ of North Dakota School of Medicine, Grand Forks*

A marked increase of N P N, urea, uric acid and creatinine in the blood follows, within one or two weeks, the oral or parenteral administration of 30-50 mg/kg of adenine to dogs receiving a complete normal diet before and during the experimental period (RASKA, S. B. *Science* 105 1947, *J Biol Chem* 165 743, 1946, *Federation Proc* 8 130, 1949). A definite relationship exists between the degree of nitrogen retention and the dosage and duration of adenine administration. A decrease in the daily dosage causes a less pronounced nitrogen retention and discontinuation causes a gradual lowering of the nitrogen retention. The maximum nitrogen retention in mg % ranged N P N, 160 to 260, urea, 100 to 200, uric acid, 3 to 8, creatinine, 4 to 10. Creatinuria was often present. As previously reported parenchymatous degeneration of the kidneys was found at autopsy. Similar blood and autopsy findings as reported for dogs were also present in rats. Larger daily doses of adenine (e.g. 100 mg/kg) produces in dogs, within one or two weeks, mucosal and submucosal hemorrhages, ulceration of buccal mucosa and tongue, conjunctivitis, scleritis, opaqueness of cornea, dermatitis and tremors of the skeletal muscles. Many of the lesions are simi-

lar to those described for experimental multiple avitaminosis. These lesions can be cured or prevented by intramuscular injection of penicillin. Administration of adenine to animals would seem to provide a means for producing experimental uremia. The possibility exists that a prolonged increased concentration of adenine or its metabolic products in the blood and tissues due to a faulty nucleoprotein or purine metabolism might play an important role in pathologic states, especially those associated with nitrogen retention and kidney diseases.

Comparison of procaine and tridiurecaine G. CARL RAU* AND B. A. WESTFALL *Dept of Physiology and Pharmacology, Univ of Missouri Med School, Columbia*

A comparison of the toxicity, general analgesia and degree of local anesthesia produced by a new compound, tridiurecaine hydrochloride (a procaine substituted diurethan), with procaine hydrochloride in rats and dogs has been made. The LD₅₀ values (intraperitoneal injection) of tridiurecaine and procaine were found to be approximately equal in rats. Unanesthetized dogs injected intravenously with tridiurecaine (1 mg/kg/5 min) showed a degree of general analgesia as great as dogs receiving equal doses of procaine. Over dosage of tridiurecaine in both rats and dogs produced mild sedation followed by respiratory paralysis and death instead of excitation, convulsions and death, characteristic of procaine. The anesthetic potency of tridiurecaine injected intradermally (0.2 cc of 1% solution per wheal) in dogs was approximately the same as the anesthetic potency of procaine (0.2 cc of 1% per wheal). Intravenous injection of tridiurecaine (1 mg/kg) into dogs anesthetized with pentobarbital sodium produced a small rise in blood pressure. Larger dosage (3 mg/kg) did not alter blood pressure significantly, but inhibited respiration, but about 5 mg/kg produced respiratory paralysis followed by a gradual circulatory failure and eventual death due to the respiratory difficulty.

Effects of ACTH on synovial membrane potentials in rheumatoid arthritis C. I. REED, NORMAN R. JOSEPH, IRVING E. STECK,* AND M. M. MONTGOMERY* *Univ of Illinois, Chicago Professional Colleges, Chicago*

In work previously published a method has been described for determining the potential difference between the inner surface of the synovial membrane and a subcutaneous reference junction in anesthetized dogs. Also a tentative interpretation of results has been presented. This technic has been modified and adapted for similar studies on intact, unanesthetized human subjects.

with uncomplicated rheumatoid arthritis, and also on healthy control human subjects. The reference junction was applied to the skin. In the normals, the resting potential ranged from 0 to +5 mv. Patients with rheumatoid arthritis showed potentials of the order of 20 to 60 mv positive at the joint. Intramuscular injection of 25 mg of ACTH in all cases so far studied caused a sharp reduction within 5 to 20 minutes, sometimes by 50%, sometimes to the normal range. Further treatment on a schedule of 25 mg every 6 hours invariably reduced the potential to near zero. After 12 doses, placebos were substituted. An upward trend of positive potential was apparent usually within 3 to 4 days, in general correlated with return of subjective symptoms and limitation of mobility.

Directional effect of electric current on secretion of chloride ions by the dog's stomach WARREN S. REHM, *Dept of Physiology, Univ of Louisville, Louisville, Ky*

Previous results have demonstrated that the production of H^+ ions by the secreting stomach can be controlled by the directional application of current (*Am J Physiol* 144:115, 1945). On the basis of these and other findings a theory of HCl production was formulated (*Gastroenterology*, In press). Analysis of this theory leads to the prediction that the effect of current application on Cl^- ion production should be in the opposite direction to its effect on H^+ ion production. Experiments were performed to test this prediction. The technique described in the above reference was used. An isotonic $NaNO_3$ solution made contact with the mucosal side and was replaced at definite intervals. The Cl^- ion and H^+ ion content of this fluid was determined. It was found (5 dogs) that from 6-26 microequivalents of Cl^- ion/gm dry weight of stomach/10 min was secreted by the resting stomach. Application of current from serosa to mucosa resulted in a decrease in the Cl^- ion secretion of the resting stomach, and application of current in the opposite direction resulted in an increase. In the secreting stomach the above prediction was borne out since it was found that current sent from serosa to mucosa resulted in an increase in the secretion of H^+ ions and in a decrease in the secretion of Cl^- ions, and also that application of current in the opposite direction resulted in a decrease in H^+ ion secretion and an increased Cl^- ion secretion.

Skeletal muscle as a source of plasma potassium during the removal of potassium by vivodialysis ROGER M. REINECKE AND FRANCIS L. STUTZMAN, **Dept of Physiology, Univ of Minnesota, Minneapolis*

A skeletal muscle, the gastrocnemius or the

cranial part of the sartorius, was isolated except for its blood and nerve supply in a dog anesthetized with sodium pentobarbital. The plasma potassium level was then reduced by vivodialysis (*Am J Physiol* 157:401, 1949). Under these circumstances there was an arteriovenous increase in the plasma potassium concentration of blood flowing through the muscle.

Relation between stroke index and time of diastole in the intact dog JOHN W. REMINGTON, *Dept of Physiology, Univ of Georgia School of Medicine, Augusta*

For a dog showing sinus arrhythmia, the stroke indexes of 86 consecutive pulses were plotted against the time of previous diastole. The points describe a definite curve, with standard deviation of $\pm 10\%$, 240 pulses taken from newly anesthetized dogs before and after vagotomy or atropinization show a similar curve, with S.D. $\pm 15\%$. Both curves show a linear relation from diastolic lengths (T_d) of 1200 to 700 msec, a curvature toward reduced stroke indexes from 700-300, and stroke indexes scattering about a fairly constant minimal T_d value of 160-200. Pulses taken from dogs after hemorrhage follow an almost parallel curve but at lower stroke index values. A curve taken at the height of an epinephrine pressor response shows sub-normal stroke indexes, with greater discrepancy at high T_d values than at low. All curves show maximum cardiac output at heart rates of 130-170. Stroke indexes calculated from the carotid pulse records of Y. Henderson (*Am J Physiol* 23:345, 1909) agree with his cardiometer volumes within an ave. of $\pm 12.5\%$. Neither set of data seem to support his setting 210 as the optimum heart rate. A major part of the flow decline seen in late hemorrhage seems referable to the fast heart rate, which is congruent with the small heart volume and rising venous pressure found at this time.

Renal tubular excretory mass and reabsorption of sodium, potassium and chloride in testosterone-treated and estradiol-treated female dogs J. A. RICHARDSON* AND C. R. HOUCK, *Dept of Pharmacology, Medical College of South Carolina, Charleston, and the Dept of Physiology, Univ of Tennessee, Memphis*

The effects of various doses of testosterone propionate and of alpha-estradiol benzoate on the renal tubular reabsorption of sodium, chloride and potassium and the tubular excretory mass (TmPAH) in normal female dogs have been studied. Testosterone propionate in 10 to 12 daily intramuscular doses of 3, 6, and 10 mg/kg had no significant effect on TmPAH, or on the tubular reabsorption of sodium, chloride and potassium. In animals receiving alpha-estradiol benzoate in sufficient, daily, intramuscular doses to produce

estrus in approximately 10 days (daily doses of 80-2000 rat units/kg) glomerular filtration rate was unchanged or reduced for varying periods after the injections. Whereas the tubular reabsorption mechanisms for sodium and chloride remained normal, the percentage of filtered potassium reabsorbed decreased as much as 50% during a variable time following estrogen administration. A return to normal occurred over a period of several weeks. In untreated as well as treated animals, the tubular reabsorption of sodium and chloride bore a directly proportional relationship to filtration rate. In the estradiol-treated dogs, TmPAH was moderately to markedly reduced (15-50%) over a period of several weeks following administration of the estrogen, probably as a result of a non-specific depression of the enzymes that control PAH transport. A gradual return to normal occurred during a recovery period of several weeks. With both testosterone propionate and estradiol benzoate no significant changes were noted in the microscopic structure of the kidney or in the kidney weight/body weight ratio.

Changes in hemoglobin during metamorphosis in the bullfrog (*Rana catesbeiana*) AUSTEN F. RIGGS, II (introduced by GEORGE WALD) *Biological Labs, Harvard Univ, Cambridge, Mass*

McCutcheon has reported that tadpole hemoglobin has an affinity for oxygen several times greater than that of the adult bullfrog, and that during metamorphosis the oxygen equilibrium function also changes in shape from a hyperbola to sigmoid—i.e., the value of n in Hill's equation increases from 1-2 (*J Cell & Comp Physiol*, 8: 63, 1936). This could indicate either that 1) tadpole hemoglobin possesses only one heme, and has perhaps a small molecular weight like that of myoglobin, or 2) it is of normal constitution, but its hemes do not interact. Examination of tadpole hemoglobin in the ultracentrifuge, with the cooperation of Prof. J. L. Oncley, shows that its molecular weight is like that of other vertebrate hemoglobins. Its sedimentation constant, s_{20} , when extrapolated to zero concentration, is about 4.9. With new techniques developed in cooperation with Prof. J. Wyman, the oxygen equilibrium function has been remeasured accurately in bullfrog tadpoles and adults. The function is sigmoid in both instances, the value of n in Hill's equation does not appear to change with metamorphosis. McCutcheon's observation that the oxygen affinity increases greatly during metamorphosis, however, is confirmed. These relationships are being explored further.

Mapping electrokymographic responses over the cardiac silhouette GORDON C. RING, A. SOKALCHUK* AND G. J. NAVIS*

The electrokymographic (EKY) records of x-ray density changes of the heart in general show

thinning during ejection and thickening during filling. Often, however, thinning is indicated during a portion of the filling period and thickening during a part of ejection. These paradoxical effects must be due to changes in position of the heart—a thinner portion of the heart moving into the x-ray beam during diastole and a thicker portion moving in during systole. In order to gain a better understanding of these effects, the EKY responses over each square centimeter of the cardiac silhouette have been carefully mapped. With the dog lying on his right side, EKY records from the sternal side of the heart show a peak which occurs after ejection begins. As one moves toward the spinal side of the silhouette these peaks appear earlier in systole and the amplitude of the responses increases. These changes in pattern can be explained by the changes in position of the dog's heart.

Analysis of value 'K' in Bazett's formula JANE S. ROBB

Dept of Pharmacology, Univ of Syracuse College of Medicine, Syracuse, N. Y.

All investigators find that the duration of electrical systole (QT) varies with cycle duration. The average value of 'K' is 0.37 for normal men. If one calculates K for the values of QT tabulated by Ashman and Hull for normal men, K does not prove to be 0.37 for all cycle lengths. If K values are again related to cycle, a smooth curve results. Experimentally, it is possible to keep cycle almost constant and to alter K. When K is calculated for 533 athletes and for 250 presumably normal medical students, graphs relating K to cycle do not overlap. Moreover, if the athletes are classified according to their major sport, certain sports seem to have a greater effect on K than do others. These facts make one doubt that the QT/cycle relationship is simply that of one dependent and one independent variable. To imply that 'K' represents 'QT corrected for cycle duration' is misleading. An alternate explanation is that some phase of cellular activity determines both QT and heart rate, that both are often changed proportionately thus producing an apparent rather than a real relationship. K seems to be a very sensitive indicator of the 'state of the heart muscle' as was suggested for QT by Blair, Wedd and Young; nevertheless its full significance is elusive. For instance, is an athlete's heart whose K is lower, better or worse than the heart of a medical student who has a higher K at the same cycle duration?

Blood vessels of nerves, as seen in living animals with quartz rod illumination and their response to drugs including cortisone JOSEPH T. ROBERTS, H. R. MURDOCK, JR.,* J. L. CAMPO,*

C. J. TANNER,* AND J. A. PAPARELLA.* *V. A. Hospital, Batavia, and Univ of Buffalo School of Medicine, Buffalo, N. Y.*

Vessels in small and large nerves of frogs and

small mammals, *in situ*, change calibre and flow volume in response to drugs, stimulation with electrodes, shock, stretching, compression and other states. Observations were made during life with the quartz rod apparatus. Rhythmical contractions, especially of arterioles of larger size, were seen. Large fusiform dilatations (sinusoids) of transverse branches were seen often in vasa nervorum of rabbits, these may serve as regulatory shunts to control vascularity of nerves. Constriction of vessels in nerves followed injection or application of epinephrine, or electrical stimulation of the nerve's field. Dilatation of these vessels followed injection of histamine or inhalation of amyl nitrite. Other drugs, including cortisone, and other procedures are being evaluated as to their effects upon the vasa nervorum, using somatic and visceral nerves and ganglions. Sludging of blood has been observed in the vasa nervorum (as noted by Knisely and others), and efforts to correlate this with systemic or generalized sludging as well as neural functions are being made. The vasa nervorum are rather abundant throughout the thickness of each nerve and are believed to have a significant role in the function of the nerve, as shown in earlier experimental and clinical studies. The occurrence of spasm and dilation of the vasa nervorum observed here supports the hypothesis of a reflex arc (proposed by Roberts, 1948) which produces 'referred pain' by ischemia of the somatic nerves in the areas of referred pain when a stimulus arises in a visceral organ associated centrally with these nerves. 'Trigger areas' in somatic nerves may be due to ischemia of the nerve due to spasm, infarction or other vascular lesions within the nerve.

Permeability of *Escherichia coli* R. B. ROBERTS AND R. T. NIESET (introduced by S. R. M. REYNOLDS) *Carnegie Inst of Washington, Dept of Terrestrial Magnetism, Washington, D. C. and Tulane Univ., New Orleans, La.*

Measurements of the permeability of cells of *E. coli* to ions of sodium and potassium have been reported (COWIE, D. B., R. B. ROBERTS AND I. Z. ROBERTS *J. Cell & Comp. Phys.* October, 1949). Using the same methods we have extended these observations to ions of rubidium, caesium, cobalt, phosphate, sulfate, and sulfide. In all cases permeability was found to be high. The ionic concentration of the cells was found to be at least 75% of the concentration in the medium, reaching equilibrium in less than 5 minutes under conditions of low metabolic activity. It appears that 75% of the cell volume is 'water-space' and that ions are freely exchanged between this water space and the external medium. In the presence of high metabolic activity enormous concentrations of the ions in the cell are observed, although the amount in the water space remains about con-

stant. A high permeability was also found in other cells which are reported to show effects of osmotic pressure.

Body fluid redistribution in induced hypothermia and hyperthermia S. ROBBARD, HIROSHI SAIKI* AND ARTHUR MALIN* *Cardiovascular Dept., Medical Research Inst., Michael Reese Hospital, Chicago, Ill.*

Numerous studies have suggested that hemodilution occurs in hyperthermia, and hemoconcentration occurs in hypothermia, but these deductions have been inferred from changes in the osmotic pressure or the plasma protein concentration of the blood. No data are available, as far as we are aware, on the actual changes in the blood volume and its relation to changes in the extracellular and intracellular fluid compartments. Our studies on physiological effects induced by changes in body temperature were therefore extended to investigate possible fluid shifts between these volumes, in order to assay more adequately the effect on the cardiovascular system. Rabbits and chicks were used. Lowering the body temperature to 25°C resulted in only a slight increase in hematocrit and specific gravity. However, plasma and blood volumes and thiocyanate space were all reduced 30% below normal levels. Rewarming resulted in a return to normal values. Induced hyperthermia resulted in a slight decrease in hematocrit and in plasma specific gravity, plasma and blood volumes were slightly reduced, but thiocyanate space was increased as much as 30%. These data suggest that induction of hypothermia results in a marked shift of fluid from the plasma and the interstitial spaces to the intracellular phase. The fact that the hematocrit and specific gravity do not rise commensurate with the decreased volumes suggests that blood cells and plasma proteins are removed from the circulating plasma and stored. The reverse processes are brought about by rewarming to normal temperature levels. Induction of hyperthermia causes water to leave the tissue cells, and causes commensurate quantities of plasma protein and blood cells to be released to the circulating blood stream.

Cardiodynamic effects of shunts from the great arteries to the auricles S. ROBBARD, S. KRAUSE,* R. REYNOLDS,* M. LOWENTHAL* AND J. SCHACK* *Cardiovascular Dept., Medical Research Inst., Michael Reese Hospital, Chicago, Ill.*

Cardiodynamic effects of surgically produced shunts connecting either the pulmonary artery or the aorta to either of the auricles have been studied in acute and chronic preparations. The effects of a connection between the pulmonary artery and the right auricle have already been reported. Anastomosis in acute experiments of the pulmonary artery to the left auricle, with a flow

through the shunt of 500 cc/min, results in reduction in the oxygen saturation of the systemic arterial blood, the right auricular pressure remains unchanged, the pulmonary arterial systolic pressure increases while the pulmonary diastolic falls resulting in an increase in pulmonary pulse pressure, the left auricular pressure rises and the central aortic pressure remains unchanged. In chronic experiments a calculated shunt of 50% of the cardiac output has been maintained as long as 3 months. Anastomosis of the subclavian artery to the left auricular appendage with flows through the shunt of as much as 3 l/min produced little changes in the right auricular or pulmonary arterial pressures, the left auricular pressure increased and the central aortic pulse pressure increased markedly. These experiments demonstrate that the heart can accommodate to enormously increased input loads without signs of dilatation or failure provided the insult is not placed too suddenly. Sudden overloading with even relatively small amounts of shunted blood led to dilatation and failure, while gradual increase of the input load permitted adaptation to inputs which at least doubled the cardiac output.

Muscle potentials in various insects during flight

KENNETH D ROEDER *Dept of Biology, Tufts College, Medford, Mass*

Pringle (*J Physiol* 108: 226) has shown that potentials (5–10 mv) recorded from the thoracic muscles of flies occur at a lower frequency than the wing movements. He suggests that the indirect flight muscles constitute a resonant system in which the contraction of one muscle group is the direct result of mechanical stretch caused by contraction of the antagonists. Periodic excitation of nervous origin serves to initiate contraction and to maintain the system in a state where it is capable of oscillation. Intrathoracic potentials and thoracic vibrations have been recorded simultaneously in various insects during stationary flight. In the cockroach (*Periplaneta*) and a moth (*Agrotis*) with wing rates of 20–40 per second one muscle potential occurs during each wing cycle, and there is at present no evidence that each excitation of the wing muscles is not of nervous origin. In a wasp (*Vespa*) and in flies (*Lucilia*, *Calliphora*, *Tabanus*) with wing rates of 120–180 per second there may be 4–15 wing cycles to each intrathoracic potential. Removal of the wing load may double the wing rate and decrease the potential frequency so that the ratio may become as high as 40:1. This suggests nervous feedback determined by the amplitude of the oscillation. Spontaneous termination of flight in these forms is usually signalled by cessation of potentials followed by a gradual decline in vibration amplitude with little or no change in vibration

frequency. It is concluded that generalized slow-flying insects have a conventional mechanism of muscle excitation, while specialized insects with high wing rates show the novel excitatory mechanism described by Pringle.

Neuromuscular excitation in the cockroach

KENNETH D ROEDER AND ELIZABETH A WEIANT * *Dept of Biology, Tufts College, Medford, Mass*

Electrical and mechanical changes following indirect excitation of one of the tergo-trochantal muscles of the cockroach, *Periplaneta americana*, were recorded from electrodes and a piezo-electric stylus placed on the exposed muscle surface. The electrical sign of muscle excitation is a positive potential of 4–5 msec duration, which begins 1.2 msec after the arrival of the nerve action potential at a point where the motor nerve penetrates the muscle substance. Contraction begins 3.0 msec after the onset of the muscle potential. The preparation behaves as a single motor unit, neither electrical nor mechanical response being subject to facilitation or gradation. Contractions begin to fuse at stimulus rates above 40–50 per second, which is well above the wing rate (25–30 per second) for this insect during flight. The muscle potential elicited by the second of a pair of liminal stimuli can be detected until the stimulus interval is less than 2.0 msec, though it becomes progressively smaller as the stimulus interval is reduced from 20 msec. Neuromuscular transmission is unaffected by curare, though the muscle becomes completely inexcitable 3–5 days after surgical transection of the motor nerve. It is concluded that conduction in this muscle depends entirely upon many branches of a single motor nerve fiber, the muscle potential being the sum of local potentials developed simultaneously in several muscle fibers, and in sequence at many motor endings on each muscle fiber. The potential change during activity of cockroach muscle appears to be similar to the end-plate potential of vertebrate muscle.

Recovery of glomerular and tubular function, including p-aminohippurate extraction, following two hours of renal artery occlusion in the dog

BETTY S ROOF,* HENRY D LAUSON, S THEODORE BELLA* AND HOWARD A EDER * *Hospital of Rockefeller Inst for Med Research, New York City*
Hamilton, Phillips and Hiller (*Am J Physiol* 152: 517, 1948) produced severe but reversible kidney damage in dogs by 2 hours of renal artery occlusion. Urea clearance returned to normal in about 35 days. The present study extends this observation to include clearances of creatinine (Cr) and p-aminohippurate (CPAH) and TMPH

in 3 dogs. In dogs 2 and 3, with single explanted kidney, measurement of PAH extraction permitted calculation of actual plasma flow (RPF). Within 1 hour after clamp release, clearances measured 2, 10 and 26% of control in dogs 1, 2 and 3, respectively. Recovery thereafter proceeded as follows. Dog 1 Progressive improvement in all functions began immediately. $CPAH$ reached 89% of control by the 35th day, $TPAH$ reached 90% only by the 78th day, with CCr and C_{urea} still under 70%. Dog 2 RPF was above normal for 1 week and moderately subnormal afterwards. Clearances varied around 20% and rose only after the 11th day. PAH extraction reached 89% of control on the 23rd day, but by the 32nd day, CCr , $CPAH$ and $TPAH$ measured only 30, 42 and 48%. Dog 3 Showing least initial impairment, CCr , $CPAH$ and $TPAH$ all reached about 60% by the 26th day with RPF slightly subnormal throughout. PAH extraction was normal by the 15th day. In all 3 dogs, $CCr/CPAH$ decreased slightly. The data indicate that the decreased clearances and $TPAH$ during the first weeks after 2-hour clamping are due mainly to factors other than concomitant reduction of RPF.

Effect of thiocarbonamide derivatives in alloxan diabetes I N ROSENBERG AND M S RABEN (introduced by E B ASTWOOD) *New England Center Hospital and the Dept of Medicine, Tufts Med School, Boston, Mass*

A curative action of thiouracil in mild alloxan diabetes has been reported by Houssay and Martinez (*Rev Soc argent de biol* 24 63, 1948). In the present study, observations were made on the effect of a variety of thiocarbonamide and other sulfur compounds on the daily weight, food and water consumption, urine volume and glycosuria of moderately and severely alloxan diabetic rats. The test substances were administered admixed with the food for periods of 3 to 19 days. Parallel experiments were performed on a similar group of diabetic animals which received, in addition to the test compounds, a constant daily suboptimal dose of insulin. Thiourea, 1,1,3-trimethylthiourea, 6-*n*-propylthiouracil, 6-cyclopropylthiouracil, 6-*tert* butylthiouracil, 6-*isobutyl*-thiouracil, 6-trifluoromethylthiouracil, 6-carboxy-thiouracil, dihydrothiouracil, 2-mercaptoimidazole, 2-mercaptothiazole, 5,5-dimethyl-2-mercaptothiazoline, 5,5-dimethyl-2-thiothiazolidone, 5-vinyl-2-thiothiazolidone, 3-phenyl-2-thiothiazolidone, 2-mercaptobenzimidazole, 4-phenylthiosemicarbazide, tetramethylthiuramdisulfide, tetraethylthiuramdisulfide, 5,5-diethylthiobarbituric acid, 5,5-diethyl 2,4,6-trithiobarbituric acid, tetrahydro-2-thiopyrimidone, methylene blue, cysteine, and cystine were tested. When judged on the ability to produce an insulin-like

effect of diminution of glycosuria associated with weight gain, none of the substances was found to be of intrinsic benefit in either group of animals. The decreased glycosuria, polyuria and food consumption observed in some animals were coincident with weight loss, and interpreted as toxic effects of the substance administered, prolonged treatment with those substances which have antithyroid effect sometimes induced similar changes, possibly due to myxedema. Food restriction alone diminished glycosuria and caused weight loss proportional to the degree of reduction in food consumption. It is concluded that the substances investigated neither improve carbohydrate tolerance nor enhance insulin-sensitivity of diabetic rats under these conditions.

Effect of temperature-change upon round window response in hamster and bat WALTER A ROSENBLITH,* LAWRENCE KAHANA,* AND ROBERT GALAMBOS *Psycho-Acoustic Lab, Harvard Univ, Cambridge, Mass*

The body temperature of hamsters and bats under barbiturate anesthesia was reduced in a manner simulating their normal hibernation. The electrical response to acoustic clicks was recorded from the round window over the ranges 40°-18° C (hamster) and 30°-8° C (bat). At ordinary temperatures the round window response resembles in all important respects that recorded from the cat and the guinea pig. Microphonic and neural components are readily distinguished. The essential findings accompanying drop in the body temperature of the hibernators are: 1) progressive reduction in amplitude of both microphonic and neural components below about 30° C; with 2) ultimate disappearance of the neural components. The greater the intensity of the acoustic stimulus the lower the temperature at which this disappearance is observed. With our most intense click (about 85 db above human threshold) the neural component in the bat could not be observed below 11° C. 3) A constant latency for the microphonic component over the entire temperature range, and 4) a striking progressive increase in latency of the neural components as the temperature is reduced below about 30° C. The changes in amplitude and latency are essentially reversible as an animal is cooled and warmed through a temperature cycle.

Comparative physiology of cardiovascular pressor reflex mechanism under head-to-tail acceleration (negative G) SHELDON ROSENFELD AND CHARLES F LOMBARD (introduced by CHESTER HYMAN) *Dept of Aviation Medicine, School of Medicine, Univ of Southern California, Los Angeles*

Studies of the effect of negative acceleration upon four species of animals indicate that certain

species are not suitable for consideration relative to man. Studies of 28 experiments including 10 goats, 10 dogs, 5 monkeys and 3 rabbits and compared with data available for man indicate that the monkey and the dog respond in a manner which most closely parallels the reflex cardiovascular response elicited in man during negative *g*. The goat does not simulate this response pattern. The major changes to cephalic circulation during negative acceleration, as measured from the carotid artery and external jugular vein connected to Statham strain gauges at the level of the brain, include the following. At the onset of radial acceleration the arterio-venous pressure difference in man, monkey and goat is slightly increased but in the dog slightly decreased. During radial acceleration of 15-second duration the arterio-venous pressure difference diminished gradually in man (30%), monkey (12%), and dog (65%), but increases in the goat (65%), and is associated with a bradycardia of various degrees. At the termination of radial acceleration within 1 to 4 seconds the arterio-venous pressure difference drops markedly in man (65%), monkey (40%), dog (60%), and drops to the normal prerun level in the goat. It is believed that at the termination of acceleration the animal or subject experiences the most harmful insult to cerebral circulation. Factors which tend to maintain minute cardiac output, as well as factors preventing generalized vasodilatation should be investigated for possibilities of offering greater tolerance to negative acceleration.

Effects on normal subjects of smoking cigarettes with varying concentrations of nicotine GRACE M. ROTH, *Section on Physiology, Mayo Clinic, Rochester, Minn*

Most investigators agree that smoking of tobacco causes elevation of blood pressure, increase in pulse rate, and vasoconstriction of the peripheral blood vessels of the extremities. Recently Haag and Larson studied a new 'low-nicotine' tobacco which when it was smoked produced much less effect on blood pressure and pulse rate than ordinary cigarette tobacco produced. One hundred ninety-two standard smoking tests, including simultaneous blood pressure, readings, pulse rates and skin temperature measurements, were carried out on 29 normal subjects. Six cigarettes made from the new low-nicotine tobacco were smoked at different times, and the main stream of smoke contained respectively 0.23, 0.55, 1.25, 1.83, 2.47 and 3.00 mg of nicotine. Neither the subjects nor the technicians making the test knew which cigarette was used. As a control, cornsilk cigarettes were smoked. The results were analyzed according to the initial basal heat production of each individual. The

effect on the skin temperatures of extremities of smoking two cigarettes containing the two lowest concentrations of nicotine in the main stream of the smoke was similar for the toes and somewhat greater for the fingers than the effect produced when cornsilk cigarettes were smoked. As the concentration of nicotine increased these effects increased. The nicotine content, therefore, should be decreased by at least 50% to prevent the vasoconstriction produced by the smoking of standard cigarettes. Likewise, a definite increase in the blood pressure and pulse rate occurred with increasing concentrations of nicotine in the cigarettes.

Potentiation of the gastric secretory response to other stimuli by caffeine JAMES L. A. ROTH (introduced by JULIUS H. COMBROE), *Dept of Gastroenterology, Graduate School of Medicine, and the Harrison Dept of Surgical Research, Univ of Pennsylvania, School of Medicine, Philadelphia*

Caffeine has been shown to potentiate histamine-induced gastric secretion in cat and man (*Am J Physiol* 142:107, 1944). In cats anesthetized with ether and prepared with a gastric cannula, the effect of caffeine upon other stimuli to gastric secretion was investigated. Urecholeline, 0.01-0.1 mg i.v., had little or no stimulating action, but evoked a marked secretory response when given after caffeine. This occurred whether the dose of caffeine itself was sufficient (35 mg base/kg) or insufficient (20 mg base/kg) to stimulate. Similar observations were made with liver extract (gastric instillation 30 units for 30 minutes) and caffeine. When the secretory rate had declined in response to repeated injections of urecholeline (0.02-0.1 mg intravenously every 15 minutes), a superimposed single injection of caffeine markedly augmented the output of acid secretion. These results have important implications in the dietary management of ulcer patients and in understanding their sustained gastric secretory response to caffeine.

Early divergence of pathways of aerobic and anaerobic glucose metabolism in yeast A. ROTHSTEIN, L. HURWITZ* AND R. MEIER* *Div of Pharmacology, Dept of Radiation Biology, Univ of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

Inhibition of glucose consumption of yeast by uranium is associated with formation of a stable complex between uranyl ion and certain cell-surface loci necessary for initial reactions between the cell and this substrate. Although relatively low concentrations of uranium completely inhibit both aerobic and anaerobic metabolism of glucose, marked differences have been observed in the inhibition curves. With uranium concentrations

below 10^{-6} M (yeast concentration, 5 mg/ml), there is no appreciable effect on anaerobic metabolism, but aerobic metabolism is inhibited up to 30%. With uranium concentrations between 10^{-6} and 10^{-2} M, the anaerobic inhibition curve passes from essentially zero to 100%, whereas the aerobic curve passes from 30% to only 70% crossing the anaerobic curve at about 60% inhibition. Only at 10^{-4} M uranium is aerobic metabolism completely inhibited. A marked difference has also been found by kinetic studies. The relationship between glucose concentration and rate of metabolism obeys the Michaelis-Menton equation both anaerobically and aerobically. In the presence of an inhibiting concentration of uranium, anaerobic metabolism still obeys the Michaelis-Menton equation but aerobic metabolism is quite different, following a complex hyperbolic function. It can be concluded that the aerobic and anaerobic pathways of metabolism diverge at or before the reactions inhibited by uranium. These reactions are known to occur on the cell surface and to involve the initial steps in glucose metabolism.

Movements of the dog's heart in the closed chest

H W RUDEL,* A SOKALCHUK,* G C RING
AND G J NAVIS * *Temple Univ Med School,*
Philadelphia, Pa

In order to better interpret the electrokymographic records obtained over the heart, movements of the dog's heart have been studied. Markers, opaque to x-rays, were placed on the surface of the right and left ventricles. The pericardium and chest walls were then closed so that the conditions for cardiac contraction were approximately normal. Our observations with this preparation differ somewhat from those made by Burchell and Visscher on the isolated heart. The base of the heart moves predominantly toward the apex and the posterior portions move toward the sternum. Early in systole, these sternal movements are much greater on the right than on the left side. This suggests a clockwise rotation of the heart (apical view) at this time. Rotatory ballistic forces in the dog and man are being investigated to further clarify these observations. The first rotatory ballistic force in the dog and man is clockwise.

Stethography of the heart sound intensity envelope

ROBERT F RUSHMER *Dept of Physiology and Biophysics, Univ of Washington School of Medicine, Seattle*

Heart sounds have been recorded as a logarithmic intensity envelope by incorporating a delay of 0.03 sec in the response of a logarithmic amplifier. Since the resulting record corresponds to a continuous line joining the wave peaks on a standard stethogram, a direct-writing penmotor (80 cycles/sec) can respond uniformly to the

amplitude of frequencies ranging from 30 to 2000 cycles/sec. By means of a series of filters, the frequency components of heart sounds and murmurs are analyzed. A major portion of the sounds appear between 30 and 150 cycles/sec, but both sounds and murmurs have components between 150 and 350 cycles/sec. A high pass filter attenuates sounds below 300 cycles/sec, to disclose high-pitched, low-intensity murmurs. Timing of murmurs and identification of adventitious sounds are facilitated by simultaneous recording of the log intensity envelope, an electrocardiogram, a pneumogram and a linear stethogram (frequency range 5-40 cycles/sec on a 4-channel, direct-writing instrument). The linear stethogram has proved useful for timing murmurs, since a high amplitude vibration is associated with the first sound at the apex and with the second sound at the base. Even the loud murmurs in congenital heart disease do not obscure these prominent excursions which appear to have a constant relation to the heart sounds.

Phosphate turnover in the liver in alloxan diabetes

JACOB SACKS AND BARBARA DAMAST *
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The effect of alloxan diabetes on the partition of the acid-soluble phosphorus compounds of liver and on their turnover rates has been studied in rats of the Sprague-Dawley strain. The tracer experiments were carried out several weeks after the administration of the alloxan, when the animals showed marked hyperglycemia, polyuria and glycosuria. They were not losing weight. Access to food and water was allowed up to the time of injection of the tracer phosphate, which was given subcutaneously. The inorganic phosphate content of the liver in these animals was about 50% higher than the normal range. The ATP-ADP and glucose-1-phosphate contents were reduced below normal. In the tracer experiments it was found that the P^{32} was removed from the plasma at a much greater initial rate than in normal animals. In the first few hours after the injection of the tracer phosphate, the relative specific activity of the P of the glucose-1-phosphate fraction was higher than that of the labile P of the ATP-ADP. This is the reverse of the situation found previously in the normal animal.

Factors influencing contraction of embryonic heart cultures (motion picture)

MACHTELD E SANO (introduced by DEAN A COLLINGS) *Dept of Tissue Culture, Temple Univ Med School and Hospital, Philadelphia, Penna*

Embryonic heart of the mouse can be used to study the effect of environmental changes such as heat and cold as well as the effect of different chemicals on the contraction of the heart muscle. This method gave consistent results with frag-

ments removed from 150 different embryos of approximately 7-8 days. Cinemicrophotography of the effect of heat and cold and toxins illustrates these findings. The medium is composed of Tyrodes solution, chicken plasma and mouse embryonic extract. Grown for 3 days the fragment shows good regular contractions. After 5 days toxic effects are noted. Contractions are irregular and decreased in amplitude. After washing and refreshing a more normal beat is gradually acquired. Lowering of the temperature to 24°C prolongs contraction for 24 hours. Lowering the atmospheric temperature to zero apparently arrests contraction. However, the cinephotographic record reveals areas of contraction which missed when studied under the microscope. Maximum length for which this fragment can be maintained at 0°C is 5-6 days. On reheating contractions are resumed. Calcium produces decrease in amplitude but increased duration of contraction period. Digitalis in a dilution of 1-100 cat units produces increased duration of contraction. Tissue culture of embryonic mouse heart is a simple, reliable and inexpensive method for testing the influence of physical and chemical factors on heart muscle contraction.

Effect of adrenalectomy on 'salt' hypertension

LEO A. SAPIRSTEIN,* WILBUR BRANDT* AND DOUGLAS R. DRURY, *Dept. of Physiology, Univ. of Southern California School of Medicine, Los Angeles*

Substitution of hypertonic sodium chloride solutions for the drinking water induces an arterial hypertension in the rat (Sapirstein, L. A., W. Brandt, and D. R. Drury. In press). In studying the mechanism of this hypertension we have investigated the role of the adrenal cortex in its maintenance by removing both adrenal glands after the hypertension was well established. Rats made hypertensive with hypertonic sodium chloride were used. After hypertension was established, the experimental animals were bilaterally adrenalectomized, and the controls unilaterally adrenalectomized. Blood pressures were followed with the tail plethysmograph for 3 weeks after the procedure, the animals continuing to receive hypertonic sodium chloride as a substitute for the drinking water. The average systolic pressure in the experimental group was 145 mm Hg before the procedure and 147 mm Hg after the procedure. The control group showed an average systolic pressure of 142 before the procedure and 146 after the operation. No adrenal tissue was found at autopsy. These results indicate that 'salt' hypertension is not dependent on the presence of the adrenal cortex.

Effects of ureteral ligation on renal function in the anesthetized dog

O. W. SARTORIUS (introduced

by W. D. LOTSPEICH) *Dept. of Physiology, Syracuse Univ. Med. College, Syracuse, N. Y.*

The alterations in renal function of the anesthetized dog produced by ureteral clamping have been investigated. When catheters, inserted into each ureter, are separately clamped for periods approximating one hour in duration urine flow from the clamped side is found to increase with removal of the clamp in spite of lowered filtration rate. No significant change occurs in renal plasma flow nor in electrolyte excretion. It would appear that ureteral ligation of such duration partially blocks the active reabsorption of water for periods ranging up to 2 hours after the clamp is removed. Posterior pituitary inhibition is not a factor in the resultant diuresis as the contralateral control kidney is not altered by the procedure.

Pharmacological blockade of pituitary activation in the rabbit and the rat

CHARLES H. SAWYER, J. E. MARKEE AND JOHN W. EVERETT* *Dept. of Anatomy, Duke Univ., Durham, N. C.*

A study was made on effects of blocking agents of adrenergic, cholinergic, ganglionic and central synapses on neurohumoral control of LH release from the adenohypophysis. With the chloroethylamine derivatives SKF-501, Dibenamine and 2-dibenzylaminoethanol, a positive correlation was found in the rabbit between ability to block the cortical stimulus from reaching the hypophysis and capacity to protect against twice-lethal dosages of epinephrine. A negative correlation was observed between central excitatory activity of these agents and their potency in blocking pituitary activation. The rat pituitary-activation mechanism reacted to these drugs in a manner almost identical to the rabbit response. The results are consistent with the hypothesis that these agents prevent pituitary activation by adrenergic blockade. The imidazoline derivatives, Prisco and C-7337, failed to block pituitary activation in either species, although C-7337 develops rapid protection against epinephrine toxicity. The failure cannot be attributed to inability of these agents to reach the effector cells, it would appear more likely that imidazoline derivatives fail because they lack the particular chloroethylamine property which blocks the *natural* adrenergic mediator (not necessarily epinephrine itself). The anti-cholinergic drug, atropine, was successful in blocking pituitary activation in both species, the central anesthetic, Nembutal, was effective in the rat but not in the rabbit, the ganglionic blocking agent, tetraethylammonium, failed in both species. Assuming that Nembutal failed in the rabbit because of temporal considerations, these collective data suggest that the site of atropine-block is located between the hypothalamus and the hypophysis.

Cerebral metabolism in hyperthyroidism and myxedema PRITZ SCHINBERG (introduced by EUGEN A. STFAH, JR.) *Dept of Medicine, Duke Univ School of Medicine, Durham, N C*

Cerebral blood flow and metabolism were measured by means of the nitrous oxide technique in 9 subjects with hyperthyroidism and in 8 subjects with myxedema. Three patients with myxedema were restudied after clinical improvement on thyroid therapy. The subjects with hyperthyroidism showed no significant variation from normal in any of the measured cerebral metabolic functions. This is of considerable interest in view of the 35% increase in cardiac output and 60% increase in splanchnic oxygen consumption that is known to occur in a similar series of hyperthyroid patients. The patients with myxedema showed reductions in cerebral blood flow (38%) and oxygen consumption (27%) commensurate with the fall in cardiac output and total oxygen consumption that is known to occur in these patients. Cerebral glucose consumption decreased in proportion to the decrease in cerebral oxygen consumption. Cerebral vascular resistance was increased almost 100%, and definitely decreased following treatment. The cerebral metabolic functions returned toward normal, in conjunction with clinical improvement, in the 3 patients restudied after thyroid therapy. These data indicate that in hyperthyroidism the brain does not share in the general increase which occurs in body metabolism, and that the clinical signs of mental dysfunction so commonly observed in myxedema may be accounted for by the decreased cerebral metabolism in this disease.

Site of action of Dramamine and Benadryl in correction of forced circling movements and of convulsant brain wave patterns induced by di-isopropyl fluorophosphate (DFP) M. SCHIFF,* W. G. ESMOND* AND H. E. HIMWICH *Medical Div., Army Chemical Center, Md*

The intracarotid injection of di-isopropyl fluorophosphate (DFP) produced an adverse syndrome turning of the head and forced circling, away from the injected side. Section of the 8th revealed that in the rabbit and cat the peripheral vestibular receptors exerted a conditioning influence while in the monkey the response remained unaltered by the 8th nerve section (C. F. Essig, J. L. Hampson and H. E. Himwich). These results afford evidence for a central cholinergic mechanism for the forced circling, a conclusion supported by the fall of cholinesterase activity observed in cerebral structures and particularly in the homolateral caudate nucleus with the smaller turning doses. In the present experiments on cats DFP in subconvulsant doses was 1) injected through the tympanic membrane 2) placed against the

round window 3) injected through the round window and uniformly failed to evoke forced circling, proving the dispensability of the peripheral vestibular receptors for this syndrome. Dramamine, 5 mg/kg, injected i.v., corrected the forced circling movements in 8 rabbits. Similarly Benadryl, 5 mg/kg, restored normal behavior in 1 other rabbit previously exhibiting the syndrome. Dramamine, 10 mg/kg, prevented forced movements in 4 rabbits subsequently injected with DFP.

DFP, in convulsant doses, was used to induce grand mal-like electrocorticograms in curarized artificially respired rabbits. Dramamine or Benadryl administered intravenously after DFP restored the normal electrocorticogram, and injected before DFP, prevented the seizure pattern. It is suggested that the atropine-like action of these 2 antihistamines, exerted centrally, is the basis for their therapeutic effects.

Kidney physiology of the desert rat *Dipodomys merriami* BODIL SCHMIDT-NIELSEN *Kettering Lab., Univ. of Cincinnati, Cincinnati, Ohio*

The kidneys of the desert rats *Heteromyidae* have been found to be able to perform unusual osmotic work. Electrolyte concentrations up to 1.2 M and urea concentrations up to 3.8 M have previously been reported. A closer study of the kidney physiology and histology was undertaken. Studies of the creatinine clearance indicate that the glomerulus filtration is proportional to the urine flow, and that the reabsorption of water in the tubuli in the desert rats may be 2-3 times as high as the reabsorption in the tubuli in the white rats.

Evidence for physiological activity in nerve membrane in response to subthreshold stimulation OTTO H. SCHMITT AND PETER A. STEWART* *Depts. of Zoology and Physics, Univ. of Minnesota, Minneapolis*

The electrical admittance of nerve membrane has been measured by the complex attenuation method. For the squid giant axon, the results agree with those of Cole for the frequency regions where both methods are reliable. As the attenuation method is not limited to single fiber analysis and does not require large fibers, its use is being extended to other than squid nerves, and to other cylindrical cells. In the low frequency region, 50-500 cps, which is not readily accessible by conventional methods, anomalous characteristics intimately associated with the physiological state of the nerve have been measured and to some extent interpreted in terms of physico-chemical mechanisms. It is established rather convincingly, for example, that gross structural characteristics of the fiber determine speed of propagation while undetermined physico-chemical membrane factors

account for the anomalous impedance. Even at levels of stimulation only a small percentage of threshold is there evidence for a physiological energy-contributing process, which is strongly time dependent and highly responsive to usual nerve reagents. Preliminary measurements show frog nerve to have average membrane properties similar to those of squid fibers, but these properties are almost completely obscured if the nerve is not freed of its external membranous sheath. A mechanized nomographic complex-plane computer which permits immediate evaluation of admittance results from raw experimental data has been devised and will be described if time permits.

Relation of stress to certain biophysical alterations of the blood in the normotensive and hypertensive subject ROBERT A. SCHNEIDER (introduced by HAROLD G. WOLFF) *Cornell Univ. Med. College, New York City*

This is a preliminary study of the effect of stress on blood-clotting time, blood viscosity, the erythrocyte sedimentation rate and the hematocrit. Subjects included healthy volunteers, hypertensive and nonhypertensive patients. The stress situations included physical exercise, a modified cold-pressor test, a half-hour period at rest where the subject was asked not to move or talk, and finally a stressful interview. Blood was drawn with a silicone treated syringe and needle prior to, immediately after, and finally one hour after stress. Clotting times were measured in siliconized Wassermann tubes (method of Barker and Margulies). The blood viscosity was measured on heparinized specimens using the method of Tang and Wang. The sedimentation rates and hematocrit percentages were both measured in Wintrobe tubes. The sedimentation rates were corrected values. Exercise in healthy adults always resulted in a marked shortening of clotting time, an increase in the blood viscosity, an increase in hematocrit and a decrease in sedimentation rate. Nearly identical findings occurred both in the cold-pressor test, only in the presence of a pressor response, and with the stress interview but again only in those subjects experiencing a pressor reaction. No significant changes occurred in these experiments in the absence of a pressor response. The immobility experiment failed to elicit a pressor reaction and produced changes in the blood opposite to those found after exercise. The significance of these findings as they relate to Cannon's work and to the problem of the hypertensive state is discussed.

Threshold electrotonic potentials of nerve and muscle at different temperatures GORDON M. SCHOEFFLE *Dept. of Physiology, Washington Univ. School of Medicine, St. Louis Mo*
In a frog sciatic nerve, stripped of perineural

sheath, brief conditioning and testing shocks of 0.08 sigma duration elicit electrotonic potentials at the stimulating cathode which summate to a fixed intensity at threshold, regardless of the interval between shocks. The decline of this threshold potential subsequent to the test shock follows an identical time course in each instance. These data are consistent with those previously reported concerning stimulation of stripped nerve or fiber bundles by threshold rectangular currents of various durations. Rheobase is elevated by cooling, but chronaxie and the time parameters of the electrotonic potential are not appreciably altered by a 15° drop in temperature. However, if the sheath is not removed the time parameters of both excitation and electrotonic potential are appreciably increased by cooling, the reciprocal chronaxie with a Q_{10} of 1.15. These data provide strong additional evidence supporting the view that local attainment of a critical outwardly directed potential difference across the distributed membrane capacity is sufficient for excitation of nerve in the absence of accommodation. Stimulation of isolated bullfrog muscle fiber bundles by means of rectangular currents gives rise to electrotonic potentials at the cathode which decline in magnitude as duration of threshold rectangular current stimulus is decreased. However, the time parameters of electrotonic potential and excitability are apparently of the same order of magnitude.

Photometric ninhydrin method for the measurement of proteolysis THEODORE B. SCHWARTZ* AND FRANK L. ENGEL *Dept. of Medicine, Duke Univ., Durham, N. C.*

It has been shown by Moore and Stein (*J. Biol. Chem.* 176:367, 1948) that the color produced by the interaction of an improved ninhydrin reagent with the free amino groups of amino acids and peptides conforms with Beer's Law over a broad range. These findings have been applied to the measurement of the rate of enzymic hydrolysis of a synthetic peptide substrate, L-leucylglycylglycine (LGG). Enzymic cleavage of LGG into L-leucine and GG results in a progressive increase in free amino groups and, consequently, a continuing enhancement of ninhydrin color yields in serial samples withdrawn from an hydrolysis mixture. Theoretical considerations suggested that a direct proportionality exists between percentage hydrolysis of the substrate and the optical density of the ninhydrin color produced in any given sample. Experimental confirmation of this hypothesis has been obtained by measuring the color yield of mixtures containing LGG, GG and L-leucine synthetically prepared to represent varying degrees of hydrolysis. This method is relatively simple and especially time saving when large

numbers of analyses are required. It permits accurate evaluation of enzyme kinetics and gives results that compare favorably with those obtained with the Van Slyke manometric technique. It theoretically may be employed to measure the enzymic hydrolysis of any synthetic peptide substrate.

Comparison of cardiac output determined by the Fick procedure and a direct rotameter method
ROBERT D. SEELY,* WILLIAM E. NERLICH* AND DONALD E. GREGG *Med. Dept. Field Research Lab., Fort Knox, Ky.*

As an accurate standard of reference with which the Fick procedure might be compared, a method was devised for use in open-chest dogs, whereby total cardiac output could be directly and continuously recorded by measuring flow through the pulmonary artery. Such a method avoids the error inherent in other direct procedures (flow meters in aorta and venae cavae) which fail to measure coronary flow. Cardiac output, recorded optically over a 6-minute period by a rotameter connected to the pulmonary artery, was compared with simultaneous measurements made with the Fick method. A modified Benedict-Roth spirometer was used to measure oxygen consumption. Arterial and venous (pulmonary artery) samples were taken continuously over the 6-minute test period and were analyzed in duplicate for oxygen content by the method of Van Slyke and Neill. Duplicate samples were required to check within 0.08 volumes %. In a few experiments, Fick flow values in closed-chest dogs were compared with flows simultaneously measured by a rotameter in the venae cavae. The average variation between the 2 series of measurements was $\pm 4\%$. Twelve of the 13 comparisons made agreed within less than 8%. This small deviation is within the range of summated errors which might exist on the basis of known technical inaccuracies.

Behavior of renal clearance mechanisms under pentobarbital sodium anesthesia
EWALD E. SELKURT AND KIRKWOOD F. GLAUSER* *Dept. of Physiology, Western Reserve Univ. Med. School, Cleveland, Ohio*

Study of renal clearances in dogs under pentobarbital anesthesia, where use of untrained dogs was desired, or in connection with surgical intervention, has made desirable reinvestigation of the possible influence of this anesthetic agent on renal function. We were particularly interested in its possible effect on sodium clearance. After control periods in trained female dogs, full anesthetic doses of pentobarbital were given and clearances of creatinine, PAH, and sodium were followed for as long as 52 hours. It was found that no significant change in CC_r , $CPAH$ and FF oc-

curred during time intervals averaging 73 minutes. During the next 117 minutes, CC_r increased, and $CPAH$ decreased both by small but probably significant degrees so that FF increased significantly. This trend continued to a more marked degree during the final 90 minutes. Sodium clearance was increased about 5-fold during the total time of observation, but emphasis must be placed on the fact that control clearances only averaged ca. 0.25 cc/min. With regard to impairment of tubular efficiency, this indicated only about 2% decrease in reabsorption of the filtered load. A marked effect was however noted on urine flow which decreased to an average of about 30% of the control value during the periods immediately following administration of the anesthetic agent.

Compensatory changes in the rat following depletion of extracellular electrolytes
ROBERT E. SEMPLE (introduced by WALTER S. ROOT)
Dept. of Physiology, College of Physicians and Surgeons, Columbia Univ., New York City

Daily water intake and urine output were measured in a group of young adult rats on a diet made complete by addition of sodium chloride. After one week of daily observation, the animals were given an intraperitoneal injection of 9 cc/100 gm body weight of isotonic glucose solution. One hour later 90% of the injected volume was recovered, and its chloride content determined. After this time sodium chloride was omitted from the diet. For the next 24 to 48 hours the animals exhibited a marked increase in urine output with a concurrent decrease in water intake. After 48 hours a normal relationship between fluid intake and output was reestablished. Fluid loss by way of the kidneys during the period of negative water balance was comparable to the volume of fluid that would be given by up the extracellular fluid compartment if the chloride concentration therein were restored to normal. This predicted loss in extracellular fluid was calculated from the amount of chloride removed in the intraperitoneal glucose solution. Serum chloride concentrations returned to normal within three days. The fluid loss was accompanied by a proportionate loss in body weight. These results indicate that electrolyte depletion in a rat by intraperitoneal glucose is followed by fluid loss sufficient to restore the plasma chloride concentration to normal.

Influence of histamine and anti-histaminic compounds on water imbibition by striated and smooth muscles and gastric mucosa
V. SENCINDIVER,* E. M. LARSON* AND M. H. F. FRIEDMAN *Dept. of Physiology, Jefferson Med. College, Philadelphia, Penna.*

The influence of histamine and anti-histaminic compounds (neohetramine, thephorin) on imbibition

tion of water by straited muscle, smooth muscle from the stomach and gastric mucosa was studied Using fresh tissue derived from the rabbit and the frog, the water imbibed over a period of 24 hours under controlled conditions of pH, temperature, and tonicity was determined The water uptake by rabbit smooth muscle and gastric mucosa was equal to that of similar weights of corresponding frog tissues The water uptake by rabbit striated muscle, however, was much less than by frog striated muscle The water imbibition by rabbit isolated smooth muscle was depressed and often reversed by the addition of histamine On the other hand, antihistaminic compounds either did not inhibit water uptake or at times accelerated it The anti-edemic action of histamine was not related to its motor effects on smooth muscle Other smooth muscle stimulants did not influence water imbibition Rabbit isolated gastric mucosa behaved similar to smooth muscle Pretreatment of the animal with anti-histaminics was not found to affect the rates of water imbibition by the isolated tissues These results do not support the views of certain French investigators on the anti-edemic properties of anti-histaminic compounds

Results suggesting endocrine participation in experimental renal hypertension ROGER W SEVY,* DOROTHY HAASCH CHESSE* AND GEORGE E WAKEBLIN *Dept of Physiology, Univ of Illinois College of Medicine, Chicago*

Crude beef anterior pituitary extract was administered 1 m (0.5–1.0 gm of wet gland equivalent/per/kg of body weight/per day) to 4 renal hypertensive dogs for 2–5 months and to 4 normotensive dogs for 1–6 months preceding and up to 1 month following bilateral renal artery constriction Of the 4 renal hypertensive dogs, 2 showed an excellent and one a moderate antihypertensive response after 2 months of treatment, the 4th showed no response The antihypertensive response in one dog was temporarily reversed by desoxycorticosterone acetate overdosage Of the 4 normotensive dogs, one, which received the longest course of treatment, showed no rise in blood pressure following constriction of the renal arteries despite marked reductions in TmPAH and the renal clearance of PAH and creatinine Two showed no rise in blood pressure following constriction of one renal artery but died, with the clinical and pathological picture of malignant hypertension, following constriction of the contralateral renal artery The 4th developed typical chronic hypertension Further possibility of endocrine involvement in experimental renal hypertension is suggested by the finding of a decrease in sensitivity to insulin and a somewhat lower fasting blood glucose level in 10 renal hypertensive as compared to 9 normotensive dogs

Potassium shifts in nerve A M SHANES *Dept of Physiology and Biophysics, Georgetown Univ School of Medicine, Washington, D C*

Electrical studies of *Rana pipiens* sciatics and *Libinia emarginata* leg nerves indicate that resting potential fluctuations are due largely to potassium movement A sensitive method to detect such potassium shifts is presently being employed It consists of collecting 1 ml samples of medium successively placed in contact with paired sets of 3 or 4 nerves Potassium analyses of the individual samples are made with the Beckman flame spectrophotometer Typically, repeated collections are made first in oxygen, then in nitrogen, and finally in oxygen again *Libinia* nerves lose 20 μ M potassium/gm wet weight/hr in oxygenated sea water whether glucose is present or not, anoxia doubles or trebles this loss, and 50–100 mM glucose reduces this increment, return to oxygen either completely stops potassium escape or causes an absorption of potassium Intact *Rana* sciatics, following equilibration for 1 hour in oxygenated $\frac{1}{2} \times$ Ca Ringer's solution, lose 0.4 μ M potassium/gm/hr, mere removal of the perineurium causes 3 of the nerve potassium to escape during equilibration, but negligible loss occurs thereafter Both types of preparation release potassium during anoxia, usually at a rate increasing with time which averages 1.4 μ M/gm/hr over 3 to 4 hours Return to oxygen causes an absorption of potassium in the absence of the perineurium (10 μ M/gm/hr), or cessation of leakage with the intact sheath Cocaine (0.1%) halves the anoxic escape of potassium without altering recovery, In normal Ringer's similar effects are observed but the magnitude of the differences appears to be less

Electrocardiographic age trends E SIMONSON *Lab of Physiological Hygiene, Univ of Minnesota, Minneapolis*

Means, standard deviations, medians and percentile distribution of 34 electrocardiographic items in the 3 standard leads and CF₄ were compared in a group (I) of 157 normal men from 17 to 25 years and a group (II) of 233 normal men from 45 to 55 years Most items showed statistically highly significant differences ($p < 0.01$) between the means of the two groups At the older age there were significant increases in the P-R interval and in KQT (= square root of R-R interval divided by Q-T interval), decreases in the QRS and T amplitudes, especially in the standard leads, and a shift to the left in the QRS and T axes The arrhythmia index (difference between the smallest and largest R-R interval) declined from a mean of 0.016 in group I to 0.044 sec in group II Both groups included men in all weight categories in a similar proportion Comparison

of partial groups of 49 younger men and 66 older men within $\pm 5\%$ of their standard weight revealed differences similar to those obtained between the total groups. The observed age trends are, therefore, not due to weight differences. The frequency distribution of most items was asymmetrical, but similar in both groups. The age differences in many items are large enough to be of importance for routine clinical interpretation. Age corrected standards were calculated from the standard deviations or percentile distribution. Statistically highly significant age trends were also observed in VR, VL, and VF.

Inactivation of thrombin and fibrinogen by tyrosinase IRVIN W. SIZER AND PHILIP F. WAGLEY *
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When commercial thrombin (Upjohn, Parke-Davis, or Armour) was incubated in phosphate buffer, pH 6.0, at 37° with commercial tyrosinase (Treemond or Synzyme) there was a marked inactivation of the thrombin. This was measured by determining clotting time after the addition of an aliquot of thrombin to a standard amount of fibrinogen (Armour) at 25°. In a typical experiment, incubation of thrombin with tyrosinase resulted, after 6 hours, in a change in clotting time from 0.4 min to 2.5 min. Although the rate of clotting was changed, the amount and nature of the final clot formed was independent of any previous treatment of thrombin by tyrosinase. Dialysis experiments indicated that the inactivation was not mediated by low molecular weight impurities in the thrombin. Similar studies were made on the inactivation of crude (Armour) and purified fibrinogen by tyrosinase. The inactivation occurred slowly at first and then at an increasingly rapid rate so that in 3 hours the clotting time, when tested by the addition of thrombin, had changed from 0.4 min to 20 min. The amount of clottable protein correspondingly decreased as a result of the inactivation of fibrinogen by tyrosinase. The clot obtained from fibrinogen oxidized by tyrosinase was very different from that obtained from normal fibrinogen. Studies with the electron microscope indicated that the fibrin from normal fibrinogen was characterized by fine fibrils, while that from fibrinogen oxidized by tyrosinase appeared completely amorphous. Preliminary studies in the ultraviolet indicate that tyrosinase will produce a change in the absorption spectrum of both thrombin and fibrinogen and perhaps of fibrin as well.

Exercise following whole-body irradiation of mice
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Medicine Inst., Natl. Insts. of Health, Bethesda, Md

In studies of metabolic factors which may be involved in the irradiation syndrome various stresses are imposed upon mice following whole-body x-irradiation. Information concerning exercise may be of practical as well as physiological importance. Mice were exercised in a cage with individual tracks 48 cm in diameter making 3 revolutions/minute. Controls tolerated this for 12 consecutive hours, or 8 hours/day on 3 consecutive days, with only transitory weight loss. The tests were considered severe enough for our purpose because many mice, after a week of thyroid administration, failed to exercise for more than a few hours, some of them dying during the test. Mice given x-ray in the low lethal dose rate (325 r) tolerated 12 hours of exercise immediately after or up to 16 days after irradiation. Mice given 400 r tolerated exercise 8 hours/day for 3 consecutive days beginning immediately after or up to 13 days after irradiation. Survival time and mortality were affected, if at all, in no more than 20% of these mice. After 600 r both irradiated controls and those exercised 8 hours/day on days 0, 1, and 2 post-irradiation died, 20% of the latter group during exercise, the remaining at about the same times as the controls. Radiation did not markedly decrease ability to tolerate exercise, nor did exercise markedly increase mortality or decrease survival time of irradiated mice. These studies are being continued.

Further observations on the action of curare

JAY A. SMITH, MELVIN POST AND SHERWIN ZALMAN (introduced by L. B. NICE) *Dept. of Physiology and Pharmacology, Chicago Med. School, Chicago, Ill*

Smith *et al* (*J. Pharmacol. & Exper. Therap.* 93: 294, 1948) show that neutralized thiamine solutions are without effect on the isolated heart and (*Science* 108, 412, 1948) that the action of thiamine is that of a curariform drug. McIntyre (*Curare*, University of Chicago Press) shows that the action of curare drugs is to raise the threshold to acetylcholine. In the present experiments on the isolated turtle heart, curare preparations abolish or reduce the inhibiting effects of acetylcholine. After atropinization of the isolated heart, curare drugs abolish the stimulating effects of acetylcholine. These results indicate that, in the absence of acetylcholine, the typical effects of curare drugs are absent. In anesthetized dogs, curare preparations (d-tubocurarine, Squibb, and thiamine) cause a fall in blood pressure, followed, in most cases, by a rise to a level higher than the control level. In addition, after the injection of curare preparations, prostigmine frequently causes a marked rise in

blood pressure and increase in the heart rate (these effects may be prevented by dibenamine and tetraethylammonium bromide). Thus, in addition to the possibility of a stimulation of the adrenergic endings by acetylcholine, these results may be interpreted to mean that the effects of curare persist longer in the parasympathetic ganglia than in the sympathetic ganglia.

Non-olfactory functions of the pyriform-amygdaloid-hippocampal complex WILBUR K. SMITH
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On the basis of the results of excitation and ablation, the pyriform-amygdaloid-hippocampal complex can now be considered to have transcended its role as a part of the olfactory apparatus and to participate in various ways in the general behavior and reaction patterns of the mammalian organism. Excitation of the posterior pyriform region in both cat and monkey produces a variety of responses mostly in the autonomic realm (*Federation Proc* 3 43, 1944, *Anat Rec* 100 713, 1948). Extirpation of this region in the monkey appears to produce no gross motor or sensory changes, but there is a remarkable alteration in behavior and in the reaction patterns of the animal. Activity is decreased so that the animal not only moves less, but more slowly than before operation. Fear and the defensive and offensive reactions associated with it have disappeared, and the animal permits itself to be handled with little or no resistance. It will now approach and take food from the observer either with its hand or mouth. Another aspect of altered activity may be designated as oral compulsive behavior. Objects placed within its reach are picked up in an apparent indiscriminate manner, brought to the nose, then placed in the mouth and if edible are ingested, if not are discarded, sometimes after chewing. Investigations designed to determine the role of the components of the pyriform-amygdaloid-hippocampal complex in the altered behavior have shown that the loss of the fear and defensive reactions can be produced without the compulsive oral behavior.

Effect of insulin-induced hypoglycemia on contraction of human gall bladder W J SNAPE, RUSSELL WIGH* AND M H F FRIEDMAN *Dept of Physiology, Jefferson Med College, Philadelphia, Penna*

The influence of vagal stimulation on the evacuation of the human gall bladder was studied in subjects without evidence of biliary tract disease. Hypoglycemia induced by insulin was used to stimulate the vagus. Evidence of gall bladder contraction was obtained by 1) radiographic examination of the visualized gall bladder and 2) determination of the bilirubin concentration of

the duodenal contents obtained by constant aspiration. In subjects previously found to show a normal gastric secretory response to histamine the production of insulin hypoglycemia resulted in evacuation of the gall bladder providing the acid gastric contents were permitted to enter the duodenum. In subjects found to be histamine-refractory the gall bladder contracted only slightly, or not at all, after the administration of insulin. It is concluded that in the human the contraction of the gall bladder may be chiefly under hormonal (cholecystokinin) mechanism.

Influence of cerebellar cortex on incoming cerebellar volleys RAY S SNIDER AND P M COOKE*
Dept of Anatomy, Northwestern Univ Med School, and Dept of Psychiatry, Univ of Illinois Med School, Chicago, Ill

The altered electrical activity of efferent cerebellar volleys in the brachium conjunctivum was visualized on a cathode ray screen (Grass Model III amplifier) following application of electrical stimuli of various frequencies and wave forms to 1) brachium restiformis, 2) cerebellar cortex and 3) central cerebellar nuclei. Electrical activation of brachium restiformis with single pulses results in modified activity at a cortical level and also in the appearance of repetitive waves approximately 30 msec apart and 30 msec duration at a conjunctival level. Similar waves have been observed following stimulation of nucleus dentatus. Effects of such physiological stimuli as 'auditory clicks' on the above systems are being studied. The most pronounced changes observed at a conjunctival level following application of single pulses to cerebellar cortex is an approximate doubling in amplitude and halving in frequency. Attempts are being made to relate these observations to certain known intrinsic cerebellar activities. Most of the above observations were made on cats prepared according to an 'isolated encephalon' technique and upon animals following ether, novocaine and beta-erythroidine narcosis. Similar observations could not be made following barbiturate anesthesia.

Changes in carbon dioxide-combining power of fetal blood FRANKLIN F SNYDER *Depts of Obstetrics and Anatomy, Harvard Med School, Boston, Mass*

A striking decrease in the carbon dioxide combining power of the fetal blood occurs as pregnancy is prolonged beyond term. In postmature rabbit fetuses, delivered at 35 days, which is the limit for survival of the fetus within the uterus, the carbon dioxide combining power averaged 31 volumes % in contrast to 40 observed in fetuses delivered at term, i.e. 31 days. At the stage of prematurity, 29 and 20 days, the fetal blood averaged 41. In previable fetuses at 27 days, the

blood averaged 39. Observations were made upon a series of 85 fetuses operatively delivered at various stages of pregnancy ranging from pre-viability to postmaturity. Determinations of the carbon dioxide combining power with the Van Slyke apparatus were carried out on 0.5 cc samples of plasma obtained from neck veins.

Effect of acute experimental polycythemia on renal blood flow. MERRILL P. SPENCER, KIRKWOOD F. GLAUSER AND PHILIP W. HALL (introduced by J. M. HAYMAN, JR.) *Dept. of Physiology, Western Reserve Univ. Med. School, Cleveland, Ohio*

When dogs were cross-transfused with matched blood in acute experiments, it was found that after a 2-hour interval had elapsed hemoconcentration had occurred without significant increase in blood volume and with readjustment of mean arterial blood pressure to approximately control values. Although the exact mechanisms of the compensatory changes are obscure at present, the increase in blood viscosity predicated on the increase in the hematocrit value offered an interesting problem in renal hemodynamics. It was found that effective renal blood flow, as measured by PAH clearance and the hematocrit percentage, had changed little. Further, glomerular filtration rate as measured by the creatinine clearance was on the average not significantly different from the control values. However, marked decrease in the effective plasma flow as measured by the PAH clearance had occurred (-22 to -51%). This gave significantly elevated filtration fraction values. When proper allowance was made for the expected increase in viscosity resulting from the increased hematocrit value, the well-maintained total renal blood flow suggested dilatation in the renal vascular circuit. Since such dilatation was accompanied by an elevation of the filtration fraction, the conclusion was reached that it occurred predominantly in the afferent arteriole.

Ratio of myosin to actin in synthetic actomyosin. S. S. SPICER AND J. GERGELY (introduced by LOUISE H. MARSHALL) *Experimental Biology and Medicine Inst., Natl. Insts. of Health, Bethesda, Md.*

Addition of adenosine triphosphate (ATP) to a colloidal suspension of myosin and actin at 0.13 M KCl and pH 7.5 causes superprecipitation. It has been assumed that these 2 components combine in almost any proportion to form actomyosin (A. SZENT-GYÖRGYI *Muscular Contraction*, N. Y., 1947). The ratio in which the 2 proteins combine during superprecipitation with ATP has been investigated. Increasing amounts of actin were added to a constant amount of myosin and vice versa. The supernate after removal of the precipitate by centrifugation was tested for presence

of both myosin and actin, and the total protein in precipitate and supernate was determined. One ratio was always found in which the supernate contained no myosin or actin. This indicates a stoichiometric ratio of myosin to actin. Myosin (or actin) added in increasing amounts beyond the stoichiometric point appeared part in the supernate and part in the precipitate up to a certain limit. All of the protein added beyond this limit was found in the supernate. This indicates a loose secondary association of the 2 components. Electrophoretic analysis showed less than 2% impurity in the myosin, but 30% or greater impurity in the actin. Therefore, protein left in the supernate at the stoichiometric point probably derived from the actin. Subtracting this protein from the actin added at the stoichiometric ratio gives a myosin:actin ratio of roughly 4:1 by weight.

Attempts to record human electro labyrinthogram.

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Dept. of Exper. Neurology, Temple Univ. Med. School, Philadelphia, Penna.

In order to pick up the electrical discharges of the statokinetic labyrinth and/or of the 8th nerve, various electrode positions were tried such as intermeatal, meatal-mastoid, meatal-retromastoid, meatal-suboccipital combinations. The meatal-retromastoid combination seems most promising, with the meatal electrode close to that part of the posterior wall of the external auditory meatus which is connected by the antrum bridge to the external semicircular canal. In order to study the question whether one picks up labyrinthine discharges, the electrogram was recorded before and after rotation in cases in which the 8th nerve had been cut or destroyed by tumors unilaterally and in which the sensory receptors of the labyrinth probably were also degenerated due to interruption of the circulation of the internal auditory artery on the operated side. In order to minimize artifacts due to the postrotatory nystagmus the patient looked after rotation in the direction of the slow component of the postrotatory nystagmus, if a nystagmus of central origin was intensified in this direction, the patient looked straight ahead. The eyes were kept open in order to suppress α waves of cortical origin. Under these experimental conditions, discharges could be picked up, which showed a definite postrotatory increase of the amplitude compared to those recorded in the resting stage, at least in single instances, this increase was limited to the side of the intact labyrinth or intact 8th nerve.

Effects of lesions of human thalamus in region of dorsomedial nuclei. E. A. SPIEGEL, H. T. WYDIS,* H. SHAI, K. B. CONGER* AND H. K. FISCHER. *Depts. of Exper. Neurology, Neuro-*

surgery, Urology, and Fels Foundation, Temple Univ Med School, Philadelphia, Penna

Lesions in the area of the dorsomedial nuclei were produced by means of the stereotaxic method in psychotics, epileptics, and in cases of unbearable pain. The location of the lesions was checked postoperatively by roentgenologic studies determining the relationship of pantopaque droplets injected at the site of the lesion to the pineal gland. Such lesions frequently produced transitory disturbances of orientation, particularly for time, and transitory defects of memory affecting recent as well as remote events. There were no significant changes of pain threshold and no disturbances of the so-called psychoreflexes. Changes in the realm of the vegetative system, however, could be observed. Pharmacodynamic tests chiefly revealed a shift of balance between the sympathetic and the parasympathetic system in favor of the former. Studies of the glucose tolerance showed a decrease of the postprandial hyperglycemia in cases in which the peak of the hyperglycemia after ingestion of glucose exceeded 180 mg. Alterations of the blood lipides and disturbances of liver function could not be found. Incontinence of the urinary bladder or precipitous micturition developed rather frequently, and cystometric measurements revealed, at least in single instances, a hyperirritability of the urinary bladder. All these disturbances of vegetative innervation were of transitory nature. It is suspected that they are due to the loss of impulses that are transmitted by periventricular fibres from the dorsomedial nuclei to the hypothalamus.

Isolation and characterization of two adaptive enzymes formed by yeast in response to maltose S SPIEGELMAN, M SUSSMAN* AND B TAYLOR * *Dept of Bacteriology, Univ of Illinois, Urbana*

Recent work by Monod (*Compt rend Acad d sc* 227 240) and Doudoroff, *et al* (*J Biol Chem*, 179 921) has demonstrated that the enzyme formed by *E coli* in response to growth in the presence of maltose is an 'amylomaltase'. A similar examination in the case of a strain of *S cerevisiae* revealed that adaptation to maltose leads to the production of two enzymes, neither one of which is an amylomaltase. The material used consisted of fast, efficiently dried cells, harvested at 18-24 hours from a well aerated culture (6 l) incubated at 30°C. Dried preparations prepared from cells either grown in a medium containing maltose, or adapted to maltose in resting suspension subsequent to harvesting, possessed the capacity to split maltose and α -methylglucoside. Similar glucose grown preparations were inactive against both substrates. The enzyme activity is extracted with $M/15 (NH_4)_2HPO_4$ (AP)

and precipitated from the supernate with ammonium sulphate (AS) at 75% saturation. Reprecipitation with AS at 45% saturation yields a precipitate containing 65% of the activity of the original AP extract. Dialysis of the second AS precipitate against large volumes of distilled water resolves the activity into 2 components. One, a fraction which precipitates during dialysis (enzyme I) and the other remains in the supernate (enzyme II). Enzyme I was shown to be a maltase capable of splitting maltose into 2 glucose molecules, as demonstrated by quantitative recovery experiments, chemical analysis based on reducing power and paper strip chromatography. The involvement of phosphate was eliminated since the enzyme functioned equally well in the absence of phosphate. Enzyme I is completely unable to split α -methylglucoside, but can hydrolyze α -phenylglucoside. Enzyme II, on the other hand, cannot split maltose or α -phenylglucoside appreciably but is very active against α -methylglucoside. Both enzymes are activated by cysteine. It should be noted that the intact cell from which these enzymes were obtained possesses no detectable capacity to split α -methylglucoside, whether it be adapted to maltose or not. No intact cell of this strain can be adapted to utilize α -methylglucoside.

Variation of respiratory quotient during normal and deep exhalation RALPH W STACY AND GEORGE H KYDD, III * *Lab of Aviation Physiology, Ohio State Univ, Columbus*

Preliminary studies on expired air made by means of the mass spectrometer and already reported showed that the first air leaving the lungs has a higher R Q than has the end of the expiration. Experiments have been designed and carried out which prove that these changes in the R Q are not the result of instrumental or technical errors. In further experiments during which expired air was collected and flow patterns recorded, the pO_2 and pCO_2 of expired air have been measured by means of the mass spectrometer. The R Q's of different fractions of the expired air were then compared with the R Q of the collected expired air. The results of these experiments indicate that in normal breathing the R Q at the beginning of exhalation is approximately 1.0 and is progressively reduced until at the end of the exhalation it has a value somewhat lower than the metabolic R Q as determined by Haldane analysis. When the tidal volume was increased to from three to five times its normal value the R Q at the beginning of expiration was about 3.0 and fell to about 1.0 by the end of the expiration. These high R Q values may be explained on the basis of the partial pressure gradient existing between the blood in the pulmonary arteries and alveolar air.

Calculations show that the $p\text{CO}_2$ gradient at the beginning of expiration is several times that at the end. On the other hand the $p\text{O}_2$ gradient changes but little during expiration.

Influence of pancreatectomy on lipid metabolism and atherogenesis in the chick I. STAMMER,* C. BOJENF,* L. N. KATZ, R. HARRIS* AND R. PICK* *Cardiovascular Dept., Medical Research Inst., Michael Reese Hospital, Chicago, Ill.*

Effect of pancreatectomy on chick lipid and carbohydrate metabolism and atherogenesis was investigated in a series of acute and chronic studies. In acute experiments, addition of cholesterol plus cottonseed oil to a plain mash diet led to a more marked lipemia in depancreatized chicks than in unoperated controls. Both groups had similar blood glucose levels and glucose tolerance curves. A 5-day course of ACTH had no effect on blood glucose, lipid or eosinophil levels, apparently mammalian ACTH was without demonstrable activity in these chicks. Pancreatized chicks chronically fed a mash supplemented with 2% cholesterol plus 5% cottonseed oil maintained plasma lipid levels consistently and significantly greater than those of unoperated controls on the same diet. This altered pattern of response of depancreatized birds to lipid feeding was accompanied by a greater incidence and degree of cholesterol-induced atherosclerosis. On a diet of plain mash alone, pancreatectomized chicks exhibited spontaneous atherosclerosis similar in incidence and degree to that of unoperated controls. These depancreatized birds consistently exhibited normal plasma and tissue lipid levels. After 9 months, neither hypolipemia, hyperlipemia nor hepatic lipidosis developed. Blood glucose concentrations and glucose tolerance curves also remained at control levels throughout. These birds also failed to respond to ACTH. In summary, the altered pattern of lipids exhibited by depancreatized chicks fed cholesterol plus cottonseed oil and the accompanying altered incidence and degree of atherosclerosis suggest (despite the usual findings in birds fed plain mash) that the pancreatectomized chick has an altered lipid metabolism.

Use of the ballistocardiogram as a measure of the maximum force of cardiac contraction ISAAC STARR *Lab of Therapeutic Research, Univ of Pennsylvania, Philadelphia*

When the aorta and pulmonary artery of cadavers were injected in simulation of the cardiac contraction, the relative force imparted to the moving fluid can be calculated for any instant from the product of acceleration and an assumed mass. The values obtained can be compared with ballistocardiograms secured simultaneously. The spread of the ballistocardiogram, as measured by

the sum of the vertical depth of the I wave and height of the J wave is closely related to the maximal force developed during any 'systole' ($r = 0.93$, and 0.94 after correction for attenuation). The correlation between this maximum of force and the square root of I depth plus J height is not significantly different. The correlations with the areas of these waves are also significant, but both that with the area itself ($r = 0.67$) and that with the square root of the area ($r = 0.83$) are significantly inferior to 0.93 . Therefore, the maximal force exerted in any systole can be estimated from the ballistocardiogram with an accuracy the equal to that of many useful clinical methods. At present this estimation of the maximal cardiac force of any systole can be made only in relative terms, I have used the average value of healthy normal young adult males as a standard and determined the deviation of any subject from this value. Experiments leading to the estimation of cardiac force or power in absolute units are under way. Normal standards for the relative values have been constructed.

The effects of altitude on size, activity and composition of gases of the colon of dogs F. R. STEGGERDA AND T. C. BUNTING* *Dept of Physiology, Univ of Illinois, Urbana*

Into dogs' colons, made permanently opaque to x-rays, one end of an open tipped catheter tube was inserted 3-4 inches beyond the anal sphincter. The other end was attached by a T tube to an air recording pressure unit outside of the decompression chamber. By means of the other arm of the T tube samples of air could be inserted and withdrawn from the colon for analysis. A mounted x-ray tube and automatic cassette changer in the chamber provided an opportunity for taking pictures of the colon when desired. The results show that when 100 cc of air are injected into the empty colon of a 10 kilo dog breathing oxygen, there occurs a progressive increase in size and activity of the colon up to an altitude of 25,000 feet. The actual increase in both cases is about threefold. At altitudes approximating 25,000 feet and above there usually occurs an escape of the gas up into the small intestine. Room air remaining in the colon 20-45 minutes at 10,000 feet shows that the oxygen present is about 20% and carbon dioxide 4%. At 20,000 feet there is little change in this percentage composition, but at 25,000 feet occurs a consistent decrease in oxygen to about 15% and an increase in carbon dioxide to an average of 16%.

Further chronic toxicity studies of hydroquinone G. G. STERN,* S. LANG,* N. R. BREWER AND A. J. CARLSON *Physiology Lab, Univ of Chicago, Chicago, Ill.*

Additional data on the toxicity studies of hydro-

quinone in rats are presented. Three groups of 40 rats each were fed varying amounts of hydroquinone with citric acid and compared with a control group. After two years on test 7 control rats, 10 rats fed 0.1% hydroquinone plus 0.01% citric acid, 11 rats fed 0.5% hydroquinone plus 0.01% citric acid, and 20 rats fed 1.0% hydroquinone plus 0.01% citric acid were still alive. The data on body weight, tibial length, WBC, RBC, differential, revealed no significant differences.

Endocrine effects of electroconvulsive shock in the rat JAMES A. F. STEVENSON, SYLVAN J. KAPLAN AND H. ENGER ROSVOLD (introduced by JOHN S. NICHOLAS) *Lab. of Physiology and Dept. of Psychiatry, Yale Univ. School of Medicine, New Haven, Conn.*

Recent studies have emphasized changes in adreno-cortical function, and processes influenced by it, during electroconvulsive shock. The effect of a series of electroconvulsive shocks upon the adrenal and other endocrine glands in the male and female rat has been investigated. Following a series of 10 or 20 daily shocks (50 ma. for 0.2 sec.) the animals were killed 24 hours after the last shock. Controls were similarly treated except for the actual shock. In all experimental groups the mean weight of the adrenals was increased, the ascorbic acid concentration was not significantly different from that of the controls, indicating a true hypertrophy. The mean weights of the pituitaries and thyroids of the shocked animals showed a slight but persistent decrease. These changes were observed whether or not the animals lost weight during the shock treatment. The increase in adrenal size appeared to persist for at least one month after the last shock. Serum sodium concentration, and muscle water content were also measured. The relationship of these findings to studies of animal learning after electroconvulsive shock and to studies in the human after electroconvulsive shock are discussed.

Combined effect of anoxic anoxia and cocaine on dog blood sugar and hemoglobin J. CLIFFORD STICKNEY, DAVID W. NORTUP AND EDWARD J. VAN LIERE *Dept. of Physiology, School of Medicine, West Virginia Univ., Morgantown*

Cocaine, an epinephrine-potentiating-agent, when given to dogs subjected to 28,000 ft simulated altitude reduces the propulsive motility of the small intestine. It seemed of interest to determine whether cocaine would also potentiate other anoxic effects. Nine adult dogs, unanesthetized, and in the post-absorptive state, were given 15 mg/kg cocaine hydrochloride intravenously, were exposed for 15 minutes to 254 mm Hg barometric pressure (28,000 ft simulated altitude), or were subjected to a combination of the 2 pro-

cedures at weekly intervals. A second series of 8 dogs was similarly treated, except that 3 mg/kg cocaine were used. Blood sugar by the Folin-Wu and hemoglobin by the Sahli methods were determined on saphenous vein blood samples obtained immediately before and after the experimental procedures. Cocaine, alone, within 15 minutes increased blood hemoglobin concentrations by 10% (statistically insignificant) at both dosage levels, but did not affect blood sugar. Anoxic anoxia increased blood sugar by 26 and 29% ($P < 0.01$) and hemoglobin by 10 and 11% ($P > 0.20$ and < 0.01) in the 2 groups of dogs. Cocaine 15 mg/kg, plus anoxia, increased blood sugar 20% ($P = 0.017$) and hemoglobin 15% ($P = 0.07$) while 3 mg/kg, plus anoxia, caused increases of 20 ($P = 0.07$) and 13% ($P = 0.017$) respectively. Cocaine, thus, decreased slightly the blood sugar response to anoxia, while it increased slightly the elevation in hemoglobin concentration, but neither of these effects was statistically significant.

Studies on effect of anoxic anoxia on goat blood sugar J. CLIFFORD STICKNEY, DAVID W. NORTUP AND EDWARD J. VAN LIERE *Dept. of Physiology, School of Medicine, West Virginia Univ., Morgantown*

Goats, 3 female and 1 castrate male, were studied in regard to blood sugar and hemoglobin in jugular vein samples drawn after a 24-hour fast under control conditions and before and after 15-minute exposures at weekly intervals in a decompression chamber to a simulated altitude of 24,000 ft. Blood sugar was determined colorimetrically on Somogyi-Shaffer-Hartmann filtrates and hemoglobin by either the Sahli or the Andes-Northup colorimetric method. The average control blood sugar in the 4 goats was 60.6 (range 55-65) mg/100 ml. The average rise in blood sugar produced in a total of 10 exposures of the 4 goats for 15 minutes at 24,000 ft was 40.1 (range 29.3-60.5) mg/100 ml, which was significant at less than the 1% level. In 2 goats, exposures for 30 minutes to 24,000 ft produced rises that were about $\frac{1}{2}$ as great as for 15 minutes. Elevations in hemoglobin concentration produced by the anoxia were, on the whole, small, and their average, statistically, not significant.

Relation of vitamin B_{12b} to vitamin B₁₂ and the biological activities of these compounds E. L. R. STOKSTAD, T. H. JUKES, JOHN A. BROCKMAN,* J. V. PIERCE* AND H. P. BROQUIST* *Lederle Labs. Div. American Cyanamid Company, Pearl River, N. Y.*

Vitamin B_{12b} has been isolated from a fermentation product of *Streptomyces aureofaciens* as a red crystalline compound which exhibits different absorption spectra than vitamin B₁₂. Biological activities of vitamin B₁₂ and B_{12b} are the same.

for *Lactobacillus leichmannii* and for chicks. Catalytic hydrogenation of vitamin B₁₂ yielded a compound which possessed the same ultraviolet, visible and infrared absorption spectra and the same biological activities for chicks and *L. leichmannii* as vitamin B₁₂. These biological activities of the vitamin B₁₂ reduction product, described here, are in marked contrast to those of vitamin B₁₂, also produced by the hydrogenation of vitamin B₁₂ (KACZKA, WOLF, AND FOLKERS, *J Am Chem Soc*, 71 1514, 1949). Vitamin B_{12a} was reported to be approximately 10-30 % as active as B₁₂ for *L. leichmannii* and 30% as active for chicks. Evidence has been obtained which indicates that vitamin B₁₂ is involved in the methylation of homocystine to methionine in the chick. Chicks were placed on a ration composed of alcohol extracted soybean protein 25%, glucose 62.5%, cystine 0.2%, dimethylaminoethanol HCl, salts, corn oil plus vitamins A, D, and E, inositol, calcium pantothenate, niacinamide, riboflavin, pyridoxine, thiamine, pteroylglutamic acid, and biotin. On this diet, and in the presence of vitamin B₁₂, both homocystine and methionine were found to give a growth response. In the absence of vitamin B₁₂, only methionine gave a response while homocystine was without effect.

Coronary dilator activity of 1-(3',4'-dihydroxyphenyl)-2-isopropylaminoethanol (Isuprel) and other N-alkyl homologues of epinephrine O O STOLAND AND D L MARCHBANKS * *Dept of Physiology, Univ of Kansas, Lawrence*

The coronary flow was measured in dog heart-lung preparations and intact dogs. The rate of outflow was measured by a bubble flowmeter between the coronary sinus and the superior vena cava and the inflow by a flowmeter between the carotid artery and a coronary artery. Heart rate and blood pressures were simultaneously recorded. Doses of Isuprel, which lower the blood pressure and increase the heart rate, produced a fairly consistent increase in the coronary flow in the various experiments tried. Doses sufficient to cause coronary dilation markedly increased the heart rate in heart-lung preparations as well as intact animals. The Isuprel was obtained from the pharmacology department of Sterling-Winthrop Research Institute. A M Lands and others have shown that this drug is a vasodilator and bronchodilator. The therapeutic value of Isuprel as a coronary dilator would seem to us to be counteracted by the undesirable tachycardia which it develops.

Effect of tridione (3,3,5-trimethyl oxazolidine-2,4-dione) on the oxygen uptake of motor and sensory cortex of dog brain H C STRUCK, D L STUMPF* AND R J CAFFREY * *Dept of*

Physiology and Pharmacology, Creighton Univ School of Medicine, Omaha, Nebr

The effect of tridione on the oxygen uptake of dog cerebral cortex slices in Krebs-Ringer-Phosphate solution (dextrose substrate) has been studied by the Warburg technic to determine whether any difference exists in the response of the two areas to this drug. Dogs were stunned by a blow on the head, the brains removed within 2-3 minutes, and immediately placed in a moist-cold box. A total of 135 slices were obtained from motor areas and a similar number from sensory areas of 14 dogs, approximately equal numbers of slices being taken from each hemisphere. Half of the slices from each dog were placed in Ringer's solution, the other half in Ringer's solution containing 0.035 M tridione per liter. This concentration has been calculated to be within the pharmacologic range used *in vivo*. Oxygen uptake of the slices was then followed for 2 hours at 38°C using oxygen as the gas phase. The mean inhibition of oxygen uptake of the motor cortex by tridione was 9.3%, which is highly significant statistically. In contrast, the mean inhibition of all slices from the sensory cortex was 4.6%, approximately half that of the motor cortex. This is not significant statistically. These results indicate that the motor and sensory areas of the dog cortex differ in their response to this anticonvulsant drug.

Abnormal electrocardiograms of chickens produced by potassium deficiency and effects of certain drugs on the abnormalities PAUL D STURKIE *Lab of Avian Physiology, Rutgers Univ, New Brunswick, N J*

Twenty-two White Leghorn chicks were placed on a potassium-deficient ration at 4 days of age. Seven showed abnormal ECG's after 10 to 12 days and within 21 days most of them showed abnormalities or were dead. Seven chicks showed some form of second degree AV block and 5 showed complete AV block. Two exhibited SA block or sinus slowing, one premature ventricular systoles and the diagnosis of two was in doubt. Some of the birds showed more than one abnormality. Ten birds, 6 weeks of age, were placed on the K-deficient ration. All of them showed abnormal ECG's within 18 days and some as early as 10 days. Nine of the 10 birds showed in one or more of their ECG's premature systoles. One of the birds exhibited second degree AV block. Eight young chicks and 3 older birds which showed abnormal ECG's received diethylaminoethanol intramuscularly. The abnormalities included second degree AV block, complete AV block, premature systoles and possibly SA block or sinus slowing. In every case, the ECG's reverted to normal. Procaine was also administered intramuscularly to 5 birds with abnormal ECG's. It

was effective in only one case Atropine sulfate was effective in 4 cases out of 6 in reverting the ECG's to normal

Relative effectiveness of natural estrogens in stimulating early uterine growth CLARA M SZEGO *Dept of Zoology, Univ of California, Los Angeles*

The relative effectiveness of estrone, estradiol and estriol in promoting early uterine growth has been determined in immature female rats after intraperitoneal injection, and in adult ovariectomized females following intravenous administration of these compounds In both preparations the order of activity was estriol > estradiol > estrone In the immature rat, the relative effectiveness was as follows in oil estriol, 100, estradiol, 39, estrone, 7, in saline estriol, 100, estradiol, 19, estrone, 2.4 These data contrast sharply with results obtained when other criteria of estrogenic activity are employed Thus, by the vaginal smear technique, estriol is the least active of the 3 natural estrogens The effectiveness of estrone and estradiol by the uterine water method of assay appeared to be relatively independent of the vehicle used over the entire dosage range studied In contrast, estriol was found to be about 3 times more effective in stimulating the infantile rat uterus when administered in saline medium than in oil A significant response was obtained with 0.003 μ gm of estriol in saline/animal This observation is in line with a) the relatively greater solubility of estriol in aqueous media, and b) the peculiar sensitivity of the infantile rat uterus to stimulation by this estrogen Application of these results to the assay of estrogens isolated from biological fluids is discussed

***In vitro* and apparent *in vivo* solubility of acetylene**

HENRY LONGSTREET TAYLOR AND CARLETON B CHAPMAN * *Lab of Physiological Hygiene, Univ of Minnesota, Minneapolis*

It is recognized that the acetylene method gives cardiac output values about 25% below those from the direct Fick catheterization method Since the theory of the foreign gas method has not been questioned, the discrepancy between these methods must be related to acetylene solubility, recirculation and effects of rebreathing Thirty-two measurements of acetylene solubility in human blood at 37.8°C and partial pressures of 7.4 to 118.0 mm Hg were made The solubility of C_2H_2 in blood at 37.8°C and 760 mm Hg was found to be 700.19 ± 2.64 instead of the Grollman value of 740 This changed solubility accounts for 5% of the difference between the two methods The recirculation error was evaluated with 19 simultaneous determinations of CO by the catheterization and acetylene procedures Acetylene was determined in the mixed venous blood at inter-

vals between 5 and 40 seconds of rebreathing and it was found that recirculation of acetylene accounts for another 5% of the discrepancy It was further found that during rebreathing the arterial oxygen content increased by 0.28 vol % Since it has been shown that venous oxygen saturation does not change during rebreathing, failure to allow for this introduces an error of not more than 7% The apparent *in vivo* solubility of acetylene calculated by equating the corrected acetylene data with the catheterization output gives an apparent solubility of acetylene *in vivo* which is 10% less than that *in vitro* This discrepancy may be explained by a) a diffusion gradient between alveolar air and blood or b) failure to account for all the errors in the acetylene method

Effect of polarization on conduction velocity of frog nerve and its modification by KCl ROBERT E TAYLOR (introduced by H A BLAIR) *Dept of Physiology, Univ of Rochester, Rochester, N Y*

The effects of polarizing currents on the conduction velocity of nerve have been investigated The most excitable A fibers of the sciatic-peroneal nerve of the frog were studied and measurements were made after the initial transient and before any appreciable long time effects of current flow had occurred Current, applied longitudinally and increasing linearly with distance for 5 cm along the nerve, results in an applied transverse membrane current which is virtually independent of distance except near the ends of the chamber, and produces a region of constant polarization about 3 cm long For any given amount of polarizing current the velocity of conduction is constant throughout this region The curve of velocity versus polarizing current has its maximum at weak cathodal currents, falling away on either side until blocking occurs at about $\frac{1}{2}$ the normal velocity Relief of KCl block by anodal polarization does not restore the velocity to the normal unpolarized value, but to about the value shown by normal nerve subjected to a comparable polarization The complete mathematical solutions for the usual core conductor model and for a saltatory model have been derived The steady state value of the membrane current in the midportion of the chamber is almost independent of distance, and completely independent of the membrane impedance in so far as it may be represented by a linear passive network

Effect of section of the corpus callosum on experimental convulsions R D TEASDALL* AND G W STAVRAKY *Dept of Physiology, Faculty of Medicine, Univ of Western Ontario, London, Canada*

It was previously shown that partial isolation of spinal neurones increases their irritability (Cannon, 1939, Stavrakys, 1943, Drake and Stav-

raki, 1948, Tersdall and Stavray, 1948, *et al*) It seemed interesting to ascertain whether partial denervation would have the same effect on the highest levels of the central nervous system. With this in mind, in 20 cats the corpus callosum was aseptically cut and after recovery from operation the animals were periodically convulsed with metrazol. Beginning 6-9 months after the operation the threshold of the animals to convulsant doses of metrazol was diminished by 20 to 50% and the convulsive pattern was altered in a characteristic way. In normal cats metrazol produces a clonic-tonic-clonic convulsive sequence. This sequence was maintained after section of the corpus callosum but the duration and the severity of the clonic phases of the convulsion was greatly increased. After 4 months of weekly injections of metrazol the corpus callosotomized animals became partially refractory to small doses of both metrazol and acetylcholine, this depressed state lasting for about 3 months. The sensitivity of normal control cats became increased instead of being depressed after a similar 4 month period of weekly injections of metrazol. The observed changes in the susceptibility to metrazol and to acetylcholine convulsions and in the convulsive pattern which occurs after section of the corpus callosum are attributed to the sensitization of the cerebral cortical neurones, which taken place following section and degeneration of the commissural fibres joining the cerebral hemispheres.

Effects of purified hypophysial gonadotrophins on the cholesterol ester content of rat testis

JAY TEPPERMAN, HELEN M. TEPPERMAN* AND JANET M. DEWITT. *Dept. of Pharmacology, Syracuse Univ. College of Medicine, Syracuse, N. Y.*

In a previous study (*Endocrinology* 41: 187, 1947) it was shown that injection of mixed gonadotrophins of sheep pituitary origin (Gonadophysin, Searle) resulted in an increase in testis cholesterol ester per 100 gm. of rat. A commercial chorionic gonadotrophin (Follutein, Squibb) was without effect on this parameter in untreated rats, but in Gonadophysin primed animals Follutein injections produced a reduction in testis cholesterol ester in association with enhanced androgen production. The suggestion was made that some constituent of Gonadophysin, possibly FSH, was responsible for the cholesterol ester increase. On the basis of studies with purified hypophysial gonadotrophins (prepared by McShan and Meyer) it is now possible to state that FSH has no apparent effect on the absolute amount of testis cholesterol ester whereas two purified pituitary LH preparations have consistently yielded the type of cholesterol ester increase seen following Gonadophysin administration. These studies were made on intact weanling rats as well as on male rats implanted with stil-

bestrol pellets according to the technique of Nalbandov and Baum (*Endocrinology* 43: 37, 1948).

Temperature and convulsive activity. PAUL TESHCHAN* AND E. GELLHORN. *Lab. of Neurophysiology, Dept. of Physiology, Univ. of Minnesota, Minneapolis*

The effect of heating a part of the cerebral cortex was studied comparatively on convulsive and nonconvulsive cortical areas of the cat. The animals were anesthetized with dial-urethane and the convulsive potentials were induced by topical application of picrotoxin. Results were as follows: 1) Convulsive potentials disappear at a lower temperature (in experiments involving progressively increasing temperatures) or earlier (in experiments in which the elevated temperature was kept constant) than normal potentials. Before the spike potentials disappear they show in most experiments a progressive decrease in amplitude and frequency. This increased sensitivity of the convulsive neuron is thought to be the result of the combined action of increased temperature and convulsive activity on the metabolism of the neuron. This interpretation accounts for the great similarity between these experiments and the earlier work of Gellhorn and Heymans on the action of anoxia on convulsive potentials. 2) Under the combined effect of elevated temperature and topically induced convulsive activity (A) more neurons are damaged than under the influence of heat on normal neurons (B). This is indicated by the fact that on recovery the amplitude of the remaining normal potentials is much less under A than under B and also that application of a convulsant drug after heating evokes spikes of higher amplitude and frequency under B than under A. 3) Repeated heating at short intervals to a moderately increased temperature (44-46°) is accompanied by a marked increase in frequency of convulsive discharges with each heating. However, this excitatory effect declines gradually as does the amplitude of the spike potentials.

Discrimination of weights by men with penetrating lesions of parietal lobes. HANS-LUKAS TEUBER*, MORRIS B. BENDER AND WILLIAM S. BATTERSBY*. *Psychophysiological Lab., Dept. of Neurology, New York Univ., College of Medicine, New York City*

Ability to judge lifted weights was analyzed in 16 men with history, surgical and x-ray evidence of loss of brain substance in the parietal regions. These men were drawn at random from a list of 250 veterans of World War II with gunshot wounds of the brain. Discrimination of weights was tested by the method of constant stimuli. Each subject compared a standard weight with another weight

by successive lifting with the unsupported hand. Twenty different weights were used. Thresholds of discrimination based on a minimum of 200 judgments were obtained separately for right and left hand. Results showed that ability to judge lifted weights may be unimpaired in men with trauma of parietal regions, as long as there are no signs of motor deficit. That is, thresholds obtained separately for the hand contralateral to the cerebral lesion were not significantly different from thresholds obtained for the other hand, nor were they different from thresholds obtained in normal controls. This was true even for cases in which other somatosensory functions, such as two point discrimination, were found to be impaired on the side opposite to the trauma. However, thresholds based on simultaneous lifting of one weight in each hand were significantly different from those obtained in normal controls. Under these conditions, the patients consistently underestimated the weight held in the affected hand. The significance of these results for theories of sensorimotor function is discussed.

Semi-automatic recording of electrical skin resistance patterns PRICE E THOMAS* AND IRVIN M KORR *Kirksville College of Osteopathy and Surgery, Kirksville, Mo*

In recent years electrical skin resistance (ESR) explorations have found considerable application in experimental and clinical investigation. In our laboratories ESR patterns on the human trunk have been segmentally correlated with other factors (*Federation Proc* 7 67, 1948, 8 87, 1949). The simple device herein described permits rapid, almost automatic explorations, with photographic recording of the ESR patterns, and eliminates many artefacts inherent in 'hand' exploration. The needle movement of a modified microammeter is used to actuate the variable arm of an electrolytic rheostat. The current passed by this circuit is of sufficient magnitude (0-800 ma AC) to vary the brightness of a 6V lamp proportionately to the microammeter readings (0-50 μ a). The bulb is housed over the exploring (roller) electrode and illuminates a rectangle identical in size and shape with the area of electrode contact. This assembly, supported from a rail, is propelled in the long axis of the body by a constant-speed motor. At the end of each strip the assembly is moved laterally the width of the electrode by means of a pawl and notch arrangement, and the motor is reversed for the next strip. The explorations are conducted in a dimly illuminated room with camera-shutter open throughout. Thus, strips of light are photographed which vary in brightness according to ESR variation in corresponding strips of skin. An outline of the subject's body and any bony landmarks previously marked on his

skin, appear on the same film in accurate relation to the record. A calibration strip is also recorded.

Functional connections between hypothalamus and medulla W C THOMPSON* AND L M N BACH *Depts of Physiology and Psychiatry and Neurology, Tulane Univ School of Medicine, New Orleans, La*

Studies were carried out on the effects of medullary lesions upon hypothalamically induced alterations in blood pressure, respiration, and knee jerk. Cats, anesthetized with sodium pentobarbital (Nembutal), were stimulated by electrodes oriented stereotaxically in the posterior hypothalamus. Lesions were placed in various parts of the medullary reticular formation by stereotaxically directed electrodes in an attempt to alter the recorded responses. Several points in the mid-brain and posterior hypothalamus were mapped which, when stimulated, usually produced simultaneous enhancement or depression of blood pressure, respiration, and the knee-jerk. Stimulation of the medial inhibitory reticular formation of the medulla contraverted the usual vasopressor response resulting from simultaneous stimulation of the posterior hypothalamus in a consistent but limited fashion. Lesions placed in the medial inhibitory reticular formation of the medulla caused enhancement of the hypothalamically induced effects on the 3 functions whereas when the intactness of the lateral facilitatory reticular formation was destroyed, the hypothalamically induced enhancement was depressed or abolished. These results indicate that the hypothalamic facilitation or inhibition of the knee-jerk acts in a parallel manner on blood pressure and respiration and that these effects are mediated by the lateral facilitatory reticular formation and the medial inhibitory reticular formation of the bulb respectively.

On electrically induced optical and structural changes in certain nerves and Elodea cells JULIAN M TOBIAS AND SIDNEY SOLOMON * *Dept of Physiology, Univ of Chicago, Chicago, Ill*

Whole nerve polarization (continued or tetanic) (crayfish, lobster, frog) causes anodal shrinkage, increased opacity and probably increased rigidity, and cathodally causes swelling, increased transparency and increased surface stickiness. These changes are reversible, re-reversible etc., slowly on standing and more rapidly following polarity reversal. The phenomenon, though visible, has been photometrically objectivized and quantified. It occurs with either platinum or non-polarizable, wick electrodes, with the nerve in mineral oil, Ringer or moist air. As opacity increases anodally, light scattering also increases there, suggesting particle or fiber size change. Calcium ion excess inhibits, and potassium ion excess may accentuate

the electrically induced change. Calcium ion excess, independently of any impressed electrical field, increases nerve opacity whereas potassium ion seems to increase transparency. There is evidence that nerve water moves toward the cathode. Polar peculiar changes in size and configuration are also seen in single frog nerve axons. Experiments are in progress to determine the relationship, if any, of these phenomena to propagated impulses. Measurements of changes in nerve viscosity as a function of activity are also being made, in terms of damping of high frequency sound transmitted along the nerve long axis (collaborating with William Fry and Frank Fry, Dept of Electrical Engineering, Univ of Illinois). Using Brownian movement as a viscosity measure, it has been found that polarization increases viscosity of the hyaline, cytoplasmic matrix in *Elodea* cells, anodally. This change too is reversible. It is felt that these findings may reflect changes in protoplasmic structure which underlie excitation.

Distribution and assay of inhibitors and accelerators of blood coagulation in extracts of organs of normal and hemophilic man L. M. TOCANTINS AND R. T. CARROLL * *Dept of Medicine, Jefferson Med College, Philadelphia, Penna*

Portions of tissues removed from the bodies of the 2 subjects within 2 hours after death, were washed free of blood and dried with acetone. The powders from each organ were extracted in pairs (one normal and one hemophilic) with absolute methanol, ethyl ether or 0.85% NaCl and the respective extracts were assayed against standard human brain thromboplastin, cephalin, thrombin and lipid antithromboplastin preparations, using silicone contacting surfaces and stable normal human plasma as substrate. The relative content of antithromboplastin in the tissues from both subjects was in the following order: brain (highest), adrenal, liver, spleen, lung, heart, tongue, kidney, pancreas, skeletal muscle, testes, duodenum, pituitary, colon, diaphragm, thyroid, skin (lowest). Chief differences in antithromboplastin content (units gm of tissue) between normal and hemophilic tissues: Testes normal 25, hemophilic 59, skeletal muscle normal 37, hemophilic 56, Brain normal 1145, hemophilic 1644, Heart normal 72, hemophilic 93. All hemophilic tissues assayed greater unitage of antithromboplastin than normal tissues, excepting the lung, in which the difference between the 2 extracts was non-significant. The most potent thromboplastins were obtained from saline extracts of brain. Lung and kidney extracts were less potent. Extracts of these hemophilic organs had essentially the same thromboplastic activity as those of normal organs. Normal skeletal muscle behaved as thrombo-

plastin, while hemophilic skeletal muscle had antithromboplastin, but no thromboplastin activity. Cephalin fractions extracted from brain and lung of the 2 subjects had about equal potency as clot accelerators. Antithrombins were found in normal and hemophilic testes and in normal pancreas.

Effect of lymphatic necrosis on neuro-muscular function (action potential, acetylcholine synthesis) of hypophysectomized animals CLARA TORDA AND HAROLD G. WOLFF *New York Hospital, Kingsbridge Hospital (V A), and Depts of Medicine and Psychiatry, Cornell Univ Med College, New York City*

In the absence of the adrenocorticotrophic hormone of the pituitary gland (ACTH) in hypophysectomized animals the neuro-muscular function is impaired. Two measurable components of this dysfunction are a decline of action potential during repetitive stimulation of the nerve and a decrease of acetylcholine synthesis. Both the action potential and the acetylcholine synthesis return to normal during the injection of ACTH to hypophysectomized animals. Since the decline of action potential and acetylcholine synthesis found in myasthenia gravis can be counteracted by either administration of ACTH or removal of the thymus it became of interest to investigate whether removal of lymphoid tissue of hypophysectomized animals restores the function of the neuro-muscular system to normal. Hypophysectomized rats were either injected with nitrogen mustard or exposed to x-ray treatment. After significant necrosis of the lymphoid tissue occurred action potential measurements and acetylcholine synthesis tests were performed. Necrosis of the lymphoid tissue did not restore the action potential and the acetylcholine synthesis to normal levels. Although the possibility that the negative results are the outcome of general debilitation due to combined hypophysectomy and nitrogen mustard and x-ray treatment cannot be excluded, the results suggest that the effect of ACTH in restoring the neuromuscular function of hypophysectomized animals is due to a mechanism other than a simple reduction of the mass of lymphatic tissue.

Effect of altered breakfast habits on physiologic response W. W. TUTTLE AND KATE DAUM * *Depts of Physiology and Nutrition, State Univ of Iowa, Iowa City*

An experiment involving 10 women was performed to show the effects of various breakfast patterns on physiologic response. The subjects lived in a University dormitory and ate all their meals in the hospital dining room supervised by the Department of Nutrition. The breakfast patterns employed were 1) basic breakfast repre-

senting approximately one-fourth the total daily caloric requirement (600 Cal), 2) light breakfast (270 Cal), 3) heavy breakfast (1000 Cal) and 4) 'no breakfast' Responses studied were 1) neuromuscular tremor magnitude, 2) capacity to do work, 3) choice reaction time and 4) strength endurance Each breakfast pattern lasted 5 weeks Comparison of data collected twice weekly for each breakfast pattern justified the following conclusions No differentiation by way of tremor magnitude, capacity to do work, choice reaction time or strength endurance could be established following regular consumption of heavy and light breakfasts respectively, of ordinary breakfast items In comparison with omission of breakfast, consumption of a 600 Cal breakfast consisting of fruit, breakfast cereal, milk, sugar, toast and butter resulted in a significant reduction of tremor magnitude in all cases, choice reaction time in 60% of the cases and an increase in maximum work output in 80% of the cases during the period when food was consumed at breakfast time Strength endurance was unaffected

Effect of water diuresis on electrolyte excretion in normal man JOHN R. URBACH, M. D. PHELPS, WILLIAM A. STEIGER, HARRY GOLD AND SAMUEL BELLET (introduced by PAUL R. DUMKE) *Heart Station, Philadelphia General Hospital and the Robinette Foundation, Univ of Pennsylvania, Philadelphia*

Reports on the effect of water diuresis on excretion of urinary solutes conflict Upon reinvestigation significant changes were found Preliminary work was done on dogs, definitive experimentation on 3 normal humans Endogenous creatinine clearances were obtained as relative measure of glomerular filtration, since exogenous clearance techniques exerted effects of their own Creatinine, chloride, phosphate, urea, CO_2 content, sodium, and potassium determinations were done on blood and urine, pH on urine only After several control periods, diuresis was induced with distilled water (50 cc/kg) by stomach tube, and excretion of these constituents measured at regular intervals Glomerular filtration varied only slightly and small variations in plasma concentration of solutes did not influence their excretion Total CO_2 excretion was directly proportional to urine flow (each expressed as clearance ratios), with high statistical correlation ($r = 0.846$) Since pH did not vary with diuresis or CO_2 excretion, the bicarbonate fraction also increased, but neither sodium nor potassium rose concomitantly Sodium and chloride excretion paralleled each other but did not appear to be related to any other factor Potassium excretion also varied, and U/P ratios below one were obtained, but again no constant correlation with any other variable could

be found Urea clearance remained constant in 2 human experiments, and varied markedly in the 3rd Phosphate excretion did not vary significantly

Effect of acclimatization on motility of small intestine during anoxia EDWARD J. VAN LIERE, J. CLIFFORD STICKNEY AND DAVID W. NORTHUP *Dept of Physiology, West Virginia Univ School of Medicine, Morgantown*

It has been shown in our laboratory that the propulsive motility of the small intestine in rats is decreased by anoxic anoxia It was reasoned that if rats were acclimatized to low oxygen tensions, exposure to altitude would no longer produce this effect A group of 17 albino rats was acclimatized by subjecting them for 3 hours, on the average, each day to a pressure of 303 mm Hg (simulated altitude of 24,000 ft), 8 were exposed for 26 and 9 for 62 days These acclimated rats were paired with normal rats (unacclimated) which were as nearly alike in weight and age as possible They were fasted for 24 hours A pair of these rats was given 2 cc of a charcoal-acacia mixture by stomach tube After allowing 10 minutes for some of this material to enter the small intestine, both rats were placed in a low-pressure chamber at a pressure of 254 mm Hg (simulated altitude of 28,000 ft) At the end of 30 minutes they were removed from the chamber and decapitated The small intestine was removed, slit open and the distance the charcoal had traversed it measured It was observed that the propulsive motility was significantly less in the unacclimatized than in the acclimatized animals This was interpreted to mean that the effect of anoxic anoxia on the propulsive motility of the small intestine could be used as another criterion for acclimatization

Metabolism of I^{131} during acute adaptation to anoxia LESTER VAN MIDDLESWORTH *Dept of Physiology, Univ of Tennessee College of Medicine, Memphis*

Rats fed a stock diet and a low iodide diet have been rapidly adapted to severe anoxia by exposure to 258 mm Hg barometric pressure (equivalent 27,000 ft altitude) at 11–14°C for 2–4 hours After this exposure the low iodide rats were injected intraperitoneally with 10 microcuries of carrier-free I^{131} The barometric pressure was then reduced to 177 mm Hg (equivalent 35,000 ft altitude) for 8–12 hours Urine collections were made throughout the exposure The animals were then returned to sea level, blood samples withdrawn and thyroid glands excised Plasma proteins were precipitated with $\text{BaOH}-\text{ZnSO}_4$ reagents and the radioactivity of precipitate and filtrate determined separately The thyroid glands were digested in 2 N NaOH and an aliquot was analyzed for I^{131}

while another aliquot was neutralized, extracted with normal butyl alcohol and the alcohol solution submitted to 2-dimensional paper chromatography with 2,4,6 collidine-2,4 lutidine—water and butyl alcohol—ammonium hydroxide solvent mixtures. Suitable sea level control rats were similarly studied with I^{131} . Animals adapted to severe anoxia demonstrated less than 2% of plasma I^{131} in protein-bound fraction, while 20–30% of plasma radioactivity was in the protein-bound fraction of controls. The thyroid glands of the anoxic series contained only $\frac{1}{2}$ as much radioactivity as controls. Radioautographs of the paper chromatograms demonstrated at least 7 radioactive compounds in the anoxic thyroid hydrolysates, but the quantity of all these compounds appeared to be less than that shown in similar chromatograms made from thyroids of control rats.

Protein metabolism of fracture callus and muscle trauma studied with radioactive methionine
LESTER VAN MIDDLESWORTH *Dept of Physiology, Univ of Tennessee, Memphis*

Rats on a high and a low protein diet (27% and 8% casein, respectively) were subjected to standard fractures of one fibula and a 1.5-inch incision produced and sutured in the abdominal musculature. After 7 days 7 mg of radioactive methionine (4×10^7 counts/min), furnished by D. L. Tabern of Abbott Laboratories, was injected intraperitoneally. After 4 additional days the animals were killed. Analyses were made for radioactive sulfur and for nitrogen in the muscle scar, normal muscle, fracture callus and normal fibula. Results were then expressed as S^{35}/N . The S^{35}/N in normal tissues of the low protein animals was 1.5 to 3.3 times greater than in rats on the high protein diet. This is possibly referable to the isotope dilution offered by greater methionine content of the high protein diet. Muscle trauma had healed well in high and low protein groups. Muscle trauma of the low protein cases showed twice the concentration of S^{35} shown by normal muscle tissue of low protein rats. However, the high protein rats showed no localization of S^{35} in the muscle scar tissue. Fracture healing was quite different, the high protein rats had 10 times more S^{35} in the fracture callus than in unfractured fibula and the callus was well formed. The low protein animals showed negligible healing of the fracture and no localization of S^{35} in the fracture scar. It therefore appears as though more dietary protein was necessary for healing of the bone fracture than for healing muscle trauma.

Acceleration of growth with early sexual development in the female monkey (*Macaca mulatta*)
G. VAN WAGENEN *Dept of Obstetrics and Gynecology, Yale Univ School of Medicine, New Haven, Conn*

cology, Yale Univ School of Medicine, New Haven, Conn

Androgen treatment of the young male rhesus monkey induced early bone union of the skeleton, an increase in muscular development and precocious spermatogenesis (VAN WAGENEN, *Federation Proc* 6: 219, 1947). It has now been possible to double the growth rate and reduce, by half, the age of the young female monkey at time of menarche (first menstruation). In a monkey colony of 5 generations, the average age at menarche is about two years, when the weight is around 3500 grams. Crystalline testosterone propionate dissolved in vegetable oil was given intramuscularly to two young monkeys weighing 1410 and 1600 grams. The dosage was 7.5 mg/kg/week. Injections began at 5 months, inducing an immediate rise from the normal curves for body-weight and length. At one year the treated animals reached the weight and length of two-year-olds and menarche occurred exactly at the end of a year in one animal and at one year, 19 days in the other. Androgen was then discontinued. This was a true beginning of sexual maturity and not just a spurious bleeding because 6 and 7 menstrual cycles, irregular as typical of adolescence, have followed, accompanied by the external skin changes characteristic of the maturing monkey. This great augmentation of growth with differentiation has not been analyzed. One is inclined to interpret it as a generalized increase in protein anabolism brought about by the nitrogen and salt retention effect of the hormone but the release of hypophyseal hormones or some other intervening mechanism may be involved.

Sensitivity of x-rayed animals to infusion of histamine, adenosine and trypsin
K. D. VENTERS* AND E. E. PAINTER *Univ of Illinois, College of Medicine, Chicago, Ill*

Control and x-rayed rabbits and dogs given an LD₅₀ dosage have been infused in the manner described previously (*Federation Proc* 7: 90, 1948) and 'threshold values' have been established for exogenous histamine, adenosine and trypsin. It appears that sensitivity of rabbits to adenosine is not increased after radiation, but sensitivity to histamine increases markedly. Maximal changes in sensitivity are directly correlated with other physiological findings occurring at the same time after radiation and must be a function of damage of tissue. Marked susceptibility of x-rayed rabbits to trypsin has made it impossible to record changes with this substance. It is apparent from the table below that dogs show their maximal increase in sensitivity to all 3 substances (approximately 50% change) at 2–2½ hours after x-radiation. These findings are correlated exactly in time with the main clinical sign of vomiting in the dog.

Our studies suggest that more than one breakdown product may be involved in the early biochemical disturbance to account for the symptoms of radiation sickness

Threshold Values for Maintaining 5.8 mm Hg Drop in Blood Pressure

Control rabbits		Post radiation in hours				
		1	1½	2	2½	3
		<i>ugm/kg/min</i>				
Histamine	6.7 (14)	7.0	3.1	2.0	2.2	2.7
Adenosine	111.2 (12)	119.0	192.0	208.0	281.0	188.0
Trypsin	107.0 (10)	No threshold could be established after radiation				
Control dogs		Post radiation				
		<i>ugm/kg/min</i>				
Histamine	1.2 (17)	1.0	0.6	0.5	0.4	1.1
Adenosine	120.4 (21)	94.0	81.2	81.4	93.0	118.0
Trypsin	113.2 (16)	96.0	89.0	55.3	49.3	73.0

Anti-ulcer action of estrogen in experimental animals FRANK E. VISSCHER AND ADRIAN P. TAZELAAR * *Labs of the Upjohn Company, Dept of Pharmacology and Endocrinology, Kalamazoo, Mich*

The incidence of peptic ulcer is greater in men than in women by a factor of about four, while in human pregnancy peptic ulcer is rarely, if ever, seen. These observations suggest that estrogen may have an anti-ulcer action. Such action has been demonstrated in two experiments on starvation ulcers in male rats. The degree of rumenal ulceration was greater in groups of 8 male rats receiving, as pre-treatment, subcutaneous injections of estrone, 200 γ in an aqueous suspension per week for three weeks. The anti-ulcer action was not evident at 40 γ estrone per week nor with 200 γ estrone and 2 mg progesterone in combined dosage, per week. Rats showing anti-ulcer action of estrogen grew less rapidly as compared with control rats and with rats not benefitted by estrogen. Results of paired feeding experiments on rats receiving 200 γ estrone per week, and results with other estrogens will be reported.

Subharmonics generated in ears of humans and animals at intense sound levels HENNING E. VON GIERKE (introduced by H. O. PARRACK) *Aero Med Lab, Air Materiel Command, Wright-Patterson Air Force Base, Dayton, Ohio*

Subjective undertones have never been found at usual sound levels although many investigators searched for them. We discovered that subharmonics are heard by subjects exposed to sound intensities above 140 db ref 0.0002 dynes/cm².

The frequency of the subharmonic was exactly one-half the frequency of the stimulating signal and its loudness was 70 db below an exposure signal of 9 Kcps. The subharmonic was radiated from the subject's eardrum and its level measured outside the auditory canal was 60 db below the stimulating signal. This proves the subharmonic is generated mechanically in the middle ear. Other tones, lower in pitch and loudness, were sometimes heard. Discomfort and possible hazard to hearing limited observations on humans to 2 frequencies. From the ears of anesthetized guinea pigs subharmonics were radiated at exposure frequencies from 3.5 to 23 Kcps. Other sounds, sometimes not harmonically related to the fundamental, were observed at frequencies and intensities below the subharmonic. A subharmonic becomes measurable at the guinea pig ear at an exposure level of 95 db at 9 Kcps and is 50 db below the fundamental. With increasing exposure level the subharmonic increases more rapidly, becomes unstable or interrupted above 115 db exposure level, and becomes a crackling or fluttering noise above 135 db. Subharmonics have also been produced by the ears of rats, cats, and dogs. These observations may be important to theories of middle ear mechanics and to an explanation of the behavior of the human ear in intense sound fields.

Conduction velocity of ulnar nerve in human subjects of different ages and sizes IRVING H. WAGMAN AND HENRY LESSE * *Dept of Physiology, Jefferson Med College of Philadelphia, Philadelphia, Penna*

Conduction velocity measurements of the most rapidly conducting ulnar nerve fibers supplying muscles of the hypothenar eminence were made on human subjects whose ages ranged from 3 to 80 years. The measurements were made by recording muscle action potentials obtained by percutaneous stimulation of the nerve at two points, namely, the wrist and the elbow. Results show that the maximum velocity of these nerve fibers found in adults (average = 59 meters/sec for a group ranging in age from 20 to 35 years) is attained as early as the third year of life. This bears out histological studies of Rexed (*Acta Psych Neurol Suppl* 33, 1944) who showed that in the human the ventral roots reach full development between the ages of 2 and 5 years. From the present functional measurements, it is shown that the time consumed by the motor impulse in traveling from the spinal cord to the periphery increases as the individual grows. It is also demonstrated that this time utilized by the motor impulse is less in small than in large individuals of the same age. As a consequence similar considerations may hold true for total reflex time. The present analysis

points to the size of the individual as the main factor determining the relations discussed here. However, from a limited number of measurements obtained on persons past 60 years of age, it seems that there may be a slowing of conduction velocity in old age and, therefore, a resultant lengthening of reflex time.

Interruption of bulbocapnine catalepsy in rats by environmental stress HENRI N. WAGNER, JR. AND JAMES W. WOODS (introduced by CURT P. RICHTER) *Psychobiological Lab., Phipps Psychiatric Clinic, and the Dept. of Physiology, Johns Hopkins School of Medicine, Baltimore, Md.*

Since 1910 considerable attention has been focused upon the effects of the drug, bulbocapnine, which experimentally produces in animals practically all of the motor manifestations seen in human catatonia. Several studies have been made in this laboratory to determine by what means the catalepsy of bulbocapnine can be abolished, with the hope that the information obtained might be of therapeutic importance to man. Previous studies by one of us have shown that, although cataleptic as a result of injection of bulbocapnine, monkeys, cats and rats remain alert to sensory stimuli of all types, vision and hearing in particular have been studied with the use of conditioned galvanic skin responses. The animals thus are in rapport with their environment but are unable to move. In this respect the animals under bulbocapnine resemble human catatonic patients, in whom a rapport with their environment has frequently been demonstrated. In this report we have described the finding that various stressful environmental factors, namely, intensive auditory stimulation, electric shocking in a situation which results in vicious fighting, and the immersion of the animals in deep water, result in a transient but complete disappearance of the cataleptic state. During the periods of stress, most of which may be considered life-threatening situations, the rats behaved in a manner indistinguishable from that of normal animals under the same circumstances.

Effects of dicumarol on peripheral circulation in patients with vascular disease KHALIL G. WAKIM, MARTIN S. KLECKNER, JR.,* NELSON W. BARKER* AND WALTER F. KVALE* (with the technical assistance of ADRIEN N. PORTER*) *Mayo Clinic, Rochester, Minn.*

From their experimental work on animals and on the empty beating heart, Gilbert and Nalefski (*Proc. Central Soc. Clin. Research* 21:5, 1948) concluded that dicumarol has a definite effect on blood flow and that the favorable results in cases of coronary occlusion are primarily due to

the action of dicumarol in increasing the coronary blood flow. We are studying by means of the venous occlusion plethysmograph with the compensating spirometer recorder and by use of cutaneous thermocouples the effects of dicumarol on the skin temperature and on the blood flow in the extremities of patients who are receiving the drug for anticoagulant therapy. After the establishment of controls, dicumarol is administered orally in therapeutic doses for several days, during which blood flow and cutaneous temperatures are recorded and compared with the control values. So far, our findings in 5 cases of arteriosclerosis obliterans consistently indicate that even though dicumarol produces a significant prolongation in the plasma prothrombin time, the accompanying changes in cutaneous temperatures over several regions of the body and in blood flow in the upper extremities are insignificant. However, there was a slight but statistically significant reduction rather than increase in blood flow in the lower extremities.

Effect of epinephrine and nor-epinephrine on whole blood coagulation JEROME M. WALDRON (introduced by I. J. PINCUS) *Dept. of Physiology, Jefferson Med. College, and Division of Medicine, Pennsylvania Hospital, Philadelphia*

Previous investigators have stated that epinephrine *in vivo* accelerates whole blood coagulation but that epinephrine *in vitro* had no effect on blood coagulation. All of these investigations had been performed in glass tubes. This problem was reinvestigated by measuring the coagulation time of fresh whole blood in collodion lined tubes. Very dilute solutions of epinephrine were added *in vitro* or injected intravenously. In addition, the effect of nor-epinephrine *in vitro* and *in vivo* on the coagulation time of fresh whole blood was investigated. Both epinephrine and nor-epinephrine *in vitro* accelerate the coagulation time of human and canine fresh whole blood. A dilute solution of nor-epinephrine when injected intravenously in dogs also accelerates the coagulation time. The adrenolytic drug, piperidylmethyl benzodioxane, when added *in vitro* inhibits both the *in vitro* and *in vivo* clot-accelerating effect of epinephrine and nor-epinephrine. Piperidylmethyl benzodioxane does not prolong blood coagulation when added to whole blood *in vitro*. This fact together with its effect on the coagulation of blood to which epinephrine or nor-epinephrine has been added *in vitro* or *in vivo* indicates that its action is directly against these sympathomimetic compounds. Furthermore it suggests that the accelerating effect of these sympathomimetic compounds on whole blood coagulation is one similar to a catalyst and not due to a change in the reacting compounds.

Relation of quick stretch and twitch response in skeletal muscle SHEPPARD M WALKER *Dept of Physiology, Univ of Louisville School of Medicine, Louisville, Ky*

The *in situ* triceps surae of male rats weighing 150 to 200 gm and the isolated sartorius muscle of the frog were arranged for optical recording with an isometric lever. The frog muscle was stimulated directly and the rat muscle was stimulated through the cut sciatic nerve. The muscle was stretched by displacement of the fixed end with a strong spring. Observations were made on muscle at initial lengths varying from 20% below to 20% above the length required for a maximal twitch. At lengths equal or above that required for a maximal twitch, quick stretch decreased twitch tension. At shorter lengths adequate quick stretch increased twitch tension up to, but never above, maximal twitch tension. Twitch tension was determined by subtracting the tension induced by quick stretch. Twitch contraction at the shorter lengths markedly increased the resistance of the muscle to quick stretch. Twitch contraction at lengths equal or above that required for a maximal twitch usually decreased the resistance of the muscle to quick stretch. Resistance is directly proportional to the amount of deflection of the isometric lever by the stretch. It was concluded that 1) quick stretch modifies twitch tension in a manner predictable from the length-tension diagram, and 2) the effect of twitch contraction on the resistance of the muscle to quick stretch is dependent upon the initial length of the resting muscle.

Effects of electrical stimulation of temporal lobe in the rhesus monkey P D WALL AND G D DAVIS (introduced by J F FULTON) *Lab of Physiology, Yale Univ School of Medicine, New Haven, Conn*

Stimulation of an area of cortex extending from the tip of the temporal lobe to the interaural plane on the inferior and medial surfaces of the temporal lobe produces marked changes of blood pressure and respiration. Similar changes could be evoked from the amygdala but not from ammon's horn. The responsive area of the temporal cortex differs from that on the orbital surface in the direction and intensity of blood pressure response and in the participation of the hypothalamus in the mediation of the response. Some evidence indicated that the hypothalamus was not involved in the pathway from the temporal cortex which passed directly to the lateral part of the midbrain. Areas controlling blood pressure did not completely coincide with those controlling respiration. Stimulation of exactly the same area may produce either hyperpnea or apnea and a hyperpneic response may be converted to

an apneic by administration of bulbo-capnine. Motor movements have been reported from stimulation of the temporal lobe but most of these appear to be due to direct stimulation of the 5th nerve.

Volume and enzyme response of the transplanted pancreas to foodstuffs in the intestine C C WANG* AND M I GROSSMAN *Dept of Clinical Science, Univ of Illinois College of Medicine, Chicago*

In 3 chronic dogs with a portion of the pancreas transplanted subcutaneously, a basal level of secretion was maintained by continuous intravenous injection of purified secretin. When the volume rate and enzyme output (amylase) had become constant, the test substance was introduced through a metal cannula in the duodenum and the effect on secretory rate and amylase output noted. The results may be summarized as follows (in this list the first number indicates the number of tests performed, the second, the average percentage increase in volume rate over the control period, and the third, the average percentage increase in amylase output over the control period): 50 cc distilled water, 5, +20, +34, 50 cc saline, 6, +5, +16, 20 cc 0.5% HCl, 9, +168, +162, 20 cc 5% Bacto Protone, 7, +82, +313, 20 cc 5% amino acids solution, 4, +52, +340, 10 cc corn oil, 2, +10, +71, 20 cc 5% sodium oleate, 3, +36, +50, 20 cc 5% dextrose, 2, +0, +16, 20 cc 10% maltose, 3, +0, +0. Since nervous connections between the intestine and the pancreas are absent in these dogs, increases in volume rate may be attributed to secretin release and increases in enzyme output to pancreozymin release. On this basis, HCl is the most potent stimulator of secretin release and peptone and amino acids are the most potent for pancreozymin release, although both hormones are released to some extent in both instances. These experiments constitute crucial physiologic proof for the existence of pancreozymin.

Further evidence for thyroid regulation of gastric function R N WATMAN* AND E S NASSET *Dept of Physiology and Vital Economics, Univ of Rochester School of Medicine and Dentistry, Rochester, N Y*

It was reported earlier that thyroidectomy reduces the survival time of guinea pigs with histamine-induced peptic ulcer. Death due to perforated peptic ulcer and peritonitis occurred in the normal animal in 135 ± 20 hours and in the thyroidectomized animal in 56 ± 8 hours. Administration of thiouracil in doses which lowered the oxygen consumption to the level of the thyroidectomized animals did not alter survival time (125 ± 29 hours). Administration of thiouracil to thyroidectomized animals failed to improve

their survival time. The results of thiouracil treatment suggest that thyroxin may not be concerned in the thyroid effect on gastric function. Auto-transplantation of the thyroid failed to affect survival time, indicating that anatomical integrity is not a significant factor. Crystalline thyroxine was administered to thyroidectomized animals in the amount adequate to restore the oxygen consumption to normal ($\pm 10\%$). This did not improve the survival time as compared with the untreated thyroidectomized group. Administration of whole desiccated thyroid substance, however, to thyroidectomized animals in adequate amount restores to normal both the oxygen consumption and ulcer survival time. These studies suggest that 1) The thyroid has an effect on gastric function which may be independent of the action of thyroxin. 2) In addition to thyroxin, the thyroid may produce an active principle which regulates gastric function. 3) These effects are not due to damage to the parathyroids, vagi or other structures. This is indicated by the auto-transplantation studies and the fact that adequate substitution by thyroid substance alone was demonstrated.

Interaction of fibrinogen and thrombin DAVID F. WAUGH AND BETTY J. LIVINGSTONE * *Dept of Biology, Massachusetts Inst of Technology, Cambridge*

Fibrinogen and thrombin (Armour) interaction has been examined over the ranges 0.036 to 0.35 mg clottable N/ml at 0.045 U thrombin/ml and 0.009 to 0.454 N I H U/ml thrombin at 0.18 mg C N/ml. Aliquots of fibrinogen and thrombin were mixed in paraffined tubes. At appropriate times the reaction was stopped by retracting the clot and adding formaldehyde to 1.8% final concentration (7 sec). After centrifugation, the supernatants were analyzed by ultraviolet absorption ($\lambda = 280 \text{ m}\mu$) for their contents of non-clottable protein and residual fibrinogen. When plotted as pseudo first-order reactions all have an initial non-linear portion extending to approximately $\ln \theta^{-1}$ equals 0.4 ($\theta =$ fraction residual fibrinogen). Beyond this point linearity (vs time) is observed. An extrapolation of the linear portion passes through the origin. The initial non-linear portion is considered to represent the accumulation of activated fibrinogen, the linkage of activated fibrinogen to form a retractable clot and the formation of a sufficient number of fibrin fibrils to keep the concentration of activated fibrinogen at a low and negligible value. The linear portions of all of the reactions so far examined are described by the equation
$$-d\phi/dt = \frac{10.21 Th_0}{1 + 22.8 \phi_0} \phi$$
 where Th_0 and ϕ_0 are the initial concentrations of thrombin and fibrinogen, respectively. The interpretations of this equation will be discussed.

The interpretations of this equation will be discussed.

Measurement of hepatic circulation by clearance of radioactive sodium in man RICHARD L. WECHSLER,* LOUIS SOKOLOFF* AND SEYMOUR S. KETY *Dept of Physiology and Pharmacology, Graduate School of Medicine, Univ of Pennsylvania, and Diabetic Coma Project, Philadelphia General Hospital, Philadelphia, Penna*

The clearance of radioactive sodium from the liver has been measured by a method previously described for the gastrocnemius muscle (KETTY, S. S. *Am Heart J* 38: 321, 1949). In this study, $5 \mu\text{C}$ of Na^{24}Cl in 1 cc of isotonic saline was injected into the liver through a 22 gauge, 4-inch needle with the patient in the supine position. The injection site, 2 interspaces below the appearance of liver dullness in the right midaxillary line, was sterilized and then anaesthetized with procaine hydrochloride. The patient was instructed to exhale completely and refrain from inhaling during injection of the needle. The Na^{24}Cl solution was injected as the needle was withdrawn. Slight discomfort was the only complaint upon injection. A Geiger-Müller counter was placed over the liver at the level of injection. Counts were recorded at half-minute intervals until a final plateau was reached. Counts per half minute less the final background were plotted semilogarithmically against time and a straight line resulted. The slope of this line yields the value for k or the clearance constant. This constant, representing the ability of the hepatic circulation to remove or supply nutritional substances, is a measure of the effective circulation through the liver tissue. Hospitalized patients, without circulatory or hepatic disorders (normal liver function tests) were chosen at random. Values for the hepatic clearance constants in this group ranged from 0.344 to 0.483 with a mean of 0.402. This mean is 8 times the mean clearance constant of the resting gastrocnemius muscle ($k = 0.050$).

Effect of auricular fibrillation on cardiac output, coronary flow and arterial blood pressure RENÉ WÉGRIA, CHARLES W. FRANK,* GEORGE A. MISRAHY,* ROBERT S. SIOUSSAT,* LEONARD SOMMER* AND GEORGE H. MCCORMACK, JR * *Dept of Medicine, College of Physicians and Surgeons, Columbia Univ, and the Presbyterian Hospital, New York City*

The effect of auricular fibrillation on the cardiac output, coronary blood flow, and mean arterial blood pressure was studied in dogs anesthetized with pentobarbital. The cardiac output of the left ventricle and the blood flow in the left descending anterior coronary artery were recorded continuously with two rotameters. The mean

arterial blood pressure was recorded continuously with an optical manometer. Thirty-three bouts of electrically induced auricular fibrillation lasting from one-half to five minutes were studied in 9 dogs. At the onset of fibrillation, there is an abrupt fall in cardiac output, blood pressure and coronary flow. After a few seconds, there is some rise in all three toward control. At the termination of fibrillation, there is a sudden increase in all three to levels greater than control. Following the shorter bouts of fibrillation, cardiac output, coronary flow and arterial blood pressure return to control simultaneously. In a few of the shorter bouts and in most of the longer ones, the coronary flow is still increased above control level when both cardiac output and blood pressure have returned to control values.

Blood changes in guinea pigs during deficiency of anti-stiffness factor VIRGINIA WEIMAR* AND ROSALIND WULZEN *Dept of Zoology, Oregon State College, Corvallis*

Guinea pigs raised on a diet adequate in minerals and in the known vitamins, with the exception of the anti-stiffness factor, develop a deficiency disease characterized by muscular stiffness in the early stages and later by heavy calcium deposits in many organs (WULZEN AND BAHR *Physiol Zool* 9: 508). During the deficiency there was produced a hypocythemic, normochromic and normocytic or macrocytic anemia. The number of young red cells in the circulating blood was decreased. Erythrocytic sedimentation was more rapid, and, in the early stages of the deficiency, the increase in sedimentation rate paralleled the development of wrist stiffness. Deficient guinea pigs showed a leucocytosis of 16,700/cu mm as compared with 7,700 for non-deficient guinea pigs. Male guinea pigs fed the deficient diet for 10-14 weeks had an increased percentage of neutrophils and a decreased percentage of lymphocytes and Kurloff cells. Males deficient for 24-110 weeks had abnormally high percentages of neutrophils and eosinophils, a decreased percentage of lymphocytes and the complete disappearance of the Kurloff cells. Stiff male and female guinea pigs fed the deficient diet 24-110 weeks showed a persistent eosinophilia, the degree of eosinophilia increasing with the stiffness. In only 2 out of 85 cases was severe stiffness not accompanied by eosinophilia, and one of these had a 6% basophilia.

Studies on heparin release in anaphylactic shock dogs RICHARD P. WHITE, PARKE H. WOODARD (introduced by O. O. STOLAND) *Dept of Physiology, Univ of Kansas, Lawrence*

Since the theory that heparin appears in the circulation of anaphylactic dogs has received support, it was thought worth-while to study heparin concentration in the blood and thoracic

duct lymph in both actively and passively sensitized dogs using the protamine titration method. Ten dogs were used as donors. They were sensitized to horse serum and at times varying from 4 to 32 days afterward bled enough blood to passively sensitize normal dogs. These were anesthetized, and blood pressure recorded. Both blood and thoracic duct lymph samples were obtained. Records were obtained before and after the injection of shock doses of horse serum. Some dogs were donors more than once. Seventeen attempts at passive immunization were made. As indicated by the characteristic fall in blood pressure, 7 were unsuccessful, 7 were successful, 2 of which gave anaphylactoid responses with recovery on the transfusion of blood and then responded by a drop in pressure to the injection of horse serum, and 3 gave anaphylactoid responses only. On the injection of horse serum only one showed any immediate change in blood coagulation time or indication of heparin in the blood. In 4, lymph coagulation was markedly slowed and more heparin appeared than in blood. Perhaps the lymph serves as an entrance of heparin into the blood. Seven donor dogs were similarly studied. The results are essentially the same. Lengthening of coagulation time occurred first in the lymph, often not appearing in the blood at all and heparin concentrations were always found higher in lymph.

Inhibition of catabolic effect of ACTH on nitrogen metabolism by a high potassium diet JOHN E. WHITNEY* AND LESLIE L. BENNETT *Division of Physiology, Inst of Experimental Biology, and the Division of Medicine, Univ of California, Berkeley and San Francisco*

Four groups of 5 'plateaued' female rats of the Long-Evans strain were maintained on a constant daily food intake. In one group 600 mg of KCl were added daily to the diet of each animal, and in another group 720 mg of KCl were added daily. The remaining 2 groups served as the normal controls. Individual urine specimens were collected daily and were analyzed for total nitrogen. The period of ACTH administration was preceded and followed by control periods of from 9-18 days. The addition of the above amounts of KCl to the diet did not alter the nitrogen balance. When ACTH at a dose of from 6-9 mg/day was given to the rats receiving the 600 mg/day of KCl their average daily urinary nitrogen excretion rose from 337 to 354 mg/rat/day while that of their controls rose from 337 to 381 mg/rat/day. When a larger dose of a less active ACTH preparation was given to the animals receiving 720 mg KCl/day the average daily nitrogen excretion changed from 388 to 381 mg, while that of their controls rose from 377 to 423 mg/rat/day. In

neither experiment prior to the administration of ACTH was the urinary nitrogen of the control animals significantly different from that of the animals fed KCl. In each experiment the urinary nitrogen during ACTH administration was significantly higher for the control rats than for the rats fed KCl.

Glucose formation in eviscerated rabbit ARNE N WICK,* DOUGLAS R. DRURY AND EATON M. MACKAY *Scripps Metabolic Clinic, La Jolla, Calif*

Recent work has shown that after removal of the liver the kidneys form sugar. It is important to know whether this sugar is made from non-carbohydrate sources or whether it is formed by rebuilding fragments of broken down glucose molecules. We have investigated this question by injecting a quantity of C^{14} labeled glucose into an eviscerated non-nephrectomized rabbit. After allowing 30 minutes for mixing, a sample of blood is taken. The concentration of glucose in the sample is determined using fermentation for identification. The radioactivity of the CO_2 produced by the fermentation is also determined. No further glucose is administered and the blood sugar steadily declines. After 3 or 4 hours and when the blood sugar is quite low another blood sample is taken and the determination repeated. If new glucose has been formed from any source other than broken down glucose molecules, this would be indicated by an increase in the ratio of blood glucose to the radioactivity of the CO_2 liberated by fermentation. We have found this to be the case although calculations indicate that a significant part of the glucose formed by the kidneys comes from former glucose fragments. The other sources of glucose will be discussed.

Effect of increased temperatures on certain constituents in the blood of the turtle CHARLES G. WILBER AND JOSEPH F. SCHANNO * *Biological Labs, St. Louis Univ., and Dept. of Biology, Stonehill College, St. Louis, Mo*

Samples of blood were removed from 10 turtles (which had been kept at 22°C for 24 hours) of the species *Chrysemys picta* and analyzed for glucose, urea and uric acid to obtain 'normal' values. The mean values for these substances in milligrams per 100 cc of blood are as follows: glucose, 65.1, urea, 27.0, uric acid, 4.8. Groups of turtles (10 in each group) were exposed to temperatures of 32°C and 38°C respectively. After 24 hours' exposure, blood was removed directly from the ventricles and analyzed for the constituents mentioned above. It was found that the glucose in the blood of turtles exposed to these increased temperatures shows a definite increase above the so-called normal values as given previously. Similarly, an increase of urea above the

so called normal was found. On the other hand, no increase was found in the amount of uric acid in the blood of turtles exposed to 32°C and 38°C respectively. It might be concluded that the increase in glucose and urea with increase in temperature indicates that the liver is stimulated to increased activity by these elevated temperatures. On the other hand, the constancy of the uric acid levels in the turtle under conditions of temperature stress indicates that purine metabolism is not markedly changed under the conditions of the present experiments. These results are in accord with other work (*Anat. Rec.* 105:97, 1949) on blood lipids of the turtle. Increased temperatures bring about a significant increase in BMR of the turtle. Whether or not the reported changes persist for a long time is not clear. Work is now in progress in an attempt to ascertain whether turtles exposed to elevated temperatures for periods up to one month show a consistent change in blood constituents.

Studies on blood pressure in dogs using an auscultatory method C. M. WILHELMJ AND E. B. WALDMANN * *Dept. of Physiology, Creighton Univ. School of Medicine, Omaha, Nebr.*

We sought a method whereby daily or multiple daily readings could be made on unanesthetized dogs for months or years. Since the animals were to be subjected to various types of stress it was felt that arterial puncture would be infeasible. We have been using the method of F. M. Allen with the difference that the dogs are recumbent. To date 11 dogs have been intensively studied. Careful training is essential. The training is in two stages: 1) the *objective* stage during which the animal learns to lie quietly on the table for the prescribed period of time, 2) the *subjective* period during which the animal loses fear and apprehension and becomes accustomed to the procedures. The time necessary for complete training varies from a few weeks to several months. In untrained animals great variations in pressure and sounds occur. In properly trained animals two types of curves appear: 1) low pressures with small daily fluctuations, 2) high pressures with large daily fluctuations. Readings must be taken under basal conditions. Shivering, panting, movement of muscle tension must be avoided. The presence of strangers, loud noises, unusual manipulations or changes in routine often cause elevations in blood pressure. The training and conditions necessary to secure basal blood pressure are similar to those necessary to obtain true basal metabolism. The same person should take the readings on each dog. Tests with mecholyl and pitressin have shown the expected changes. Two different observers have been found to obtain readings in satisfactory agreement.

Adrenalectomy on frog skin potentials MARTIN W WILLIAMS* AND CLIFFORD A ANGERER
Dept of Physiology, Ohio State Univ, Columbus

This laboratory is interested in certain physico-chemical properties of cells and tissues as influenced by changes in the concentration of certain steroid hormones. Initial work on frogs show that isolated skin increases its permeability to certain radioactive isotopes and changes certain of its bioelectric properties following adrenalectomy. The P D across the skin was determined by a null-point potentiometer in conjunction with the following cell, calomel/Ringer-Agar/Ringer's solution/skin/Ringer's solution/Ringer-Agar/calomel. Male *Rana pipiens* were divided into 3 groups: Adrenalectomized, renal damaged (sham-operated), and unoperated frogs. The latter 2 groups served as controls. All ventral skins were removed and kept in Ringer's solution buffered with phosphate solution at pH 7.4 from $\frac{1}{2}$ to 1 hour prior to potentiometric study. Zero time was measured from the close of this immersion period. At the end of one hour (i.e., $1\frac{1}{2}$ –2 hours after removal of skin) of recording the potentials at 10-minute intervals there was a decrease in the mean value of 58% for skins of adrenalectomized frogs compared with controls. No significant change was observed for mean values of skins from renal damaged and normal frogs. Further studies are underway.

Reactions of diet-induced fatty livers of rats to an acid diazo dye W LANE WILLIAMS AND DAVID W HOLANDER * *Dept of Anatomy, Univ of Minnesota, Minneapolis*

Large amounts of parenterally injected acid diazo dyes are not immediately hepatotoxic, are not excreted by, and do not stain the cytoplasm of normal hepatic parenchymal cells of rats and mice. Centrilobular cells of livers damaged by CCl_4 are immediately stained (cytoplasm) by such dyes and remain stained until they undergo lysis (*Anat Rec* 101:133, 1948). Fatty livers were produced by a low protein (4%)-high fat diet during the 5th–10th weeks of life. Terminally, subcutaneous or intraperitoneal injections of 1 to 2 cc of 0.5% aqueous trypan blue were given for 3–7 days. Macrophages were free of fat but contained large amounts of dye. The large, fat-filled, parenchymal cells were not stained by trypan blue. Such atypical cells were not permeable to dye and no significant amount of fat was deposited in active or potential macrophages. A few small areas of inflammation were observed in which liver cells contained dye and fat. The local tissue reactions indicated that an additional injurious process was active in such sites. These rats weighed 40–60 gm initially and the diet permitted no increase in body weight. Terminally

parenchymal cells contained large amounts of fat. Slightly older rats (100–120 gm) fed the same diet showed no increase in weight but liver cells were not distorted and liposis was limited to small cytoplasmic droplets. Such cells were also free of dye while adjacent macrophages were filled with granules of trypan blue.

Ventricular fibrillation in delayed treatment of TEPP poisoning J H WILLS, B P McNAMARA* AND E A FINE * *Pharmacology Section, Med Division, Army Chemical Center, Md*

In the dog poisoned by intravenous injection of TEPP, delay in administration of atropine for longer than 4–5 minutes may result in rapid death of the animal from ventricular fibrillation following injection of the atropine. If respiratory paralysis has occurred before atropine administration, ventricular fibrillation follows that therapy in every case. This action is not a specific property of atropine, resulting also from the use of the diethyl aminoethyl ester of 1-phenyl cyclopentyl monocarboxylic acid (Panparnit). In the dog with paralyzed respiration, administration of artificial ventilation before atropine therapy allows recovery of the animal. It is believed, therefore, that the fibrillation following delayed treatment with atropine of TEPP poisoning depends upon a sudden increase in the work demanded of a severely anoxic heart.

Activity of inhibitors of acetylcholine esterase as function of pH IRWIN B WILSON AND FELIX BERGMANN (introduced by DAVID NACHMAN-SOHN) *Dept of Neurology, Columbia Univ, College of Physicians and Surgeons, New York City*. The inhibition of acetylcholine esterase by various inhibitors has been investigated as function of pH. Hereby information may be obtained as to the mechanism of interaction between the enzyme protein and the inhibitors and as to the nature of the structure hydrolyzing acetylcholine.

The inhibition by eserine decreases linearly with increasing pH, prostigmine action is virtually unaffected. The results show that the cationic form is the stronger inhibitor. They suggest a negatively charged structure as part of the active surface. A completely different type of pH-dependence is found with TEPP. Since this electrically neutral compound remains unchanged within the pH range of enzyme activity, all variations of inhibition must be ascribed to changes of the active structure of the protein. TEPP inhibition as function of pH shows a maximum close to 7, the shape of the curve being similar to that of the pH activity curve of the enzyme itself. Assuming that TEPP does not interact with the anionic but with the other part of the active center, the parallelism suggests that the pH-activity curve of the enzyme reflects the changes of this second

part The pH effects can best be interpreted as the dissociation of 2 acid groups These results will be discussed in the light of observations on salt effects

Time of consciousness at high altitudes J W WILSON AND ELIZABETH COMFORT * *Aero Med Lab, Air Materiel Command, Wright-Patterson Air Force Base, Dayton, Ohio*

It has been observed that time of consciousness following oxygen mask removal at a given altitude is considerably longer than the time available following rapid decompression to the same altitude In view of the importance of the observation in connection with the establishment of safety procedures in pressurized aircraft, a comparison of these two sets of conditions has been made in 6 resting individuals for an altitude range of 25,000-40,000 feet These experiments have demonstrated that the time reserve is 27-49% longer in the mask removal than in the rapid decompression series With individuals working mildly (1800 ft lb/min, bicycle ergometer) the time of consciousness following rapid decompression was reduced by 25-50% below that of resting individuals By use of our data on air-breathing subjects at rest and of data obtained by Hornberger on resting individuals breathing oxygen (*U S Air Force, AMC, Engineering Division Memorandum Report TSEAA-660-83-E, 5 February 1946*), a graphic comparison has been made which gives some indication of the importance of the 'nitrogen-dilution effect' (*Proc Soc Exper Biol & Med* 70 185, 1949) in determining the duration of consciousness of air-breathing individuals after explosive decompression to high altitudes The validity of applying the widely used term 'time of useful consciousness' to both of the two practically important situations in aviation in which consciousness may be lost as a result of hypoxic hypoxia, is questioned, since the victim may be entirely unaware of the threatening danger

An analysis of cerebral control of reflex pupillary dilatation in the cat and monkey WILLIAM CROSBIE WILSON (introduced by A G MULDER) *Dept of Physiology, Stritch School of Medicine, Loyola Univ, Chicago, Ill*

It was found that cerebral reflex pupillary dilatation follows adequate cortical and subcortical excitation of the 1) gyri proeus, genualis, and subcallosus in cats, and 2) the cruciate gyrus (area 8) in the Rhesus monkey Partial denervation of the pupil, by sympathetic chain section in some experiments, and in others by ciliary ganglionectomy in cats and monkeys demonstrated Frontal lobe reflex pupillary mydriasis in cats is due to active inhibition of parasympathetic activity, in monkeys to predominately sympathetic activity Response to the light reflex and a study

of ciliary ganglion action potentials before, during and after frontal lobe stimulation substantiates the resulting mydriasis in cats to be due to inhibition of para-sympathetic tone In addition evidence indicates that centrifugal frontal lobe fibers controlling the light reflex by active inhibition of parasympathetic activity pass through the hypothalamus to the Edinger-Westphal nucleus

Changes in endocrine organs induced by bacterial pyrogens W F WINDLE, H H WILCOX,* RUTH RHINES,* AND C CLEMENTE * *Dept of Anatomy, Univ of Pennsylvania School of Medicine, Philadelphia*

We studied histologically with controls most organs of 2 series of female rabbits, one receiving Baxter Pyromen, PsP, (of pseudomonas origin) in 100-200,000 γ dosages over periods of 19-303 days, and the other receiving Baxter Pyromen, PVP (of proteus vulgaris origin) in dosages of 500-20,000 γ /kg thrice weekly for 3-6 weeks (lb 50, about 75,000 γ) Neither series showed vascular, renal or intestinal lesions resembling exhaustion stages of the general adaptation syndrome The PsP-treated rabbits exhibited hyperplasia and hyperactivity of lymphatic and reticuloendothelial tissues Administration of PVP induced only slight changes in such tissues Both preparations produced blood picture changes like those after stress or administration of ACTH Certain changes suggestive of involution after an earlier phase of marked growth stimulation were found in the adrenal cortex of both series Other controlled series of rabbits and rats were given PVP, 1 γ /kg daily or 5 γ /kg thrice weekly for 12 hours-122 days Most endocrine organs underwent changes suggestive of increased cytoplasmic activity The anterior lobe basophils and cells of pars intermedia of the hypophysis showed increased cytoplasmic vacuolization within 12 hours Similar changes appeared in the adrenal cortex Stroma cells of the virgin ovary became swollen Administration of PVP for longer periods led to widening of the adrenal zona reticularis without total cortical hypertrophy Prolonged high doses of PVP caused reduction in the anterior lobe basophil population and involutional changes in the zona reticularis

Influence of cortisone and related steroids upon spreading effect of hyaluronidase CHARLES A WINTER AND LARS FLATAKER * *Merck Inst for Therapeutic Research, Rahway, N J*

The spread of the dye T-1824, after intradermal injection both with and without added hyaluronidase, has been studied in mice and rats In mice, as Opsahl has shown, adrenalectomy increases the area of the spread, but in rats maintained in good condition with 1% NaCl to drink, a difference between the spread of dye in normal and adrena-

lectomized animals can be shown only if saline is withdrawn and the animals fasted overnight. In both species, and in both adrenalectomized and intact animals, cortisone acetate or adrenal cortex extract (ACE), if administered intramuscularly prior to the test, strongly inhibits the spreading action of hyaluronidase, and moderately inhibits the spread of the dye in saline. ACE is also effective in inhibiting the spread if injected locally (intradermally, mixed in the syringe with the dye) in amounts as small as 0.025 ml, but cortisone in quantities up to 0.25 mg is completely ineffective when administered in this way. Steroids which inhibit the spreading factor when injected intramuscularly 0.5 mg daily for 3 days prior to test include the following: cortisone acetate, testosterone propionate, Δ 4-androstenedione-3,17, and Δ 4-3,20-diketo 17 hydroxy-21-acetoxypregnene. Steroids similarly administered which do not inhibit the spread include desoxycorticosterone acetate and Δ 4-androstenedione-3,11,17. No clear relationship is so far apparent between chemical structure and activity in inhibiting the spreading factor. Anesthesia has been found to influence the results, since Evipal exerts a marked, though temporary, inhibition of the spreading factor, while ether is without effect.

Relation of constituents of gastric juice to occurrence of mucosal erosion and bleeding STEWART WOLF AND GEORGE B. JERZY GLASS * *New York Hospital, and the Dept of Medicine, Cornell Univ. Med. College, New York City*

Special interest attaches to the noxious effects of acid and pepsin on the one hand, and the protective action of the constituents of gastric mucin on the other, in determining the occurrence of injury to the gastroduodenal mucous membrane. Since the recent introduction of a method for fractionating gastric mucin which allows separation of the glandular product (mucoprotein) from that secreted by the lining columnar epithelium (mucoproteose), it has become possible to correlate quantitatively mucus secretion with acid and pepsin. In the current study, performed on the fistulous subject, Tom, a day-to-day photographic record in color of the gastric mucosa was correlated with changes in acid, pepsin, mucoprotein and mucoproteose secretion. Special attention was directed to the fragility of the membrane and to the presence of hemorrhagic spots and erosions. At the same time throughout this period a careful record of life situation, attitude and emotion was kept. After central vagus stimulation by intravenously administered insulin, or during sustained personal conflict with anxiety and resentment, HCl, pepsin and mucoprotein concentration were increased, while mucoproteose concentration was

low. Under these circumstances the membrane was red and engorged and there was the greatest evidence of susceptibility of the membrane to injury. Under resting fasting circumstances there was no constant relationship among the various secretory constituents, but high acid with low mucoproteose concentration seemed to be the circumstance most favorable to the occurrence of hemorrhage and erosion in the membrane.

Effect of estrogen on iodine turnover in thyroids of rats and mice L. F. WOLTERINK, C. C. LEE, K. OLSEN AND M. MURRAY (introduced by J. MEITES) *Dept of Physiology and Pharmacology, Michigan State College, East Lansing*

Although estrogens have very little effect on the early uptake of iodine in the rat (K. E. PASHKIS, A. CANTAROW AND W. C. PEACOCK, *Proc Soc Exper Biol & Med*, 68:485, 1948), they have been shown to retard the output of iodine from the thyroid of the chick if administered daily for a 2-week period (EPSTEIN AND WOLTERINK, *Poultry Sci* 28:763, 1949). In the present experiments, an attempt was made to demonstrate the effect of various dosages of the dimethyl ether of stilbestrol on the turnover rate of iodine in the thyroids of rats and mice. Turnover rates were computed from the average counts of I^{131} made on whole dry thyroids removed at autopsy 18 and 68 hours after injection of radioiodine. Estrogen dose rates of 1 μ gm/animal/day intraperitoneally for 3 days increased the output of I^* from the thyroid both in rats and mice. Ten μ gm and 100 μ gm doses for the same period and massive single doses usually depressed I turnover. The observation of Meites and Chandraseker (J. MEITES AND B. CHANDRASEKER, *Endocrinology* 44:368, 1949) that the mouse has a lower thyroid activity per unit body weight than the rat was confirmed.

Relationship between thyroid activity as assayed by the thiouracil-thyroxine method and by the thyroid turnover of radioiodine in pair-fed rats L. F. WOLTERINK AND C. C. LEE (introduced by W. D. COLLINGS) *Dept of Physiology and Pharmacology, Michigan State College, East Lansing*

By the method of Dempsey and Astwood (E. W. DEMPSEY AND E. B. ASTWOOD, *Endocrinology* 32:509, 1943), the thyroid secretion rate in a group of pair-fed 70-gm male Rockland rats was determined to be 5.36 D,L-thyroxine μ gm D,L-thyroxine/100-gm body weight/day. The turnover rate of I in the thyroids of the same group, computed from I^{131} counts 40 and 80 hours after I^* injection, was 25.6%/day. If an iodine concentration in the thyroid of 70 mg % is assumed (D. A. MCGINTY, *Ann N Y Acad Sci* 50:403, 1949), and if the thyroid is forming only L-thyroxine instead of the racemic mixture, the corrected

thyroxine secretion rate, computed from this turnover figure for these rats, is 5.16 μg D,L-thyroxine/100 gm body weight/day. The difference of 4% between the 2 figures is not statistically significant.

Objective method for measurement of systemic arterial pressure in intact human ear. EARL H. WOOD, EDWARD H. MORGAN* AND GABRIEL G. NAHAS* *Section on Physiology, Mayo Fdn., Univ. of Minnesota, Rochester*

Simultaneous recordings of intraradial arterial pressure at eye level and of ear opacity pulse in 20 normal subjects during exposure to positive acceleration on the human centrifuge (*Federation Proc* 5:59, 1946) demonstrated close correlation between amplitude of ear pulse and systolic pressure when arterial pressure fell below 50 mm Hg. Ear pulse uniformly disappeared and returned simultaneously with reduction of systolic pressure to zero and its recovery. Indication that blood circulating in the flushed ear was at arterial pressure led to an attempt to estimate blood pressure by measurement of the externally applied pressure required to obliterate the ear opacity pulse. A modified oximeter earpiece was used. The translucent earpiece pressure capsule was 20.5 mm in diameter. Reflected light from non-pressurized portions of the pinna was minimized by masking the photocell and ear end of the pressure capsule by 2 rings 8 mm in diameter. The photocell was connected in series to a condenser and a Moll Micro Galvanometer (sensitivity 0.006 μ amperes/mm at 4 meters). Attenuation of low frequencies caused by insertion of the condenser was 50% at 2.7 cycles/sec. Earpiece capsule pressure, ear pulse, EKG, and intraradial pressure were photographed simultaneously during determination of brachial artery pressure by auscultation in 20 normotensive and hypertensive subjects. When thickness of the ear pulse trace line was approximately 1 mm and the control ear pulse amplitude 5-10 mm, the intracapsular pressure at which the ear pulse was first discernible correlated with directly determined systolic pressure more closely than systolic pressure determined by auscultation. Correlation between the intracapsular pressure at which the ear pulse attained maximum amplitude (10-55 mm) and direct intra-arterial diastolic pressure was not significantly different from the correlation between direct and auscultatory diastolic pressures.

Antagonism of adrenocorticotrophic hormone (ACTH) and adrenal cortical extract (ACE) to desoxycorticosterone (DCA) pathological changes. DIXON M. WOODBURY,* CHARLES A. ROSENBERG* AND GEORGE SAYERS. *Depts. of Pharmacology and Physiology, Univ. of Utah and Veterans Hospital, Salt Lake City*

ACTH and ACE antagonize the following actions of DCA: 1) hypertension, 2) electroshock threshold elevation, 3) insulin hypersensitivity. Experiments were designed to test the possibility that the antagonism would also apply to pathological changes induced by DCA. Twenty-three male rats were unilaterally nephrectomized and given 0.9% sodium chloride to drink. The animals were divided into 3 groups: I, untreated; II and III, implanted with twelve 15-mg pellets of DCA; III, in addition, injected s.c. with one mg ACTH (Armour) thrice daily. Animals were autopsied 30 days after the beginning of hormone treatment. DCA induced cardiac and renal hypertrophy; ACTH did not influence action of the steroid on the heart, but appeared to have a slight inhibitory effect upon renal enlargement. DCA caused interstitial myocarditis, periarteritis nodosa (particularly in the pancreas) and focal glomerulitis in varying degrees of intensity. ACTH, given simultaneously with DCA, reduced the incidence and degree of severity of the lesions. In another experiment 16 male rats were unilaterally nephrectomized, given 0.9% sodium chloride to drink, implanted with twelve 15-mg pellets of DCA and exposed to cold 8 hours daily. Half the animals received no additional treatment, each member of the remainder was injected with one-half cc of Upjohn's lipo-extract of adrenal cortex twice daily. The cardiac hypertrophy induced by DCA was antagonized by ACE. There was a tendency for kidney weights to be less in the animals treated with the combination DCA-ACE than in the rats given DCA alone.

Membrane resting and action potentials from excitable tissues. J. WALTER WOODBURY AND LOWELL A. WOODBURY (introduced by JAMES E. P. TOMAN). *Dept. of Physiology, Univ. of Utah College of Medicine, Salt Lake City*

The insertion of a capillary micro-electrode into single cells of a tissue (GRAHAM AND GERARD *J. Cell & Comp. Physiol.* 28:99, 1946; LING, *Federation Proc.* 7:72, 1948) not only gives functional isolation *in situ* with minimal damage to the cell but also permits quantitative, simultaneous measurements of the membrane resting and action potentials of that cell. Heretofore, overshoot of the action potential has been demonstrated in skeletal muscle by Nastuk and Hodgkin (*Federation Proc.* 8:175, 1949), and in squid giant axons by Curtis and Cole (*J. Cell & Comp. Physiol.* 19:135, 1942) and Hodgkin and Huxley (*J. Physiol.* 104:187, 1945). We have made similar measurements on other tissues, using capillaries of 0.5 micron tip diameter. Frog heart ventricle gives an average membrane potential of 60 mv and an action potential of 81 mv, indicating an overshoot of 21 mv. The form is monophasic and the dura-

tion corresponds to the extrinsic QT interval Preliminary results from frog sciatic nerve trunks give fiber membrane potentials as high as 100 mv, but usually 50 to 80 mv Simultaneous recordings of membrane resting and action potentials show overshoot of the spike The form of the membrane action potential is essentially that reported by Tasaki and Mizuguchi (*J Neurophysiol* 11 295, 1948) and Schoepfle and Erlanger (*Am J Physiol* 134 696, 1941) for unimpaled single frog sciatic fibers Studies on other excitable tissues are in progress

Pattern of localization in the precentral motor cortex of *Macaca mulatta* C N WOOLSEY AND P H SETTLAGE * *Depts of Physiology and Anatomy, Univ of Wisconsin Med School, Madison*

Somatic sensory and motor areas of the rat's cerebral cortex are so organized as to form somewhat distorted body images in which the various parts are related to one another much as in the actual animal The sensory and the motor patterns are mirror images, touching one another at snout and limb apices along a line which corresponds to the central sulcus of primates (*Federation Proc* 7 137, 1948, 8 144, 1949) We have reexamined the localization pattern in the precentral gyrus of the monkey and have been surprised to find that the motor pattern of this animal also hangs together as it does in the rat and that the accepted separation of face from trunk is not a fact In essence, fingers, toes and lower face are represented within and adjacent to the central sulcus The axes of arm and leg run rostrocaudally so that proximal parts of limbs are better represented rostrally, as are also upper face, eyelids and pinna Binding together face, arm and leg representations is an area for axial musculature, which forms the rostral border of the pattern This border corresponds approximately to the rostral limit of 4S, or the boundary between Vogt's 6 α and 6 β , as judged from gross brain maps Abdominal musculature is represented between arm and leg nearer the central sulcus The results raise the question whether the usual separation of the precentral region into physiologically distinct motor and premotor fields is justifiable

Electromyographic patterns in reinnervated muscles MELVIN D YAHR, ERNST HERZ AND JOSEPH MOLDAVER (introduced by PAUL F A HOEFER) *Dept of Neurology, College of Physicians and Surgeons, Columbia Univ, and Neurological Inst, Presbyterian Hospital, New York City*

Electromyographic studies were carried out in a group of patients having complete peripheral nerve injuries with subsequent suture, 3 to 5 years prior to examination Using a differential

high gain condenser coupled amplifier, cathode ray oscillograph, and co-axial needle electrodes, two outstanding patterns were recorded 1) spontaneous discharges below 1 mv at rest with motor unit discharges of 500 uv upon volitional contraction, 2) no spontaneous activity at rest but, upon volitional contraction, large spikes varying in amplitude from 2 to 15 mv These exhibited the characteristics of motor units, maintaining their amplitude, smooth contour and constant duration throughout a contraction, but varying in frequency with strength of contraction When compared with potentials derived from normal muscles they were 20 to 30 times larger in amplitude The first pattern was obtained from muscles giving only perceptible contraction and chronaxies 5 to 10 times normal value, the second from muscles with varying strengths of contraction but chronaxies within the normal range These findings suggest correlation between the electromyographic patterns and anatomical characteristics of re-innervated muscle The first pattern described as obtained from denervated and re-innervated fibers existing side by side during the process of reinnervation The second was obtained from reinnervated muscle in which a reconstruction of the motor units has taken place This reconstructed motor unit probably contains a larger than normal number of muscle fibers which are responsible for the large spike potentials

Regional heat loss by temperature gradient calorimetry A C YOUNG,* L D CARLSON AND H L BURNS * *Dept of Physiology and Biophysics, Univ of Washington School of Medicine, Seattle*

The data described are part of those collected in field tests at northern latitudes in an investigation of possible physiologic changes occurring during acclimatization Special suits were designed to measure heat loss These consist of an inner layer, a core layer and an outer layer of insulative material Grids of nickel wire are fastened on either side of the core layer Resistance changes in the grids indicate the temperature drop, and the heat loss may be calculated The 18 separate grids in the suit enable distinction between heat loss from the head, the back, right or left body, upper or lower arm, and upper or lower leg from the right or left side, hands, feet, and the sole of the foot Heat loss from each region and the total heat loss have been recorded Tests were carried out in the field using unacclimatized and local (acclimatized) subjects The results indicate that, under similar conditions of exposure, the heat loss from peripheral regions of acclimatized persons is maintained at a higher level than that of unacclimatized subjects Total heat loss of the 2 types of subjects will be discussed

Oxygen in carotid blood and oxygen utilization of brain in pre- and post-mature fetal rabbits

I MAUREEN YOUNG (introduced by DONALD H BARRON) *Lab of Physiology, Yale Univ School of Medicine, New Haven, Conn and Sherrington School of Physiology, St Thomas's Hospital, London, England*

To obtain post-mature fetuses the does were prepared with chorionic gonadotrophine and progesterone on the 25th day of pregnancy. An average value of 50% saturation with oxygen was found for the carotid blood in pre- and post-mature fetuses, 27-35 days old, there was considerable scatter in the figures but those obtained from the first fetus exposed in any experiment were considered the most satisfactory. Fontanelle blood from 26-31 day old fetuses was very constantly around 35% saturation with oxygen, but fell rapidly to less than 10% saturation in the post-mature period. In fetuses 25-31 days old the blood emerging from the placenta in the umbilical vein was 68-85% saturated with oxygen. The constancy of the oxygen content of the fontanelle blood demonstrates that the supply of oxygen to the brain fully meets the demands of the growing organ during the last third of prenatal life. In the post-mature period this is not the case, for though the saturation of the carotid blood with oxygen does not diminish, the coefficient of utilization by the brain increases as shown by the steep fall in the oxygen content of the fontanelle blood.

Anemia in the rabbit following treatment with relaxin

M X ZARROW AND I G ZARROW *
Dept of Biological Sciences, Purdue Univ, Lafayette, Ind

Previous studies have shown that relaxin possesses the ability to cause a water retention in the rabbit. This observation plus the fact that the rabbit not only develops an antidiuresis during the last trimester of pregnancy, but also has a high concentration of relaxin in its blood stream led to an investigation of the cellular and non-cellular elements of the blood during pregnancy and after treatment with relaxin. The anemia of pregnancy was characterized by a drop of approximately 20-30% in both the number of erythrocytes and hematocrit. A decrease was also noted for the hemoglobin and non-protein nitrogen. The reticulocytes increased while the chlorides remained unchanged. The subcutaneous

injection of castrated and normal female rabbits with 250-500 guinea pig units of relaxin 3 times daily for 3-10 days also produced changes similar to those observed during normal pregnancy. A drop occurred in hematocrit, erythrocyte count, hemoglobin and non-protein nitrogen. The reticulocytes increased markedly and the blood chlorides remained unchanged. The mean corpuscular volume tended to decrease while the mean corpuscular hemoglobin concentration was not altered.

Desoxycorticosterone hypertension in relation to hepatorenal mechanisms
BENJAMIN W ZWEIFACH AND EPHRAIM SHORR
Dept of Medicine, Cornell Univ Med College and The New York Hospital, New York City

Circulatory changes which develop in the terminal vascular bed during experimental renal hypertension in rats have been shown to be related temporally to the hepatorenal principles, VDM and VEM. A series of specific criteria were obtained by directly visualizing the circulation in the mesentery of hypertensive animals and by assaying, with the rat mesoappendix technic, the blood and tissue extracts for vasotropic activity. These include 1) hyperreactivity of terminal arterioles, metarterioles and precapillaries, 2) augmented response of these vessels to renal VEM preparations, 3) hyperplasia of mesenteric capillary bed, 4) appearance of VEM and VDM in blood, and 5) formation of VEM by kidney tissue *in vitro* under both aerobic and anaerobic conditions. Similar experiments were carried out on rats made hypertensive by administration of DCA (subcutaneously and intramuscularly) together with a high salt intake. Hypertension developed in over 90% of the rats, the pressure rising within 15-20 days, to reach 145-200 mm Hg, and in partially nephrectomized animals to about 175-200 mm Hg. None of the DCA hypertensive animals showed changes comparable to those found in renal hypertensives. No VEM appeared in the blood during the acute elevation of blood pressure, no increased reactivity of the terminal vascular bed developed, no vascular hyperplasia was seen, and no metabolic lesion developed in the kidney whereby VEM was formed aerobically, as well as anaerobically. It therefore appears that the mechanisms responsible for the development of hypertension with DCA differ from those present in the renal hypertensive syndrome.

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Effects of magnesium on phosphate metabolism in vivo P H ABBISON AND EINAL AIDOUS (introduced by DEAN BURK) *Carnegie Inst of Washington, Dept of Terrestrial Magnetism, Washington, D C*

Procedures have been devised for the preparation of trace element free media by exploiting the potentialities of cation resin exchange materials. The method involves passage of all components of the medium through a resin (Dowex-50) column. All metallic cations except the alkali metals are completely removed. To date synthetic media have been principally employed, involving glucose plus inorganic salts. The purification process was checked through use of radioactive isotopes and bio-assay of the purified media. In theory and practice it is possible to obtain media absolutely freed from trace elements. The purified media have been used to study effects of magnesium on the P³² metabolism of *E coli*. In addition to an expected influence on glycolysis, the absence of magnesium has a more pronounced effect on ribonucleic acid formation decreasing the rate of synthesis by as much as a factor of ten.

Effect of incubation on the cholesterol content of rat liver homogenate W P ADAMS,* L I RICE,* M C SCHOTZ,* G F JACOBSON* AND W MARX *Dept of Biochemistry, Univ of Southern California School of Medicine, Los Angeles*

In preliminary experiments it had been observed that the amount of cholesterol extractable from liver homogenates of rats fed 1% cholesterol with the diet, decreased during incubation at 37°C (*Federation Proc* 7:171). Subsequently, however, such an effect was not consistently demonstrable, therefore, the possibility of an artifact had to be taken into consideration, in order to explain the earlier results. Upon further investigation it was observed that in some cases incubation rendered the extraction of cholesterol more difficult. As a consequence the extraction procedure was modified accordingly. In recent experiments, it was not possible to demonstrate, under the conditions so far investigated, a significant change in cholesterol concentration during incubation of rat liver homogenates.

Origin of the carbon skeleton of isoleucine EDWARD A ADELBURG* AND E L TATUM *School of Biological Sciences, Stanford Univ, California, and Dept of Bacteriology, Univ of California, Berkeley*

An isoleucine-requiring strain of *Neurospora crassa*, which accumulates in the filtrate large amounts of the isoleucine precursor α,β -dihydroxy- β -ethylbutyrate (E A ADELBURG, D M BONNER AND E L TATUM. In manuscript), was fed C¹⁴-labeled acetate. Methylene-labeled and carboxy-labeled acetate were used in separate experiments, and the isoleucine precursor produced in each case was isolated chromatographically. This material, which was found to contain high concentrations of C¹⁴, was then degraded by two different pathways: first, direct oxidation with dichromate to yield the terminal carbons as acetate, and second, cleavage with lead tetraacetate and subsequent stepwise degradation of the resulting methylethylketone and glyoxylic acid. The results of these degradations show that formation of the branched chain of isoleucine involves direct participation of acetate (probably as 'active acetate') in a coupling with a four-carbon compound, and that the latter compound is not formed directly from two-carbon fragments. Finally, the radioactive precursor obtained during these experiments has been used in investigating the utilization of this compound for isoleucine biosynthesis by other deficient micro-organisms. The significance of these findings is discussed in relation to the mechanism of biosynthesis of the carbon skeleton of isoleucine.

Studies on mechanism of acetate oxidation by bacteria SAMUEL J AJL* AND MARTIN D KAMEN *Washington Univ Med School, St Louis, Mo*

Suspensions of *E coli* adapted to oxidize acetate show heightened activity with pyruvate and C-4 dicarboxylic acids but not with α -ketoglutarate, which is attacked at the same rate by normal or adapted cells. When 2-C¹⁴-acetate is oxidized by such suspensions in the presence of unlabeled pyruvate or C-4 dicarboxylic acids, singly or in combination, considerable C¹⁴ accumulates in all

initially unlabeled substrates, except alpha-ketoglutarate. In a typical experiment with labeled acetate, unlabeled succinate, and alpha-ketoglutarate, it was found that 41% (68 micromoles) of the succinate was oxidized to acetate (or a compound in equilibrium with acetate) 25% of the alpha-ketoglutarate (30 micromoles) were decarboxylated and 25% of the acetate (44 micromoles) were metabolized. At least 7% of the acetate metabolized was assimilated while at most 52% was oxidized to CO_2 and not less than 41% condensed either directly to succinate or by way of a C-4 fragment in equilibrium with succinate. Less than 0.5% of the labeled carbon appeared in alpha-ketoglutarate. Specific activity data show that succinate carboxyl and evolved CO_2 were equilibrated. These results while not in accord with mechanisms based on a conventional tricarboxylic cycle agree quantitatively with operation of a C-4 dicarboxylic cycle condensation mechanism. Diametrically opposed data were obtained when similar experiments were performed with *M. lysodeikticus*, which attacks acetate readily. Equilibration of labeled acetate with unlabeled dicarboxylic acids was roughly 10-fold less than observed in *E. coli* and occurred to about the same extent with all substrates added, including alpha-ketoglutarate. CO_2 evolved was not in equilibrium with carboxyl carbon. Further work is planned with photosynthetic bacteria.

Phenylalanine requirements of children ANTHONY A. ALBANESE, REGINALD A. HIGGONS,* BETTY VESTAL* AND LOUKIA STEPHANSON * *Nutritional Research Lab, St. Luke's Hospital, New York City*

The available data on the amino acid requirements of man have been secured for the most part by the depletion procedure. The difference between the repletion needs so obtained and normal maintenance values increases with the duration of the depletion period which is known to involve numerous biochemical readjustments. In order to obtain amino acid requirement data which will have both practical and fundamental applications, means have been explored whereby measurements of the physiological availability of amino acids can be made under optimal dietary conditions as they prevail in life. This involves a determination of the 'saturation point' of a test amino acid which is added stepwise to the diet of subjects maintained in normal N-metabolism by natural foods fed *ad libitum*. Preliminary observations by the use of this technique show that when evaporated milk constitutes the principal source of dietary protein, the phenylalanine saturation point is reached when the diet contains 170 mg of L-phenylalanine/kg/day, for 6- to 8-month-old children. The saturation level for 3-month-old

children is reached sharply at 205 mg of L-phenylalanine. Calculations from these figures disclose that the normal phenylalanine needs of infants can be met by evaporated milk formulae which provide 4.2 gm of milk protein/kg/day for the first 3 months of life, and 3.6 gm of milk proteins for the last 6 months of the first year of life.

Nucleotide content of human blood HARRY G. ALBAUM, THEODORE CAYLE* AND ARTHUR SHAPIRO * *Dept. of Biology, Brooklyn College, Brooklyn, N. Y.*

The adenine nucleotide content of normal human blood has been studied spectrophotometrically by means of enzymes (ALBAUM, H. G. AND R. LIPSCHITZ. In press). The method utilizes perchloric acid filtrates of blood, and can be used to assay for total purine, adenylic acid, adenosine diphosphate and adenosine triphosphate on a 1 ml sample of blood. Heparinized blood remains unchanged in nucleotide content for at least 60 minutes, whereas oxalated blood, unless immediately deproteinized, changes in composition. Studies carried out on 6 normal, healthy adults, over a period of more than 3 months, indicate that the total purine level changes very little in each individual. Such bloods usually contain little or no adenylic acid or adenosine diphosphate, the greater part of the purine present is in the form of adenosine triphosphate. The nucleotide appears to be present only in the cells. Experiments are now under way on the ability of blood to synthesize ATP from added adenylic acid and other nucleotides *in vitro*.

Study of cholesterol turnover rate in using deuterium as a tracer element ROSLYN B. ALFINSLATER,* HARRY J. DEUEL, JR., MICHAEL C. SCHOTZ* AND FRANK K. SHIMODA * *Dept. of Biochemistry and Nutrition, Univ. of Southern California, Los Angeles*

Female albino rats 60 days old of the Univ. of Southern California strain were administered deuterium oxide for varying lengths of time up to 40 days. At the end of specified periods, the animals were anesthetized by injection with sodium pentobarbital (Nembutal) and sacrificed by withdrawal of blood from the heart. The following organs were extirpated: brain, lung, liver, adrenal and kidney. The cholesterol was extracted with an alcohol-ether mixture in a Soxhlet extractor. Total cholesterol was determined by a modified Schonheimer-Sperry method. The deuterium content of the isolated cholesterol was determined by combustion to water-deuterium oxide, and reduction over zinc to H_2 -deuterium. The ratio of the HD/HH was read in an Isotope-Ratio Mass Spectrometer.

Early results indicate that the greatest turnover of cholesterol in the organs investigated takes place in the adrenals. Lungs, liver and kidney follow in this order. The least amount of turnover was found in the brain. Similar experiments are in progress on rabbits.

Increased urinary excretion of choline in the phosphorus poisoned dog CARL E. ANDERSON,* C. FREEMAN LUCKEY* AND C. S. ROBINSON
Dept. of Biochemistry, Vanderbilt Univ. School of Medicine, Nashville, Tenn.

In 1935 Best and his co-workers (*J. Physiol.* 83: 275, 1934) found that choline, which protects the livers of rats from chloroform poisoning, did not prevent the fatty infiltration of the liver after phosphorus poisoning, but brought about a more rapid disappearance of fat during the recovery stage. In view of this finding, and the increase in neutral fat that occurs in the livers of phosphorus poisoned animals, it would appear reasonable to suspect an interference with the formation of transport fat. To test this hypothesis 2 healthy female dogs of average weight (8 and 12 kg.) were poisoned by the subcutaneous injection of 0.4 mg. of yellow phosphorus per kilogram body weight. The phosphorus was dissolved in peanut oil and kept in an atmosphere of nitrogen until ready for use. Urine was collected in the conventional manner. Choline was determined by both the colorimetric estimation of the Reinecke precipitate and by a manometric method to be described elsewhere. In both dogs there was found a marked increase in urinary choline as the administration of P_4 was continued. This increase was greatest just prior to death on the 8th and 10th days. Autopsy revealed typical yellow fatty livers and marked gastro-intestinal hemorrhages. When the injection of P_4 was discontinued just prior to the fatal dose and the dog allowed to recover, the excretion of choline returned to the base level. Starvation for 9 days decreased rather than increased urinary choline.

Electrometric determination of unidentified serum constituents EDNA ANDREWS* AND ADRIAN C. KUYPER
Dept. of Physiological Chemistry, Wayne Univ. College of Medicine, Detroit, Mich.

The following procedure was used for the determination of unidentified serum constituents. Protein was removed from serum by ultrafiltration, carbonate was removed by aeration of the acidified ultrafiltrate, and phosphate was removed by precipitation as calcium phosphate. The serum was ultrafiltered and the ultrafiltrate was titrated electrometrically, precision being increased by the maintenance of constant ionic strength. The identified substances present in the ultrafiltrate were determined quantitatively by accepted ana-

lytical procedures and their titration curves were calculated. The titration curve of the unidentified constituents was obtained by subtracting the curves for the identified substances from the experimentally determined curve. Eight sera from normal individuals were analyzed. The total unidentified substance titrated between pH 2.5 and 10.5 varied from 0.54 to 3.13 mEq./l. of serum. Buffer peaks were present in most of these samples at about pH 4.5, 7.0 and 9.5.

Decarboxylation of cysteic acid JAMES C. ANDREWS AND JOHN F. R. KUCK*
Dept. of Biological Chemistry and Nutrition, School of Medicine, Univ. of North Carolina, Chapel Hill

The decomposition of cysteic acid in solution under pressure yields two chief products. Alanine is produced in low yields by hydrolysis at 190°, this reaction is somewhat accelerated at higher temperatures. At 220° decarboxylation begins and as the temperature is increased the formation of taurine and carbon dioxide becomes the dominant reaction. At 240–250° fragmentation of the cysteic acid begins, producing small amounts of carbon, sulfur, hydrogen sulfide, mercaptans, sulfur dioxide and probably other products. The conversion of cysteic acid to taurine and to alanine is respectively 60–70% and less than 20%. Separation of amino acids was achieved by paper chromatography. Elution of the strips gave solutions of a single amino acid, which was determined colorimetrically with ninhydrin in the case of cysteic acid and alanine. A method for the determination of taurine in microgram quantities was developed employing the color formed by taurine, phenol and sodium hypochlorite at pH 9.5. Using an extract of rat liver as a source of cysteic acid decarboxylase (Blaschko, 1942) the disappearance of added cysteic acid from incubated mixtures was followed qualitatively with paper chromatography. The presence of appreciable amounts of preformed taurine and of substances running with cysteic acid interferes seriously with quantitative determinations.

Radioactive crystalline ribonuclease CHRISTIAN B. ANFINSEN
Biophysical Lab and Dept. of Biological Chemistry, Harvard Med. School, Boston, Mass.

Beef pancreas was sliced at the slaughterhouse as soon as possible after the death of the animal. Ten-gram portions of slices, 0.5 mm. thick, were placed in 500 ml. Erlenmeyer flasks containing 50 ml. of bicarbonate buffered medium at pH 7.4 and 38°C. (*J. Biol. Chem.*, 179: 1001, 1949). The medium included glucose at a level of 200 mg. %. Radioactivity was added as $Na_2C^{14}O_3$ through a vaccine port in the stopper of the flasks. After incubation at 38°C. for two hours, the flasks were chilled in ice and 0.5 M sulfuric acid was added to

give pH 1 Ribonuclease was isolated by a slight modification of the method of Kunitz. The final product was recrystallized once. This material was analyzed for enzyme activity, protein nitrogen and specific radioactivity. The enzyme activity-nitrogen ratios agreed with those obtained on several batches of 5 times recrystallized ribonuclease. The specific activity of the carbon dioxide liberated by ninhydrin from the washed defatted, hydrolyzed protein was about 0.1 to 0.3% that of the gas phase carbon dioxide. By using CO_2 with very high specific activity in the incubating flask it was possible to prepare ribonuclease with sufficient radioactivity to permit considerable dilution with 5 times recrystallized ribonuclease. These preparations showed constant specific radioactivity and enzyme activity upon further recrystallization. Data on phase rule criteria of purity will be presented.

Alanine as a source of acetyl groups of excreted acetyl amines in the intact animal H. S. ANKER
Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.

D- and L-alanines labeled with deuterium and carbon 14 were prepared by reduction of the corresponding phenylhydrazone of pyruvic acid with hydrogen and Adams catalyst and subsequent resolution. On feeding the labeled L-alanine together with foreign amines to rats of the Sprague-Dawley strain, identical isotope concentrations in both aliphatic and aromatic acetyl amines were found as after labeled pyruvic acid (*J. Biol. Chem.*, 176: 1339, 1948). After feeding of D-alanine the concentration of both isotopes in the acetyl group of the aliphatic amine was much higher than in that of the aromatic amine. These results account for the finding by Bloch and Rittenberg (*J. Biol. Chem.* 159: 45, 1945) that in the Sherman strain deuterio D,L-alanine gives rise to a much higher deuterium concentration in the acetyl group of phenylaminobutyric, than of p-aminobenzoic acid. It seems most likely that L-alanine is deaminated to pyruvic acid which in turn is metabolized to acetic acid, the latter acting as acetylating agent for both types of amines. For D-alanine a special mechanism to provide acetyl groups for the aliphatic amine seems to be available. It is most reasonable to assume that D-alanine reacts with a keto acid to yield an acetyl amino acid. This can then transfer its acetyl group to an aliphatic foreign amine as has been shown by Bloch and Rittenberg (*J. Biol. Chem.* 169: 467, 1947).

Procedure for the chemical estimation of free choline in plasma HAROLD D. APPLETON,* BETTY B. LEVY,* J. MURRAY STEELE* AND BERNARD B. BRODIE *Depts. of Biochemistry and Medicine, New York Univ. College of Medi-*

cine and Research Service, Goldwater Memorial Hospital, New York City

A simple method for the estimation of choline is described. It involves the precipitation of choline as an iodine complex, which is then determined in ethylene dichloride by its absorption at 365 m μ . Excess free iodine present does not interfere in the measurement. As little as 5 micrograms of choline can be accurately estimated by this procedure. Betaine, urea, creatine, creatinine, uric acid, cystine, histidine, guanidine, lysine and arginine were either not precipitated by the iodine reagent or the precipitate in ethylene dichloride did not absorb light at 365 m μ . The method has been applied to the estimation of free choline in plasma. The phospholipids are removed by acetone precipitation and the free choline estimated in the supernate. Counter-current distribution of the choline-like material measured by the procedure showed the presence of considerable material with solubility characteristics which differed from choline. This material could be separated from choline by its preferential solubility in butanol. An added step, in which the choline solution is washed with butanol prior to the formation of the iodine complex, has resulted in a specific method for the estimation of free choline in plasma. The method may also be applied to the estimation of choline in urine, in plasma phospholipids and other biological material. The free choline plasma values in normal and diseased states will be discussed.

Quantitative micro determination of thiosulfate

KAZKO ARAI, FRANK L. ALDRICH AND JOSEPH H. GAST (introduced by HOWARD B. LEWIS)
Dept. of Biochemistry, Baylor Univ. College of Medicine, Houston, Texas

Adaptation of the specific precipitation of thiosulfate as the nickel ethylene diamine salt together with the spectrophotometric measurement of the yellow iodine color at 355 m μ by the procedure of Sendroy and Alving (*J. Biol. Chem.* 142: 159-170, 1942) permits determination of isolated thiosulfate at concentrations ranging from 2.0 to 11 μg sulfur/ml. Appropriate controls must be read simultaneously to correct for dilution, oxidation and absorption by high concentrations of nickel ethylene diamine sulfate or nitrate which may be present initially or in the wash solution. Such salts show a slight absorption at this wavelength but do not reduce iodine. The conditions for reproducible precipitation require 1) alkalinity, obtained with ammonium hydroxide, 2) the presence of an equal volume of absolute ethanol, 3) at least a 300-fold excess of solid nickel ethylene diamine nitrate, 4) at least 4 hours standing at 5 to 10°C preferably overnight. Check determinations with added radioactive thiosulfate indicate

70% recoveries from urines containing concentrations of the above order of magnitude. Applications to other biological materials will also be discussed, including the problem of protein removal and losses of added thiosulfate. This procedure represents an approximately 20-fold extension of the range of thiosulfate concentration which can be determined by the usual microtitration.

Chromatographic fractionation of steroids of blood and adrenal tissue REGINALD M. ARCHIBALD AND JACQUE GENEST * *Hospital of the Rockefeller Inst for Med Research, New York City*

One volume of plasma, serum or packed blood cells is pipetted slowly into 5 volumes of 50% (by volume) acetone-alcohol, then brought to the boil and the supernatant filtered. The precipitated protein is extracted twice with 2.5 volumes of boiling acetone-alcohol. (With finely minced tissue, overnight extraction is repeated until extracts are colorless.) The combined filtrates are reduced to the original volume (hydrolyzed when desired) and extracted with 2 volumes of CCl_4 . An aliquot of this extract (equivalent to 5 cc plasma) is dried, dissolved in 5 cc benzene and chromatographed on alumina according to the technique described for urine extracts (*Federation Proc* 8: 180, 1949). Cholesterol and its esters are neatly separated from each other and from at least some other components which give color in the Liebermann-Burchard reaction. This technique shows some promise of providing simple determinations of cholesterol and of its esters in blood and tissues. Desoxycorticosterone and bis-dehydrodissynolic acid-7-methyl-ether are eluted readily from alumina by water. As compared with blood of normals, the blood of a patient with an adrenal tumor contained an increased amount of an easily eluted component. This was eluted in the same order as was the component previously reported (*ibid*) as increased in the tumor and in the acid hydrolyzed urine of a patient preceding (but not after) surgical removal of a hirsutizing adrenal tumor.

Rat growth experiments with some derivatives of phenylalanine MARVIN D. ARMSTRONG AND JOY D. LEWIS (introduced by V. DUVERGNEAUD) *Univ of Utah College of Medicine, Salt Lake City, Utah*

The metabolism of some compounds structurally related to phenylalanine has been studied by the use of the growth technique. The more readily prepared diastereoisomer of β -phenylserine, reported some time ago to be at least partially converted to benzoic acid *in vivo*, was examined for its ability to form either tyrosine or phenylalanine; it was found to be neither toxic nor beneficial when added to diets deficient in either of these amino acids. As would be expected from enzymatic

studies previously reported in the literature, glycidyldehydrophenylalanine is readily available as a precursor for phenylalanine in the diet and acetyldehydrophenylalanine cannot serve as a precursor of either phenylalanine or tyrosine when administered either by feeding or intraperitoneal injection. The effects of the isomeric 2-, 3-, and 4-fluorophenylalanines on the growth of young rats was studied and the acute toxicity of 3-fluorophenylalanine (BOYER, EVANS AND PHILLIPS, *J Pharmacol & Exper Therap* 73: 176, 1941) was confirmed. The 2-, and 4-fluorophenylalanines likewise are toxic but to a much lesser degree than the 3-fluoro isomer. Possible explanations for these toxicities will be discussed.

Lipide phosphorylation by surviving slices of the liver of rats on various diets CAMILLO ABTOM AND MARJORIE A. SWANSON * *Dept of Biochemistry, Bowman Gray School of Medicine, Winston-Salem, N C*

Slices prepared from the livers of rats previously maintained on various diets were incubated aerobically in the presence of labelled Na_2HPO_4 . The incorporation of P^{32} into the phospholipides by liver slices from animals maintained on a 5% casein diet was much lower than the incorporation by the livers of rats on a stock diet containing 25% proteins of mixed origin. This decreased synthesis of phospholipides seems due to the low protein level rather than specifically to a deficiency of choline or choline precursor in the diet of the animals. Indeed, supplementation of the low casein diet with choline did not raise the synthesis of phospholipides, whereas an increase in the casein content of the diet to 25% increased the rate to that found in the livers of rats on the stock diet. Addition of choline *in vitro* caused a marked inhibition of the phospholipide synthesis by the livers of rats on the high protein diets, whereas a certain degree of stimulation was obtained, when choline was added to the slices prepared from the livers of rats on the low protein diet. The effects of the *in vitro* addition of ethanolamine were similar, although less marked and less consistent. Betaine added to the surviving slices generally enhanced lipide phosphorylation, irrespective of the previous dietary regime of the animals.

Influence of vitamin B_{12} on a succinylsulfathiazole-induced dietary deficiency in rats CONRADO F. ASENJO *Dept of Chemistry and Nutrition, School of Tropical Medicine, Univ of Puerto Rico, San Juan, P R*

In the course of investigating the suppletory action of natural products on folic acid-deficient rats receiving a highly purified basal diet containing succinylsulfathiazole, it was noticed that many of the products tested induced a growth

response and a leukocyte regeneration at rates well above those obtained with the optimal level of folic acid. This suggested that the depleted animals suffered not only from a severe folic acid deficiency but also, perhaps, from other less severe deficiency, or deficiencies. As such a basal diet could have possibly developed a vitamin B₁₂ deficiency concurrently with the severe folic acid deficiency, it was decided to study the effect that B₁₂ supplementation could have in the depleted rats. Preliminary evidence indicates that when high levels of folic acid are given as supplement, the concurrent administration of vitamin B₁₂ significantly improved the growth-response and white cell count. At low levels of folic acid supplementation, the addition of B₁₂ did not improve the response produced by folic acid alone, though survival was a little longer. Folic acid negative controls receiving B₁₂ did not show any improvement, but they also lasted longer than the controls without B₁₂. The incidence of infarct-like splenic lesions was high in both groups of controls, in those receiving B₁₂, perhaps because they survived longer, the splenic lesions developed to such an extent that this organ was found, in several instances, adhered to those adjacent. A level of 0.5 µg/day of B₁₂ was used throughout.

Detection and identification of metabolites in tissues by means of paper chromatography
 JORGE AWAPARA (introduced by HARRY J. DEUEL, JR.) *Univ. of Texas, Anderson Hospital for Cancer Research, Houston*

Paper chromatography has been used as a quantitative method for some amino acids which are completely resolved by this procedure. The appearance of several unidentified components in chromatograms from tissue extracts has led to a system of isolating those components by means of paper chromatography, in sufficient quantity to allow identification. Thus far, ethanolaminophosphoric ester has been identified in nearly all organs of the rat and some human tumors. Presently, a peptide has been detected in many organs and in blood and an unidentified amino acid in brain only. The identification of the latter is under way.

Mild dephosphorylation of pyrimidine nucleotides
 JAMES E. BACHER* AND FRANK WORTHINGTON ALLEN *Dept. of Biochemistry, Univ. of California Med. School, Berkeley*

Existing methods for the hydrolysis of pyrimidine nucleotides require strenuous conditions and lead to partial destruction of the organic radicals. In an attempt to circumvent such destruction, the following procedure was devised. To a solution of pyrimidine nucleotide is added a 4-fold m excess of La(NO₃)₃. The pH is adjusted to 10 by the addition of NaOH. The suspension is heated at 100° for 2 hours. The pyrimidine nucleosides from

uridylic acid and from cytidylic acid have been recovered in approximately 95% yields and identified by the use of a Craig counter-current distribution apparatus and the ultra-violet spectra. The method of Fiske and Subbarow for the determination of phosphate cannot be applied directly to the hydrolysates. The procedure for hydrolysis is expected to aid in the preparation and analysis of the pyrimidine constituents of ribonucleic acids.

Spectrophotometric microdetermination of methionine, based on its reversible reaction with iodine (Lavine)
 BOHDAN BAKAY* AND GERRIT TOENNIES *Inst. for Cancer Research and Lankenau Hospital Research Inst., Philadelphia, Pa.*

Among amino acids methionine is distinguished by a reversible reaction with iodine, which can be abolished by oxidation with iodate (LAVINE, *J. Biol. Chem.* 151:281, 1943). The analytical application of this principle has been modified by replacing thiosulfate titrations with spectrophotometric determination of iodine, combined with solvent extraction. Adequate determinations (precision 1-2%) on undecolorized hydrolysate samples containing 50-100 micrograms of methionine are thus possible. In one pair of calibrated test tubes a blank and a 5 cc. aliquot of unknown solution are allowed to react with iodine at pH 7, the excess iodine is extracted with amylalcohol-carbon tetrachloride, and the colors of the aqueous layers are determined. The color of the blank tube represents residual iodine, and that of the unknown, iodine and hydrolysate color. Their difference (A) represents the effect of the unknown. The aqueous phases are then acidified. The difference between the two tubes at this stage (B) represents the value (A) plus the iodine liberated. Therefore, B minus A represents the reversibly bound iodine, i.e., methionine and substances reacting similarly. In another pair of tubes, blank and aliquot are first oxidized by iodate, and then the previous procedure is applied, yielding net color before acidification (C), and net total color following acidification (D). D minus C represents iodine bound reversibly after oxidation with iodate, i.e., substances other than methionine. (B - A) - (D - C) thus yields the methionine value. Details of technique and results obtained in the analysis of proteins and liver extracts will be presented.

Transfer of purines and pyrimidines from bacterial host to bacteriophage progeny
 J. MICHAEL BARRY, MIRIAM GOLUB BANKS AND ARTHUR L. KOCH (introduced by E. A. EVANS, JR.) *Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.*

Radioactive adenine, guanine, and thymine have been isolated from *E. coli* grown on ammonium lactate medium supplemented with NaH¹⁴C₃O₃.

and used for studying the transfer of bacterial purines and pyrimidines to bacteriophage progeny. *E. coli* cells can be specifically labeled in the purine fraction by culturing them on an ammonium lactate medium supplemented with C^{14} -labeled adenine, in which case both the adenine and guanine of the cells are labeled and account for all the radioactivity of the whole bacteria. Close to 100% of the adenine and guanine of the bacteria is derived from the adenine of the medium. Analysis of the bacteriophage T₂ grown on these specifically labeled bacteria demonstrates that almost one-half of the phage purines is derived from bacterial host purines. Analogous experiments with C^{14} -labeled guanine and thymine have also been carried out.

Transamination and total free amino acid carboxyl nitrogen in induced states of growth

PAUL D. BARTLETT (introduced by OLIVER H. GAEBLER) *Edsel B. Ford Inst. for Med. Research, Henry Ford Hospital, Detroit, Mich.*

The importance of transamination in the dynamic state of amino nitrogen has been emphasized in a number of studies on protein metabolism. Since nitrogen storage induced by the administration of growth hormone is accompanied by changes in plasma amino acid concentration, it seemed of interest to study the process of transamination and the concentrations of amino acids in tissues obtained from animals in which growth had been arrested by hypophysectomy or induced by the administration of growth hormone. Transaminase activity was determined in tibialis anticus homogenates of pair-fed rats by the method of Ames and Elvehjem (*J. Biol. Chem.* 166: 81, 1946). High activity in normal adult female rats was practically unaffected in states of induced growth. Activity was somewhat lower in rats hypophysectomized at 26–28 days of age and growth hormone treatment reduced it to the level of rapidly growing normal rats fed *ad lib*. Q_T^{10} values for each of the described groups were respectively +317, +313, +297, +256, and +250/mg of homogenate nitrogen. Total free amino acid carboxyl nitrogen analyses on pooled leg muscle for each of the groups of 3 to 6 rats gave values of 19.5, 18.5, 24.3, 27.9, and 28.1 mg %. Free amino acid concentrations in pooled muscle obtained from hypophysectomized rats in induced states of growth and from rapidly growing normal rats were thus found to be higher than in states of arrested growth and appear to be inversely related to the transaminating capacity of the tissue.

Sex differences in response to alloxan

ELIOT F. BEACH, PHOEBE J. BRADSHAW* AND N. R. BLATHERWICK *Biochemical Lab., Metropolitan Life Insurance Company, New York City*

Mature albino rats were fasted 4 days and then

injected subcutaneously with 20 mg alloxan monohydrate/100 gm body weight after which food was given. To determine the effects of alloxan on blood sugar during the first 48 hours groups of rats were studied at intervals to explore the significant fluctuations. The extent of permanent alteration in glucose economy was evaluated by running oral glucose tolerance tests on each animal before and 2 weeks following alloxan and by postprandial blood sugar and urinary glucose and acetone excretion tests after 1 and 3 weeks. These tests reveal marked individual variations in response to alloxan with the females of our strain reacting more violently and developing more severe permanent distortions of carbohydrate metabolism than the males. During the early 'triphasic' response more severe hyperglycemia, followed at 20–30 hours by much deeper hypoglycemia, has been observed in the females. Of 40 females examined to date 10 developed fatal acidosis, hyperglycemia and glycosuria compared with only 3 of 40 males. Fifty per cent of the males and only 17.5% of the females treated with alloxan failed to develop persistent glycosuria. The severely glycosuric females, when compared with the severely affected males, were characterized by greater distortions in oral glucose tolerance, higher fasting blood sugars and more frequent acetonuria.

Pool of miscible uric acid in the gouty human.

JEAN D. BENEDICT,* PETER H. FORSHAM,* MARCEL ROCHE,* SIDNEY SOLOWAY* AND DEWITT STETTEN, JR. *Division of Nutrition and Physiology, Public Health Research Inst. of The City of New York, Inc., Med. Clinic, Peter Bent Brigham Hospital, Boston, and Dept. of Medicine, Harvard Med. School, Boston, Mass.*

The magnitude of the pool of uric acid which mixes promptly with, and dilutes, tracer doses of intravenously injected isotopic (N^{15}) uric acid has been measured in the gouty human subject and has been found to exceed normal values by as much as 25-fold. Subsequent to the uric acid diuresis produced in the gouty subject by protracted salicylate therapy, this pool has been observed to contract to about $\frac{1}{5}$ of its initial value without the occurrence of any marked change in the level of serum uric acid concentration. This contracture has been ascribed to the loss by the patient of that portion of the urate initially present in the solid phase which is rapidly miscible with uric acid in solution. Whereas therapeutic doses of colchicine were without demonstrable effect upon the quantities measured, the administration of ACTH, in an acute experiment, was followed by the expected diuresis of uric acid. Since this effect was accompanied by a fall in the serum uric acid concentration and no change in the rate of dilution of

isotope, it is provisionally concluded that the site of this action is at the level of the renal tubule

Metabolism of intravenously administered N^{15} -labeled compounds MAX BERENBOM* AND JULIUS WHITE *National Cancer Inst, National Insts of Health, Bethesda, Md*

Comparative studies have been made of the metabolism in rats, after intravenous administration of N^{15} -labeled ammonium glutamate (labeled in the ammonium ion), glutamine (labeled in the amide group), glycine, and L-phenylalanine, by following the 24 hour urinary excretion of N^{15} . Normal rats (200-220 gm) injected with an aqueous solution of ammonium glutamate, glutamine, glycine, or L-phenylalanine, equivalent to 5 mg of labeled nitrogen/rat, excreted 66, 66, 38, and 40% of the N^{15} respectively. Similar results were obtained when the amount of ammonia or glycine was increased to 15 mg of labeled nitrogen/rat. Young, growing rats (100-110 gm) each receiving 2.5 mg of labeled nitrogen, and rats (original weight 200-220 gm) fasted for 5 days and then each injected with 5 mg of labeled nitrogen, in the form of one of the above mentioned compounds, excreted approximately the same percentage of administered N^{15} as the normal rats. In all these experiments approximately 90% of the excreted N^{15} was recovered as urea and 2-8% as ammonia. Although the percentage of administered N^{15} excreted as ammonia was small, during the first hour a relatively large fraction of the total ammonia excreted was derived from the injected compounds.

Isolation of β -glucuronidase of calf spleen and factors influencing its activity PETER BERNFELD* AND WILLIAM H. FISHMAN *Cancer Research and Cancer Control Unit, Depts of Surgery and Biochemistry, Tufts College Med School, Boston, Mass*

Glucuronidase from calf spleen has been purified by several ammonium sulfate fractionations, mainly in an alkaline medium. A highly active enzyme has been obtained which behaves like a single protein in the electrical field at pH 4.2 and 8.6. The pure glucuronidase has an isoelectric point between pH 7.5 and 8.0 and an activity optimum at pH 4.5. During this purification, several fractions with lower glucuronidase activity have been discarded, but no fraction has been obtained showing a different pH optimum. A marked shift of the pH optimum of the pure glucuronidase to pH 5.0 or more, accompanied by a change in its activity, can be observed after addition of saccharate, gluconate, adenylate or of a non-protein fraction obtained from calf spleen. It is believed that the alkaline fractionation removes such or similar acid compounds which otherwise always

accompany the protein in an acid precipitation. This explains the observation made by other authors that several glucuronidases with different pH optima can be obtained by fractional salt precipitations in an acid medium. The existence of only one glucuronidase in calf spleen is confirmed by the identical ratio of the velocity of hydrolysis observed with two different substrates (phenolphthalein-glucuronide and menthol-glucuronide) for all fractions obtained during the purification. The activity of pure glucuronidase at pH 4.5 is increased 50% by 0.5% ribonucleate and 120% by 0.5% desoxyribonucleate. The activity of glucuronidase in tissue would appear to be greatly determined by the desoxyribonucleate present.

Effect of ACTH and other substances on the lipid metabolism of rat brain FREDERICK BERNHEIM AND HOWARD L. ZAUDER* *Dept of Physiology and Pharmacology, Duke Univ Med School, Durham, N C*

When brain homogenates are incubated aerobically and then precipitated with trichloroacetic acid, the filtrate heated with thiobarbituric acid produces a red color. It has been shown that an oxidized form of linolenic acid reacts with the thiobarbituric acid to form the colored product. The *in vitro* addition of ascorbic acid before incubation increases the values. When rats are injected with either ACTH or adrenal cortical extract some time before the removal and incubation of the brains, large increases in values are obtained both with and without the addition of ascorbic acid. A number of analgesic drugs such as morphine, meperidine, methadon and adrenaline produce similar effects.

Loss of body potassium during pergastric intestinal perfusion LIONEL BERNSTEIN,* PHILIP B. O'NEILL,* ARTHUR BERNSTEIN* AND WILLIAM S. HOFFMAN *Hektoen Inst for Med Research, Cook County Hospital, Chicago, Ill*

Perfusion of the entire intestine was carried out in 9 human subjects with preterminal uremia. The perfusing fluid contained 148 or 143 mEq sodium, 5 or 10 mEq potassium, 3 mEq magnesium, 105 mEq chloride, 51 mEq bicarbonate, and 2% glucose. The fluid was perfused through a stomach tube at a rate of approximately 2 l/hour and collected from the rectum. The gastric perfusate was also collected by suction. At the end of 8-12 hours the potassium loss from the body ranged from 2-12.7 gm. Since the total extracellular potassium was less than 3 gm, the greatest part of the lost potassium came from the cells. In all cases there was a significant drop in serum potassium concentration, in 2 instances to the pathologically low level of 3.0 mEq/l. But the serum potassium concentration could not be predicted

from the total potassium loss. The fluid obtained from the rectum had potassium concentrations ranging from 11.3–26.0 mEq/l, or from 2–7 times as high as the serum potassium concentration. Even when the perfusate contained 10 mEq/l, the potassium balances were negative. The passage of potassium from body fluids to perfusate was therefore not due to simple dialysis. But the exact mechanism involved is not clear.

Effect of amino acids on the non-enzymatic decarboxylation of oxalacetic acid. SAMUEL P. BESSMAN (introduced by JOSEPH H. ROF) *Research Foundation of Children's Hospital, Washington, D. C.*

Amino acids in concentrations approximating the total amino acid content (as equivalents of alpha amino nitrogen) of normal tissues have been found to stimulate the decarboxylation of oxalacetic acid (OAA) from 2–18 times the spontaneous rate. Conditions were acetate buffer 0.10 M, pH 5.0, temperature 37°C, OAA approximately 0.07 M, amino acid tested usually 0.05 M, in total volume of three ml. A freshly prepared solution of OAA was placed in the sacs of the Warburg flasks and the reaction was begun by tipping after a period for temperature equilibration. It was found that almost all of the amino acids stimulated the breakdown of OAA as a first order reaction with respect to OAA. The most active amino acids tested were L(+) histidine (186), DL ornithine (078), L(+) lysine (050), glycine (049), and L(+) arginine (045), the numbers in parenthesis representing the first order reaction constant expressed in reciprocal minutes. The constant for our preparation of OAA was 0.10 min^{-1} . Acetylation diminishes the activity of glycine and glutamic acid, and the amides of the dicarboxylic acids are more active than the parent amino acids. Since the tissue concentration of amino acids is sufficient to cause at least a doubling of the rate of OAA breakdown and since the stimulation is proportional to the concentration, these results suggest a physiological role of amino acids in the regulation of carbohydrate oxidation, thus offering another approach to the problem of gluconeogenesis.

Influence of choline and inositol upon the rate of deposition and removal of liver lipids. C. H. BEST, C. C. LUCAS, JEAN M. PATTERSON* AND JESSIE H. RIDOUT *Banting and Best Dept. of Med. Research, Univ. of Toronto, Toronto, Canada*

Assessment of the lipotropic activity of inositol has sometimes been complicated by the presence of choline in the basal diet and by inadequate dietary protein. The rate of change of total liver lipids and cholesterol under the influence of 1) an improved basal diet alone, 2) plus supplementary inositol alone, 3) plus choline alone, and 4)

with choline plus inositol, has been measured by analyzing livers of animals fed the test diets for periods of 3, 5, 7, 9, 14, 21 and 28 days, respectively. Rats (Wistar strain, weighing 70–100 gm) were depleted of B vitamins by feeding for 3 weeks a fat-free hypolipotropic diet consisting of casein 10, arachin 2, fibrin 2, salts 4, cellulose 2, sucrose 80, cod liver oil concentrate 0.015%. After the depletion period the rats were divided into groups and were fed the test diets (containing liberal amounts of all the commercially available B vitamins) for the periods mentioned. A similar experiment was conducted in which fat was present (12% replaced an equal amount of sucrose) in the diets during both depletion and test periods. When liver lipids were plotted against time the 7-day test period, which has been frequently used, was found to be unsuitable. This period is one of rapid change rather than of equilibrium. Inositol alone, even at maximal effective dosage, has only a limited lipotropic effect in fat-free diets and none in diets containing fat.

Thionase activity. FRANCIS BINKLEY *Univ. of Utah College of Medicine, Salt Lake City*

Preparations of purified thionase, activated with glutathione, have been found to attack a wide variety of S-substituted cysteines. The S-methyl, ethyl, propyl and butyl derivatives are attacked with the formation of neutral mercaptans identified as methyl, ethyl, propyl and butyl mercaptans respectively. The S-carboxymethyl and carboxyethyl derivatives are attacked at a low rate with the formation of acidic mercaptans. The methyl ester of cysteine and cysteinylglycine are cleaved without preliminary hydrolysis and with the formation of hydrogen sulfide. Djenkolic acid is cleaved with the apparent formation of cysteine. Certain of the above results have been checked as to whether or not the compounds will provide cysteine for the growth of the white rat. Frozen pig kidney has been found to be a suitable source of the enzyme but no activity is observed unless glutathione is added. Certain evidence has indicated the presence of a coenzyme. The purified enzyme may be dissociated into dialyzable and non-dialyzable components. The dialyzable component has a characteristic spectrum in the ultraviolet and is destroyed by heat-labile components of various tissues.

Effect of colchicine and cortisone on uric acid metabolism in gout. CHARLES BISHOP, WILLIAM GARNER AND JOHN H. TALBOTT (introduced by WILSON D. LANGLEY) *Dept. of Medicine, Univ. of Buffalo Med. School and Buffalo General Hospital, Buffalo, N. Y.*

Certain aspects of uric acid metabolism in patients with gout and other diseases are being studied in this laboratory using heavy nitrogen.

Uric acid with N¹⁵ in the 1,3 positions was injected intravenously into gouty individuals during gouty attacks both before and after therapy with colchicine or cortisone. Excreted uric acid was isolated as the ammonium salt from 6 hour samples of urine. Pool size and turnover rate were calculated by the method of Stetten (*J Biol Chem* 181:183, 1949). In one gouty patient with a uric acid pool size of 3300 mg (approximately 3 times normal), colchicine caused a 50% decrease in the pool size and an increase in the turnover rate from 0.50 to 1.01 pools/day. Another gouty patient with a comparable pool size demonstrated after cortisone therapy a 30% decrease in the pool size along with an increase in the turnover rate from 0.59 to 1.26 pools/day. A third gouty patient showed no metabolic response to either of these drugs even though his uric acid pool size was comparable to those of the other two patients studied. This latter patient, however, had been maintained on a small daily dose of colchicine for some time. The implications of these findings will be discussed.

Comparison of various techniques for hydrolysis of conjugated 17-ketosteroids in urine JOEL BITMAN* AND SAUL L. COHEN *Dept of Physiological Chemistry, Univ of Minnesota Med School, Minneapolis*

A technique for the hydrolysis of a relatively labile group of 17-ketosteroid conjugates by weakly acidic buffers was previously reported (*Federation Proc* 8:184, 1949). The 17-ketosteroids thus released contain a high percentage of 3-beta hydroxy compounds (40-60%). The hydrolysis of 17-ketosteroid conjugates with an ox spleen β -glucuronidase preparation has been found to release approximately 50% of the ketosteroids in urine. Glucuronidase hydrolysis releases 17-ketosteroids with a relatively low 3-beta hydroxy steroidal content (3-7%). Evidence that the buffer hydrolyzed conjugates are sulfates has been obtained through 1) a comparison of the buffer technique with the mild hydrolytic procedures of other workers, 2) the complete hydrolysis by this technique of pure sodium dehydroisoandrosterone sulfate, and 3) the non-effectiveness of the buffer treatment in hydrolyzing the glucuronide linkage or of the glucuronidase treatment in hydrolyzing the sulfate linkage. It has been found possible to hydrolyze $\frac{3}{4}$ to $\frac{1}{2}$ of the conjugated 17-ketosteroids of normal male urine by the use of a combination of these procedures. The remaining conjugated 17-ketosteroids which were not released by the treatments thus far attempted can be hydrolyzed by the usual HCl treatment. The combined use of buffer and enzyme techniques obviates to a great degree the destructive effects of strong acid hydrolysis.

Formation of acetate from acetaldehyde in cell-free yeast extracts SIMON BLACK *Chemical Division, Dept of Medicine, Univ of Chicago, Chicago, Ill*

Two extracts of bakers' yeast have been prepared which catalyze the simultaneous formation of acetate and ethanol from acetaldehyde. An extract of rapidly dried (1 minute) Fleischmann's yeast, after dialysis against a non-phosphate buffer, requires for its catalytic action DPN, inorganic phosphate, magnesium ions, and cystein. Under certain conditions a small accumulation of a labile phosphate can be demonstrated after incubation with acetaldehyde. An extract of frozen (liquid N₂) Red Star yeast loses its activity irreversibly on dialysis against a non-phosphate buffer. When dialyzed over-night against phosphate its activity is dependent upon DPN, magnesium ions, and an additional unidentified factor present in a yeast 'kochsaft'. The latter is prepared from a briefly dialyzed (2-3 hours with stirring) preparation of the enzyme extract. The activity of the Red Star extract is greatly increased by heating to 50° for 15 minutes. Its activity also increases markedly on storage for 1-3 days at 2° or -10°, but 1 week at either of these temperatures results in complete inactivation. The activity of both extracts remains in the supernatant when centrifuged at 15,000 r.p.m. A colorless, clear solution of the Red Star enzyme has been prepared by fractionation with acetone.

Demonstration and comparison of two glycogen fractions in liver and muscle WALTER LYON BLOOM,* MARY SCHUMPERT* AND GEORGE T. LEWIS *Dept of Biochemistry, Emory Univ School of Medicine, Emory University, Ga*

There has been considerable speculation concerning the presence in tissues of 2 types of glycogen. No adequate method has been available for the separate determination of the 2 types and no physiological significance has been described for such fractions. This report presents a simple method of distinguishing 2 fractions of glycogen in tissue. The procedure depends on differences in solubility of the 2 fractions in trichloroacetic acid extracts of homogenized tissue. The results obtained with the method are easily reproducible and have a high order of statistical significance. The 2 glycogen fractions have been shown to be present in the liver and muscle of rabbits and rats. The liver of the fed animal contains more of the acid soluble fraction (85% of the total) than the muscle (55%). Adrenaline has been shown to most markedly influence the removal of the acid-soluble fraction of muscle glycogen, and it is suggested that this fraction is the most physiologically labile component of the glycogen present in liver and muscle.

Minor role of non-hepatic tissues in plasma protein synthesis observed with the aid of lysine- α - C^{14} C G BLY,* L L MITTER AND W F BAILE Depts of Radiation Biology and Pathology, Univ of Rochester School of Medicine and Dentistry, Rochester, N Y

The surviving carcasses of normal Wistar strain rats, posterior to the second lumbar vertebra, with or without kidneys, has been found to synthesize plasma globulins from a complete mixture of amino acids containing DL lysine α - C^{14} . Although the amino acids are rapidly removed from the continuously perfused heparinized blood, the total plasma protein synthesized amounts to 5-20% of that synthesized by the liver from the same amino acid mixture in the same time (MITTER, L L, C G BLY, M L WATSON AND W F BAILE *Federation Proc*, this issue) Weight for weight, the liver is at least 200 times as active as the carcass in plasma protein synthesis. On a relative basis the skeletal muscle, kidney, and testes have produced far more tissue protein than plasma protein. The liver, on the contrary, synthesizes an amount of plasma protein of the same order of magnitude as that of the liver tissue protein.

Influence of α -amino acids on rate of enzymic conversion of phosphorylated compounds OSCAR BODANSKY, OLGA BLUMENFELD,* ELAINE ZINKER* AND BLANCHE MARKARDT *Memorial Hospital and Sloan-Kettering Inst for Cancer Research, New York City

The extent and nature of the inhibition of phosphatase hydrolysis of Na β -glycerophosphate by α -amino acids have been previously reported (BODANSKY, *J Biol Chem* 179 81, 1949, 174 465, 1948). In the present study, similar inhibition was shown to exist for the dephosphorylation of ATP and fructose-L,6-diphosphate by intestinal and bone phosphatase preparations. However, α -amino acids accelerate the dephosphorylation of ATP by fresh tissue homogenates at pH 7.4 and of fructose-L,6-diphosphate by liver hexosediphosphatase at its alkaline optimum. Acceleration of dephosphorylation of ATP by α -amino acids is greatest in skeletal muscle homogenates, but also occurs to some extent in cardiac, liver, brain and kidney homogenates. In extension of Sutherland's finding that enzymic conversion of glucose L-phosphate to glucose-6-phosphate is activated by metal-binding agents (*J Biol Chem* 180 1279, 1949), a number of α -amino acids were found to accelerate this reaction, the degree of acceleration being dependent on the nature and concentration of the α -amino acid. Hexokinase activity was affected only negligibly by α -amino acids at pH 7.4. Incubation of skeletal muscle homogenates with Co^{++} , Zn^{++} , Mn^{++} , Ni^{++} or Cu^{++} resulted in marked inactivation of the dephosphorylation of

ATP, and such activity was restored to varying extents by the addition of histidine. Data are also presented concerning the relationship between the effects of α -amino acids and the effects of metal ions for osteogenic sarcoma phosphatase, liver hexosediphosphatase, phosphoglucomutase, and hexokinase.

Studies on metabolism of 3-hydroxyanthranilic acid ANN H BOKMAN* AND B S SCHWEIGERT Division of Biochemistry and Nutrition, American Meat Inst Foundation, and Dept of Biochemistry, Univ of Chicago, Chicago, Ill

During investigations on the enzymatic destruction of 3-hydroxyanthranilic acid, an intermediate in the formation of nicotinic acid from tryptophan, it was found that this compound fluoresces, and that the fluorescence observed was a linear function of the concentration. The fluorescence of 3-hydroxyanthranilate was found to vary with the pH, and a pH of 7.0 was found to be optimum. Quinolinic acid, nicotinic acid, and N'-methyl-nicotinamide do not fluoresce under the conditions used for measuring 3-hydroxyanthranilic acid, and 3-hydroxyanthranilate could be quantitatively recovered in the presence of heat inactivated rat liver homogenates and solutions of acetone dried powders of liver. Experiments were performed both with rat liver homogenates, and with solutions of acetone dried powders of rat liver, in which 3-hydroxyanthranilic acid, buffer, and enzyme solution were incubated together at 37° and at pH 7.4 for various time intervals. It was found that the conversion of the substrate to non-fluorescent products was proportional to the enzyme concentration, and that with the enzyme concentration constant the conversion of substrate was proportional to the amount added until the enzyme became limiting. Coupled with these experiments, microbiological determinations of nicotinic acid before and after acetic acid treatment of the incubation mixtures were made and the conversion of 3-hydroxyanthranilate to quinolinic acid equivalent to 86 to 100% on a molar basis was found. These values were in accord with the amounts of substrate converted to non-fluorescent products by the test systems.

Potassium compounds associated with carbohydrate metabolism of *Escherichia coli* E T BOLTON (introduced by J B ALLISON) Dept of Terrestrial Magnetism, Carnegie Inst of Washington, Washington, D C

Studies of uptake and loss of potassium by *E coli* as well as studies of the metabolism of potassium-deficient cells have indicated the existence of potassium compounds in the cell. Since these compounds dissociate fairly readily in aqueous solution it is difficult to identify them by conventional chemical procedures. By means of paper

chromatography and radioactive tracer methods, however, it is possible to demonstrate potassium compounds in extracts of *E coli*. Comparisons of chromatograms of extracts of cells grown in the presence of K^{42} with known samples reveal the existence of compounds which show the same movement in a butyl-alcohol-acetic acid-water solvent as the K-salts of hexose phosphates. These compounds account for a large fraction of the total potassium in the cell. The biological origin of these compounds is further demonstrated by control experiments with non-radioactive cell extracts. These findings confirm the work of others in this laboratory who have shown that potassium is associated with the metabolism of hexose phosphates.

N^{15} Studies on conversion of tryptophan to niacin in neurospora DAVID M BONNER AND C W H PARTRIDGE * *Osborn Botanical Lab, Yale Univ, New Haven, Conn*

The scheme of niacin synthesis in neurospora involving tryptophan is largely predicated on mutant strains of one genetic type. Since certain observations regarding such strains are not readily reconcilable with the thesis that tryptophan serves as a major niacin precursor, a direct check of the conversion of indole to niacin has been made. Strain 10575 requires tryptophan or indole for growth, accumulates anthranilic acid, but cannot grow on niacin. Since the only source of tryptophan available to this strain is that added, the formed niacin should reflect the same N^{15} content as the indole used for culture if tryptophan conversion represents a major pathway of niacin synthesis. For ease of isolation, a double mutant was formed with 10575 and a strain known to accumulate generous quantities of quinolinic acid (BONNER AND YANOFSKY, *Proc Nat Acad Sci* 35 576, 1949). This double mutant was cultured on indole having 3.97 atom % excess N^{15} . After growth, the quinolinic acid present in the medium was isolated and found to contain 2.98% excess N^{15} . The anthranilic acid accumulated during growth of this double mutant was also isolated and its N^{15} content determined as a direct check of the tryptophan cycle recently proposed by Haskins and Mitchell (*Proc Nat Acad Sci* 35 500, 1949). The anthranilic acid was found to contain no appreciable N^{15} . One may conclude, therefore, that in neurospora, anthranilic acid is not formed from tryptophan to an appreciable extent and that niacin formation from tryptophan represents a major synthetic pathway.

Inulin clearance during pregnancy ROY W BONS-
NES AND WILLIAM A LANGE * *Dept of Obstetrics
and Gynecology, Cornell Univ Med College and
New York Hospital, New York City*
According to data in the literature, pregnant

women have inulin clearances of the same order of magnitude as nonpregnant women. The subjects for these tests were, in the main, near term. A survey by us of this function throughout pregnancy shows 13 normal women 11-37 weeks pregnant to have significantly elevated inulin clearances which average 183 ml/min/1.73 m² with a standard deviation of the sample of ± 20 compared with 119 ± 4 for 8 nonpregnant controls. However, women 38 weeks or more pregnant were observed to have inulin clearances which average 129 ml/min/1.73 m², a value not significantly different from the control value and in agreement with the data in the literature. But the standard deviation of this sample is ± 43 , indicating quite a range of values. Both groups of pregnant women were observed to have changes in simultaneously performed urea and p-amino hippurate clearances which followed the inulin clearances in direction but not in magnitude.

Mechanism of bacterial growth inhibition by hydroxylamine ERNEST BOREK, NATHAN GROS-
SOWICZ* AND HEINRICH WAELSCH *Depts of Bio-
chemistry, New York State Psychiatric Inst and
Columbia Univ, New York City*

The recent discovery of an enzyme system (glutamo- and asparto-transferases) which exchanges the amide group of glutamine and asparagine with hydroxylamine suggested a possible mechanism for the inhibition of bacterial growth by hydroxylamine. The growth inhibition was studied on *Lactobacillus arabinosus* and on *Proteus vulgaris*, the latter organism can synthesize glutamic acid and also contains glutamo- and asparto-transferases, while the former is unable to synthesize the amino acid, and the presence of the enzyme system has not been demonstrated in it. In synthetic media containing amino acids the growth of *Proteus vulgaris* is inhibited completely by 3×10^{-4} molar hydroxylamine, while *Lactobacillus arabinosus* requires about 5 times the concentration of the inhibitor. The growth inhibition of both organisms is overcome by ammonia or glutamine, or any compound from which ammonia is easily liberated. The antibacterial index with both organisms is about 1. The relationship between hydroxylamine inhibition and its reversal can be studied only within a narrow range of concentrations of the inhibitor, since a high concentration of hydroxylamine interferes with the utilization of other metabolites (keto acids, etc). These findings suggest that hydroxylamine may interfere with the formation and utilization of asparagine or glutamine.

Incorporation of C^{14} -labeled amino acids into proteins of fractions of guinea pig liver homogenate HENRY BORSOOK, CLARA L DEASY,*
ARIE J HAAGEN-SMIT,* GEOFFREY KEIGHLEY*

AND PETER H. LOWE * *Kerckhoff Labs of Biology, California Inst of Technology, Pasadena, Calif*

Three different particulate fractions and a supernatant fraction of guinea pig liver homogenate were prepared by differential centrifugation. The four fractions exhibited functional differences in the rates at which they incorporated glycine, histidine, L-leucine or L-lysine. L-lysine was incorporated into the proteins of the particulate fractions under one set of conditions and into those of the supernatant fraction under another set of conditions. Evidently the incorporation of amino acids into proteins *in vitro*, does not, in the adult cell, necessarily depend on direct participation of the nucleus. One hour after the intravenous injection of guinea pigs with 20 mg of labeled amino acid/kg the incorporation of glycine, histidine, leucine or lysine into the liver proteins was of the same order of magnitude as in the most active liver fraction *in vitro*. Each of the 4 amino acids was incorporated into the proteins of all 4 fractions. The greatest uptake in every case was in the microsome fraction. *In vitro* this fraction was the least active, this may be because optimal experimental conditions have not yet been found for the *in vitro* experiments.

Characteristics of protease trypsin inhibitor of soy beans DONALD E. BOWMAN *Dept of Biochemistry and Pharmacology, Indiana Univ School of Medicine, Indianapolis*

Present evidence further supports the protease nature of the soy bean trypsin inhibitor to which we have called attention, which is soluble in 60% alcohol and 10% trichloroacetic acid. This inhibitor differs from the globulin soy bean trypsin inhibitor in many respects. A few of the properties of this protease are indicated by the method of preparation which involves extraction of previously unheated soy bean meal with 60% ethyl alcohol, precipitation with acetone or increased alcohol, precipitation of inert material and any remaining globulin inhibitor with trichloroacetic acid, precipitation of the active material with 5% saturated ammonium sulfate and dialysis. Intravenous administration is effective in diminishing fatal pulmonary and cardiac embolism which results from the injection of small amounts of trypsin in rabbits. It also inhibits the trypsin-like protease of plasma but, like other trypsin inhibitors, it is less effective against the thromboplastic and proteolytic activities of iodinated trypsin than it is with untreated trypsin. It does not prevent the hypotensive effect of intravenous trypsin. Rabbits do not appear to become sensitized to this inhibitor. Further evidence indicates that there are various bean trypsin inhibiting fractions as we have emphasized since first calling

attention to bean enzyme inhibitors. One fraction, also having protease characteristics, is quite similar to the material used in the present work except that it is insoluble in trichloroacetic acid at room temperature.

Further properties of a tuberculostatic fraction of human plasma DONALD E. BOWMAN *Dept of Biochemistry and Pharmacology, Indiana Univ School of Medicine, Indianapolis*

A fraction of autoclaved human plasma which retards the growth of the H-37 strain of tubercle bacilli has characteristics of a lipid. It is soluble in alcohol, ether and acetone. It can be concentrated by direct ether extraction of autoclaved plasma or inert material can be precipitated from an aqueous extract of the autoclaved plasma with 90% ethyl alcohol at low temperatures with subsequent ether extraction of the residue of the evaporated alcoholic filtrate. It can be precipitated with ammonium sulfate and dialyzed. It inhibits the growth of the H-37 strain on undiluted sterile human plasma which has been coagulated with a minimum of heat. In the majority of instances growth readily occurs on such plasma in the absence of the activated inhibitor. The degree of inhibition is further estimated by determining the nitrogen content of the washed organisms grown in synthetic media. Observations suggest that the presence or the liberation of this heat stable substance *in vivo*, possibly through an enzymatic mechanism, may contribute to resistance to tuberculosis.

Biosynthesis of radioactive fatty acids and cholesterol ROSCOE O. BRADY* AND SAMUEL GURIN *Dept of Physiological Chemistry, Univ of Pennsylvania School of Medicine, Philadelphia*

We have studied the biological synthesis of long chain fatty acids and cholesterol by incubating slices of liver tissue from fed rats of the Wistar strain in the presence of C¹⁴-labeled acetate, pyruvate, butyrate, hexanoate, and octanoate as substrates. The usual procedures for the isolation of fatty acids and cholesterol were followed. Fatty acids whose chain lengths were longer than 10 carbon atoms were separated by precipitation with a copper-lime mixture and effectively freed from contaminating substrate. All of the substrates investigated contributed carbon atoms for long chain fatty acid and cholesterol synthesis *in vitro*. The addition of amorphous insulin to the incubating medium increased significantly the amount of acetate incorporated into long chain fatty acids. It would appear that all of the short chain fatty acids we investigated are as efficiently converted to long chain fatty acids as is acetate.

Metabolic competition of pyruvate and lactate in cardiac muscle slices MYRON C. BRIN AND

ROBERT E OLSON (introduced by FREDRICK J STARE) *Dept of Nutrition, Harvard School of Public Health, and Dept of Biological Chemistry, Harvard Med School, Boston*

Cardiac muscle slices from healthy ducklings 250-350 gm in weight fed an adequate diet *ad libitum* were incubated in Warburg vessels with pyruvate alone at levels of 1-5 mm/l, with lactate alone at levels of 1-5 mm/l and with lactate plus pyruvate with each varied from 1-5 mm/l, at 37° for one hour. Oxygen consumption was determined manometrically and changes in pyruvate and lactate levels during incubation determined chemically. In this range the utilization of pyruvate and of lactate was a function of initial concentration, pyruvate being used 20% more rapidly than lactate. With combinations of pyruvate and lactate as substrate, the total C₂-compound utilization was greater than that with each alone. Under these conditions, increasing concentrations of lactate had relatively little effect upon pyruvate utilization, whereas increasing concentration of pyruvate progressively decreased lactate utilization. Related isotope studies of the effect of each substrate upon the oxidation of the other will also be presented.

Some biological and chemical properties of the citrovorum factor HARRY P BROQUIST,* E L R STOKSTAD AND T H JUKES *Lederle Labs Division, American Cyanamid Company, Pearl River, N Y*

Recent studies have indicated that concentrates of natural materials active for growth of *Leuconostoc citrovorum* reverse the toxicity of folic acid antagonists for some microorganisms under conditions in which pteroylglutamic acid (PGA) is ineffective. In the present study further evidence for the ability of citrovorum factor (CF) concentrates to reverse 4-amino PGA for *L citrovorum* was found. In studies with mice it was found that CF concentrates given either in the diet or by injection could protect mice against the toxic effects of 4-amino PGA, PGA was without effect in alleviating this toxicity. In counter current distribution studies by the method of Craig, it was found that the distribution curves for CF activity or folic acid activity were indistinguishable. In other experiments it was found that CF was readily converted by exposure to HCl to a compound with properties similar to folic acid. These findings coupled with the ability of CF concentrates to reverse the toxicity of 4-amino PGA in microorganisms and mice support the view that CF is a hitherto unrecognized biologically active form of PGA.

Chronic exposure of adult mice to C¹⁴O₂ DONALD L BUCHANAN (introduced by SIMON BLACK)

Biology Division, Argonne National Lab, Chicago, Ill

Certain fractions of total carbon of tissues and of compounds in various organisms are derived from air CO₂. Such fixed carbon fractions have not been reported. If an animal is kept for an extended period in CO₂-labeled air the ultimate ratio of the specific activity (*s a*) of the carbon in a particular component to the *s a* of the atmospheric CO₂ should represent the fixed carbon fraction of the component. Mice were ventilated in 3 serially connected, sealed metabolism chambers with constantly flowing labeled air. Animals were sacrificed at intervals during a 34-day exposure and up to 45 days thereafter, and the *s a* of tissues and excreta measured. Although the C¹⁴O₂ content per unit volume of gas was equal in the three chambers, the total CO₂ concentration was not. Below 1.7% the CO₂ concentration had slight influence on C¹⁴O₂ retention. Hence, total retention of air CO₂ is proportional to its concentration below 1.7%. Urea carbon reached a constant *s a* within the first day while erythrocyte carbon failed to reach a constant level. Each of approximately 30 other components reached constant *s a* levels in 3-16 days. Fixed carbon fractions of various soft tissues varied between 10⁻⁴ and 3 × 10⁻⁵ at the normal air concentration of CO₂, while that for urea carbon was 3.5 × 10⁻³. All *s a*-time curves were more complex than the simple exponential except the erythrocyte curve which was linear. The latter showed the life span of the mouse erythrocyte to be 52 days.

Lipids of normal and pathological aortas R C BUCK* AND R J ROSSITER *Dept of Biochemistry, Univ of Western Ontario, London, Canada*

The concentration of free and total cholesterol (method of SCHOENHEIMER AND SPERRY, *J Biol Chem* 106 745, 1934) phospholipids (method of HACK, *J Biol Chem* 169 137, 1947) and total fatty acid (oxidative method of BLOOR, *J Biol Chem* 77 53, 1928) were determined in a number of normal and pathological human aortas. In aortas that appeared normal on macroscopic examination the concentration of total cholesterol and total phospholipid increased with age. From birth to 35 years the increase in cholesterol was confined chiefly to free cholesterol, the percentage of cholesterol in the ester form being low in these younger age groups. After 35 years the increase was chiefly in the ester fraction, the free cholesterol remaining fairly constant. An increase in the concentration of sphingomyelin accounted for all the increase in phospholipid with age. Cephalin, lecithin, or neutral fat (i.e. fatty acid other than in cholesterol ester or phospholipid) was not related to age. In aortas showing macroscopic evidence of atherosclerosis there was an increase in

the concentration of both free and ester cholesterol, total phospholipid and neutral fat, the increase in the concentration of these lipids being related to the severity of the lesion. Lecithin and sphingomyelin accounted for the increase in phospholipid. There was no increase in cephalin.

Hydrolysis of 17-ketosteroid conjugates HENRY J BUEHLER,* PHILIP A KATZMAN AND EDWARD A DOISY *Dept of Biological Chemistry, Saint Louis Univ School of Medicine, Saint Louis, Mo*

Our previous work has shown that bacterial glucuronidase effectively hydrolyzes steroid glucuronides. In a continuation of our studies on the hydrolysis of conjugated steroids, our attention has been focussed on the steroid sulfates. Attempts to obtain microbiological sulfatases have shown these enzymes to be highly specific but none has been found to hydrolyze the 17-ketosteroid sulfates. Refluxing aqueous solutions of sodium dehydroisoandrosterone sulfate or sodium androsterone sulfate with 15 volumes % of HCl for 10 minutes resulted in poor recoveries of both steroids. Longer periods of refluxing gave even poorer recoveries of androsterone and did not improve the recovery of dehydroisoandrosterone. Autoclaving for one hour at pH 7.3 (or higher) under 15 pounds pressure hydrolyzed the dehydroisoandrosterone sulfate almost completely but did not hydrolyze androsterone sulfate. This difference of behavior was also exhibited when the procedures of Talbot, *et al* and Bitman and Cohen for the hydrolysis of 17-ketosteroid sulfates were employed. Autoclaving at pH 7.3 produced no detectable hydrolysis of the glucuronides of estriol, menthol or phenolphthalein. Continuous extraction with ether for 24 hours at pH 0.7 or treatment by standing in 7.2 N HCl for 18 hours at 38°C effectively hydrolyzed both steroid sulfates. The glucuronides were not affected by continuous ether extraction at pH 0.7 but were hydrolyzed to the extent of 50% by the strong acid treatment. The application of these procedures to urine of normal men have shown that continuous extraction with ether at pH 0.7 liberated from 25-200% as much 17-ketosteroid as was liberated by glucuronidase hydrolysis.

Role of methionine as methyl donor for choline synthesis in the chick K A BURKE,* R F NYSTROM* AND B CONNOR JOHNSON *Division of Animal Nutrition and Radiocarbon Lab, Univ of Illinois, Urbana*

Methionine labeled in the methyl position with radiocarbon has been synthesized and fed to individual chicks. The expired CO₂ was collected at 1- to 8-hour intervals for the next 48 hours, after which the chickens were sacrificed and the choline isolated from the whole carcass. Radioactive carbon determinations made on the expired CO₂

and on the isolated choline showed both to contain C-14. Quantitative data on the oxidation of methionine-methyl groups and on the synthesis of choline from methionine-methyl groups will be reported. These findings bring the chick into conformity with the rat, man and pig.

Studies on metabolism of radioactive L-ascorbic acid J J BURNS,* H B BURCH* AND C G KING *Dept of Chemistry, Columbia Univ, New York City*

The gross metabolism of radioactive L-ascorbic acid, labeled with C¹⁴ in the carboxy position, has been studied in normal and scorbutic guinea pigs. The labeled compound was synthesized by addition of radioactive sodium cyanide to L-xylosone. Pure L-ascorbic acid was obtained in 25% yield after purification through ion exchange. Feeding experiments were carried out with normal and scorbutic guinea pigs. The radioactivity appearing in respiratory carbon dioxide during 24 hours was 20-30% of the injected dose. The urine contained 3-6% of total activity, and the feces 0.7%. Calcium oxalate, isolated from the urine, contained 60% of the total urine activity, demonstrating a precursor role of ascorbic acid in oxalate formation. The distribution of radioactivity in organs and tissues was approximately the same as found by chemical analysis for ascorbic acid. There appeared to be no marked differences in tissue incorporation in the normal and scorbutic guinea pig when compared on a unit weight basis. Studies have been made on the incorporation of radiocarbon into the petroleum ether fractions extracted from the spleen and adrenals of scorbutic guinea pigs.

An improved micro-method for sugars using direct colorimetry of ferricyanide GEORGE O BURR AND T TANIMOTO* *Dept of Physiology and Biochemistry, Experiment Station, Hawaiian Sugar Planters' Association, Honolulu, T H*

Clarification of leaf extracts with lead or Somogyi reagent fails to remove a considerable amount of non-sugar reducing material which reacts with cold alkaline ferricyanide. Activated carbon removes this material quantitatively without adsorbing measurable quantities of reducing sugars. At the same time it completely decolorizes the plant extract, making a direct colorimetric reading possible. Based upon the above observations the following micro-method has been developed. To 5 ml of alkaline potassium ferricyanide, containing 0.005 mEq of ferricyanide, is added 2 ml of clarified plant extract which contains not over 180 micrograms of reducing sugars. After heating for 25 minutes in an 80° C water bath the density of the cooled solution is read in a photoelectric colorimeter with a No. 42 filter or in

a spectrophotometer at wave length 420 m/ μ , the absorption maximum for potassium ferricyanide. At this wave length the formed ferrocyanide gives no measurable interference and the decrease in density is proportional to the amount of reducing sugar present. The method is rapid, accurate and sensitive to less than 1 microgram of reducing sugars.

Antibiotic action of the pigments of *Fusarium bos-trycoides* F. A. CAJORI, M. A. HAMILTON,* E. URBANICH* AND T. PURSHOTTAM* *Dept. of Biochemistry, Univ. of Colorado School of Medicine, Denver, Colorado Foundation for Research in Tuberculosis, Colorado Springs*

Culture fluid of *F. bostrycoides* strongly inhibits the growth of the tubercule bacillus, H-37, when tested *in vitro*. The inhibiting action was found to be related to pigment production by the mold. The pigments were extracted from the mold mycelium with hot alcohol and from the highly colored culture fluid with benzene. After freeing the extracts from fat by adsorption on alumina, a crystalline pigment was obtained with antibiotic activity toward the tubercule bacillus similar to the activity of streptomycin when tested by our assay method. This pigment, which has indicator properties, melts at 238°–239° C and is a naphthaquinone, similar to, but not identical with javanicin (ARNSTEIN AND COOK, *J. Chem. Soc.* II 1021, 1947). Experiments bearing on the possible mechanism of the inhibitory action of this material on the growth of the tubercule bacillus will be presented.

Precipitation and separation of 2-hydroxy-3-naphthoic acid hydrazones of conjugated and free ketosteroids BERNARD CAMBER (introduced by R. M. ARCHIBALD) *Hospital of the Rockefeller Inst. for Medical Research, New York City*

Conjugated ketosteroids of unhydrolyzed urine, blood serum, etc. may be precipitated readily at room temperature as hydrazones by addition of 1/10th vol of 0.4% solution of 2-hydroxy-3-naphthoic acid hydrazide (*Nature* 163: 285) in acetic acid. The water washed precipitate from urine has been resolved by paper partition chromatography in a triethylamine–t-butanol–water system into at least 10 components which fluoresce under blue light. Further resolution may be obtained in a second dimensional partition of the colored sulfanilic acid azo derivatives in a water saturated s-butanol system. Quantitation, characterization and further resolution of these precipitates and their azo derivatives is being attempted by column chromatography on alumina and on HIO₄ oxidized starch or cellulose. Transfer of the hydrazide moiety from the steroid to the aldehydic groups of the column involves a new chromatographic principle, 'group exchange'. The hydro-

phobic unconjugated ketosteroids (reference standards, desoxycorticosterone, cortisone, bile acids, urine hydrolysate extracts) dissolved in 0.8% solution of the hydrazide in acetic acid precipitate as hydrazones on addition of 20 vol of 5% methanol in water. A mixture of the hydrazones of steroids known to occur in the β ketonic fraction from acid-hydrolyzed urine (isoandrosterone, dehydroisoandrosterone, allopregnanol-3 β -one-20) has been resolved by paper partition chromatography in an isopropyl ether-triethylamine-water system at 0° C and also in an isopropyl ether-trimethylamine-methanol-water system at room temperature. These methods may facilitate corticosteroid studies in blood and urine and permit removal of ketosteroids from urine without acid hydrolysis. The hydrazide moiety apparently facilitates fractionation and permits visualization and characterization of components.

Methylation of nicotinamide with a soluble enzyme system from rat liver G. L. CANTONI (introduced by S. P. COLOWICK) *Dept. of Biological Chemistry, Univ. of Illinois College of Medicine, Chicago*

The biosynthesis of N¹-methyl-nicotinamide (NMeN) demonstrated earlier in liver slices by Perlzweig and collaborators (*J. Biol. Chem.* 150: 401, 1943) has been studied. It was found that NMeN is formed by an enzyme system obtained from rat liver. The methyl donor is L-methionine. Betaine is active as a methyl donor only in the presence of homocysteine. The reaction is probably endergonic and requires Mg⁺⁺. It was found that the energy of the phosphate bond in ATP or phosphoglyceric acid, the latter used in conjunction with catalytic amounts of ATP, is utilized for the biosynthesis of NMeN. Oxygen is not required. The reaction product was identified as NMeN by a) its ultraviolet absorption spectrum, b) conversion to a fluorescent product with alkali plus methyl-ethyl-ketone, and c) enzymatic oxidation to L-methyl-3-carboxyl-amide-6-pyridone. The enzyme system from rat liver withstands heating at 46° C for 10 minutes and can be fractionated in the cold with ammonium sulfate between 0.4 and 0.50 saturation. A preparation has been obtained which has a specific activity 1.5–2.5 times as great as the crude liver extract. Further work on the purification of the enzyme system and the mechanism of the reaction is in progress. It is suggested that the enzyme responsible for the methylation of nicotinamide by methionine and ATP be called nicotinamide methylkinase.

Oxidation of L-ascorbic acid by o-iodosobenzoic acid WENDELL T. CARANAY* AND LESLIE HELLMAN *Dept. of Physiological Chemistry, Johns*

Hopkins Univ School of Medicine, Baltimore, Md

The oxidation of ascorbic acid by *o*-iodosobenzoic acid proceeds as a second-order reaction in non-buffered aqueous solutions over the pH range from 4-8 with k_2 equal to 1.3 liters mole⁻¹ min⁻¹ at 25° (half-life 13 hours for 0.001 M solutions). The slow rate of the reaction is interpreted on the basis of internal chelation in the molecule of *o*-iodosobenzoic acid since the corresponding *m*- and *p*-iodosobenzoic acids oxidize ascorbic acid practically instantaneously. The acidic component of all buffers tested catalyzed the reaction by combining reversibly with *o*-iodosobenzoic acid to weaken normal internal chelation. An autocatalysis, observed in acetate buffer at pH 4.6 but not in phosphate buffer at pH 7, was traced to *o*-iodobenzoic acid, an end product of the reaction. Dehydroascorbic acid had no effect. A specific catalytic effect of phosphate on the oxidation of ascorbic acid was observed at pH 7. Cupric ion catalyzed the reaction in agreement with observations on the oxidation of ascorbic acid by oxygen. The reaction provides a method for the study of the controlled oxidation of ascorbic acid under various conditions, a problem of considerable interest in view of the undetermined role of the vitamin in oxidation-reduction processes and the mechanism of its action *in vivo*.

Fatty acids as biotin substitutes for *Leuconostoc*

WARNER W. CARLSON, VIRGINIA WHITESIDE-CARLSON AND KATHERINE KOSPETOS (introduced by EMMETT B. CARMICHAEL) *Biochemistry Dept., Med. College of Alabama, Birmingham*.
Previously it was found (CARLSON AND WHITESIDE-CARLSON, *Proc Soc Exper Biol & Med* 71: 416, 1949) that *Leuconostoc* required biotin in glucose and fructose media, but were free of a requirement for this vitamin in sucrose media to the degree that the disaccharide was utilized via the mechanism resulting in dextran synthesis. The ability of fatty acids to substitute for biotin in these carbohydrate media has now been investigated. The fatty acids were used in the form of monoesters of polyoxyethylene sorbitan, compounds known commercially as 'Tweens'. With all strains of *Leuconostoc* employed, Tween 80, the mono-oleate, and Tween 20, the mono-laurate, produced a higher level of growth in sucrose media than was obtained with biotin. In their presence half-maximal to maximal growth was obtained with the various strains in biotin-free glucose and fructose media. Reversal of egg white inhibition by Tween 80 and Tween 20 was observed only with *L. dextranicum* elai, a strain distinguished by giving approximately a quantitative yield of dextran from sucrose. Tween 60, the mono-stearate, and Tween 40, the mono-palmitate, were less

active than the oleate and laurate. Their ability to substitute for biotin in sucrose media paralleled the dextran synthesizing capabilities of the various strains. They could not substitute for biotin in media containing the constituent monosaccharides, and their presence increased the inhibitory effect of egg white. Although most reports stress the ability of unsaturated fatty acids, especially oleic, linoleic and vaccenic acids, to replace biotin, in the case of *Leuconostoc* it appears that lauric acid is also highly effective in the form of Tween 20.

Genesis of C₄ dicarboxylic acids in the tricarboxylic acid cycle S. F. CARSON,* J. W. FOSTER, D. S. ANTHONY* AND E. F. PHARES* *Biology Division, Oak Ridge National Lab., Oak Ridge, Tenn.*

Two recent discoveries concerning biochemical genesis of C₄ dicarboxylic acids require some re-investigation and perhaps some reinterpretations concerning the tricarboxylic acid cycle. First, the recent unequivocal proof of the '2C₂ condensation reaction,' (FOSTER, CARSON, *et al Proc Nat'l Acad Sci* 35: 663, 1949), second, the finding that metabolic CO₂ can become incorporated into carboxyl groups of preformed C₄ dicarboxylic acids, but which were synthesized by the 2C₂ condensation reaction (FOSTER, CARSON *et al Proc Nat'l Acad Sci*, in press). Krebs type malonate inhibition experiments were conducted with C¹⁴H₂COCOOH and C¹³O₂. Malate, succinate, lactate and α-ketoglutarate were isolated, purified and completely degraded, specific activities were determined for both C¹³ and C¹⁴. The results indicate that the 2C₂ condensation reaction cannot be excluded from having taken part in the synthesis of the C₄ dicarboxylic acids. It is also indicated that one may have to re-examine the quantitative significance of the role of CO₂ in the actual synthesis of C₄ dicarboxylic acids, versus the amount of labeled CO₂ which merely enters by simple reversible exchange with already existing C₄ acids. Finally, it should be pointed out that in all the malonate experiments, the controls formed as much succinate (frequently more) than the treatment containing substrate. As far as we are aware, very few, if any, isotope experiments on the tricarboxylic acid cycle have been conducted with controls. Our controls yielded malate containing as much C¹³ in carboxyl (from C¹³O₂) as the pyruvate experiments.

Structure of cerebrosides HERBERT E. CARTER, FRED L. GREENWOOD* AND CHARLES G. HUMISTON* *Division of Biochemistry, Noyes Lab of Chemistry, Urbana, Ill.*

In previous work we have shown that triacetyl-sphingosine yields acetic acid on catalytic reduction as a result of cleavage of the allylic acetox-

group The product has now been isolated and shown to be diacetylsphingine Oxidation of N-benzoylsphingine gave an optically active N-benzoylaminostearic acid which, on racemization through the azlactone, was converted into the known DL-benzoyl- α -aminostearic acid These results furnish final confirmation for the structure of sphingosine The optically active benzoyl- α -aminostearic acid is tentatively assigned the D-configuration on the basis of studies of optical rotation of acylated amino acids in various solvents Catalytic reduction of hexaacetylphrenosin yielded acetic acid but no reducing sugar Hydrolysis of the product gave sphingine The structure of cerebrosides will be discussed in the light of these data

Reaction of ferrocytochrome-c with peroxidases and peroxides BRITTON CHANCE (introduced by J M BUCHANAN) *Johnson Research Foundation, Univ of Pennsylvania, Philadelphia*

The reaction of ferrocytochrome-c with the peroxide complexes of the 4 purified peroxidases (horse-radish (HRP), verdo (VPO), lacto (LPO), and cytochrome-c (CCP) peroxidase) has been found to be very rapid, of roughly the same or greater speed than the combination of these peroxidases with other electron donors or with hydrogen peroxide Since the latter reaction has a very low energy of activation (< 2000 cal based on experiments on HRP and catalase), it is suggested that the reaction of the large cytochrome-c molecule occurs at a larger active center than the iron-porphyrin group of the peroxidases A characteristic of the kinetics of the reactions catalysed by LPO, VPO, and HRP is a saturation effect which may be caused by the formation of a peroxidase-peroxide-cytochrome-c complex of a dissociation constant of about 1×10^{-6} M Although CCP has the highest activity (k_4 for the reaction of CCP H_2O_2 with cytochrome-c is about $10^8 M^{-1} \times sec^{-1}$ and k_4 for the others is $0.6 - 1 \times 10^7 M^{-1} \times sec^{-1}$ at pH = 4.7) the activity of the other peroxidases is considerable and, for HRP and VPO, is comparable to rough estimates of the turnover of cytochrome-c in plant cells and leucocytes The activity of the enzymes is fragile, HRP and CCP are readily inactivated by the turnover of several separate additions of ferrocytochrome-c In the case of HRP, the activity is restored by the addition of p phenylene diamine

Fractionation of coenzyme A-dependent acetyl transfer systems T C CHOU,* G D NOVELLI,* E R STADTMAN* AND FRITZ LIPMANN *Biochemical Research Lab, Massachusetts General Hospital and Dept of Biological Chemistry, Harvard Med School, Boston*

Pigeon liver extract was fractionated into acetyl donor and various acetyl acceptor fractions by use

of acetone fractionation in the cold combined with protamine precipitation At pH 8.2, 40% acetone precipitates the acetyl donor fraction (ATP-acetate reaction) together with the acetoacetate- and citrate-synthesis acceptor systems The sulfanilamide acceptor enzyme remains dissolved and may be precipitated by increasing the acetone concentration to 60% The 40% acetone precipitate was dissolved and treated further with protamine Therewith, the acetoacetate acceptor enzyme was precipitated and separated from the citrate system which remained in solution As donor system with the acetyl acceptor fractions from liver extract, a microbial enzyme (*Clostridium kluyveri* or *butylicum*, *E coli*) may be used which brings synthetic acetyl phosphate into reaction With acetyl phosphate as the acetyl donor in such mixed enzyme systems, it could be shown that in the condensation with oxalacetate to citrate, the acetyl-bound phosphate is split off, and free citrate is formed It furthermore appears that 2 acetyl phosphate molecules enter acetoacetate synthesis This is shown 1) by a 2:1 ratio of acetyl phosphate disappearance to acetoacetate formation and 2) by use of carboxyl-labelled acetyl phosphate With the latter acetoacetate was obtained which was labelled nearly equally in the carbonyl and carboxyl positions

Succinic dehydrogenase—cytochrome C link in heart muscle succinoxidase HAROLD W CLARK (introduced by ELMER STOTZ) *Dept of Biochemistry, Univ of Rochester School of Medicine and Dentistry, Rochester, N Y*

Several claims have been made for an 'S-C factor,' that is, a factor necessary for reduction of cytochrome c by succinic dehydrogenase and succinate These claims have been attributed, however, to unspecific effects in the complex association of factors in the particulate matter of the succinoxidase system (KEILIN, D AND E F HARTREE, *Biochem J* 44 205 1949) By ammonium sulfate fractionation of a pig heart extract in the presence of 2% cholate, a succinic dehydrogenase preparation (i.e., one which with succinate reduces O-chloro,2,6-dichlorophenol indophenol) is obtained which does not reduce cytochrome c in the presence of succinate and KCN Neither dialysis of the preparation, nor addition of inert proteins or calcium phosphate gel established reduction of cytochrome c Addition of an ammonium sulfate fraction obtained with lower cholate concentration caused a reduction of cytochrome c, and the rate of reduction served as an assay for the 'S-C factor' activity of the added fraction Cholate itself did not affect the assay in the concentrations encountered Various other treatments caused great variations in the ratio of 'S-C' succinic dehydrogenase, indicating the sepa-

rate nature of the 2 factors, although an 'S-C' preparation has not yet been obtained entirely free of the dehydrogenase. A combination of ammonium sulfate fractionations and $\text{Ca}_3(\text{PO}_4)_2$ adsorption has thus far resulted in an 'S-C' preparation approximately 40-fold purified over the original heart muscle extract.

Effect of cortisone on protein metabolism in the rat as studied with isotopic glycine I. CLARK (introduced by R. H. SILBER) *Merck Inst for Therapeutic Research, Rahway, N. J.*

Normal and adrenalectomized rats weighing about 220 gm each received 3 mg of cortisone acetate subcutaneously daily for 5 days. They were maintained on a constant food intake and received 1.0% sodium chloride in their drinking water. On the fourth day they received a tracer dose of glycine containing 32% N^{15} . Urinary urea ammonia, total nitrogen and N^{15} were determined in the usual manner. It was found that cortisone markedly increased urinary nitrogen excretion. Its greatest influence was on the adrenalectomized animals. Since the excretion of the isotope reflects the amount of nitrogen being used for protein synthesis, the amount of protein synthesized can readily be calculated. In the animals receiving cortisone there was a decrease in protein synthesis as compared to the control animals. The significance of these findings and the effect of cortisone on the size of the metabolic pool and on protein catabolism will be discussed.

Method for the estimation of 2-keto-hexonic acids

SEYMOUR S. COHEN AND MARY C. LANNING * *Dept. of Pediatrics, Children's Hospital of Philadelphia and the Dept. of Physiological Chemistry, Univ. of Pennsylvania School of Medicine, Philadelphia*

Various 2-keto acids condense with o-phenylene diamine to form quinoxalines. The spectra of the compounds formed by this reagent with pyruvic acid and with 2-ketogluconic acid, as an example, are quite similar, showing a marked absorption maximum at 330 $\text{m}\mu$. The formation of these compounds as a function of pH, temperature, concentration of reagent and duration of heating has been examined. Conditions have been established for the specific estimation of 2-keto-hexonic acids in microgram quantities, unaffected by the presence of relatively large amounts of gluconic and 5-ketogluconic acids. Dehydro-ascorbic acid interferes markedly in this estimation. The selected conditions minimize the slight interference by ribose. Quinoxaline formation enables the ready detection of 2-keto-hexonic acids, otherwise difficultly detectable, on paper chromatograms. The application of this reaction to the study of the enzymatic degradation of 6-phosphogluconic acid will be discussed.

Studies on the synthesis of creatine in homogenates of the liver STANLEY COHEN (introduced by F. CAJORI) *Depts. of Pediatrics and Biochemistry, Univ. of Colorado School of Medicine, Denver*

As part of a study of the synthesis of creatine from amino acids by homogenates of the liver of the adult and embryonic stages of development of various species, a source of error in the analytical procedure has been found. The 'creatine' synthesized by homogenates of beef liver from guanidoacetic acid and DL-methionine was found to be almost entirely an artefact due to the formation of α -keto- γ -methiolbutyric acid from D-methionine, which gives a marked color with alkaline picrate solutions. This interference may be avoided by the use of L-methionine and adsorption of the creatine formed on Lloyd's reagent prior to the determination. The survey of the ability of homogenates of the liver of various vertebrate animals to synthesize creatine from L-methionine and guanidoacetic acid has indicated that the liver of the guinea pig is the most efficient in this respect. The reaction requires Mg^{++} and is inhibited by Ca^{++} . The details of these experiments are presented.

Separation of adenosine polyphosphates by ion-exchange and paper chromatography WALDO E. COHN AND CHARLES E. CARTER * *Biology Division, Oak Ridge Natl. Lab., Oak Ridge, Tenn.*

Methods for the separation of mononucleotides by ion-exchange and paper chromatography, previously described, have been applied to the separation of AMP, ADP and ATP from their mixtures. A quantitative isolation of each of these three compounds from the others, as well as from adenine and adenosine if present, is achieved by successive elution from a strong-base anion-exchange column with 0.01 N-NH₄Cl (bases and ribosides), 0.01 N-HCl (AMP plus monophosphorylated nucleotides, if present), 0.01 N-HCl + 0.02 N NaCl (ADP) and 0.01 N-HCl + 0.2 N NaCl (ATP). Quantities of between 0.1 and 10 mg can be handled on a 1 cm \times 1 cm² column at flow rates of up to 3 ml/cm² min, using 100 ml volumes of these reagents after absorption of the mixture from ammoniacal solution. Paper chromatograms are developed with an isoamyl alcohol-disodium phosphate mixture and the components are detected by fluorescence as previously reported. Analyses of various commercial preparations of ADP and ATP show varying amounts of the compounds. The ion-exchange separation, accomplished within 3 hours, is suitable both for analysis and the preparation of pure material.

Absorption and distribution of C^{14} labeled fatty acids in the rat JOHN G. CONIGLIO, * CARL L. ANDERSON * AND C. S. ROBINSON *Dept. of Bio-*

chemistry, Vanderbilt Univ Med School, Nashville, Tenn

The deposition of fatty acids in the liver and depot fat of rats was studied 5 and 7 hours after feeding C^{14} -labeled fatty acids obtained by biosynthesis. Five young fasted rats were fed between 83 and 275 mg of the labeled fatty acids and killed 5 or 7 hours after feeding. The highest concentration of radioactivity was found in the liver, but a significant percentage was found in the mesentery lipids. No differences were observed in the distribution of the adsorbed C^{14} in the lipids of these tissues between the 5- and 7-hour experiments. In the liver the specific activity of the triglyceride fatty acids was always higher than that of the phospholipid fatty acids. The ratio between the two was constant and averaged 2.23. In 3 experiments the fatty acids associated with the esterified cholesterol had a higher specific activity than those of the neutral fat fraction. The 'free fatty acids' of the liver had a specific activity identical with that of the phospholipid fatty acids suggesting an origin in the autolytic decomposition of the phospholipids of the excised liver. In the mesentery the major part of the C^{14} of the total fatty acids was associated with the neutral fat fraction, but in the two experiments the specific activity of the phospholipid fatty acids was higher. The free fatty acids of the mesentery had a higher specific activity than any other fraction indicating a deposition of absorbed fatty acids or soaps as such. In 5 to 7 hours an average of 80% of the ingested fatty acids was absorbed and a small fraction oxidized.

Oxidation of triphosphopyridine nucleotide by plant enzymes ERIC E. CONN* AND BIRGIT YENNESLAND *Dept of Biochemistry, Univ of Chicago, Chicago, Ill*

Fractionation of the soluble proteins of wheat germ has led to the observation that this plant source contains a number of enzymes which act upon triphosphopyridine nucleotide (TPN) as a substrate. One protein fraction causes a destruction of the nucleotide. It hydrolyzes one phosphate group very rapidly from oxidized TPN but not from reduced TPN. Pyrophosphatase activity is also present. A separate protein fraction catalyzes the aerobic oxidation of reduced TPN. At least two different proteins are necessary for the oxidation. The reaction is inhibited by cyanide and by catalase. Accumulation of hydrogen peroxide cannot be demonstrated. Cytochrome c is not a component of the enzyme system. All active preparations contain high peroxidase and cytochrome peroxidase activity. Purified horseradish peroxidase alone cannot cause the oxidation of reduced TPN, either in the presence or the absence of added hydrogen peroxide. When horse-

radish peroxidase is added to the wheat germ enzyme, however, a stimulation of the rate of oxidation of reduced TPN is observed. Reduced diphosphopyridine nucleotide is oxidized about one-fourth as rapidly as reduced TPN. The oxidation of both substances appears to occur by a mechanism different in nature from that previously postulated for hydrogen transport from pyridine nucleotides to molecular oxygen.

Metabolic fate of the isopropyl group of leucine

MINOR J. COON (introduced by D. WRIGHT WILSON) *Dept of Physiological Chemistry, Univ of Pennsylvania School of Medicine, Philadelphia*

In liver tissue, the α and β carbons of leucine split off as a two-carbon unit which is capable of condensing to form acetoacetate. Experiments have recently been carried out to determine whether the isopropyl group of the amino acid also contributes to ketone body formation. Liver slices from fasted rats were incubated at 38° in a Ringer-phosphate buffer solution containing γ - C^{14} -leucine. The acetoacetate which was produced was degraded independently by permanganate and by Denigès' reagent, and it was found to be labeled chiefly in the carbonyl carbon. In similar experiments where β - C^{14} -isovaleric acid was the substrate, the resulting acetoacetate and β -hydroxybutyrate were each degraded by standard procedures to yield acetone and carbon dioxide. From the radioactivity of the products, it was calculated that in each acid the amount of C^{14} in the β carbon was approximately 20 times that in the carboxyl carbon. It is proposed that the isopropyl group of leucine (or isovalerate) yields a two-carbon intermediate which acts almost exclusively as an acetylating agent in forming acetoacetate. A similar hypothesis has been used by Crandall, Brady and Gurin in order to explain the behavior of the terminal fragment arising from octanoic acid.

Effect of niacin and tryptophane in counteracting toxicity of crystalline borrelidin for rats J. M. COOPERMAN,* S. H. RUBIN AND B. TABENKIN* *Depts of Nutrition and Microbiology, Hoffmann-La Roche Inc., Nutley, N. J.*

The production of pellagra like signs in animals fed toxic materials has been reported by several investigators. The role of corn in the production of niacin deficiency in the rat has been described by Krehl *et al.* Berger, Jampolsky and Goldberg described the isolation from broth of a *Streptomyces* species of a rather toxic crystalline antibiotic, borrelidin, a borrelidin-like material was also found in concentrated corn steep liquor. Experiments were designed to test the effectiveness of niacin and tryptophane in overcoming the toxicity of borrelidin in the rat. The basal ration contained 20% casein, 71% sucrose, 4% salts IV,

3% cod liver oil and 2% corn oil. Vitamins were supplied in large excess (5-50 times known minimum requirements). Rats were given 10 mcg borrelidin per day orally and their growth compared to a control group receiving no borrelidin and a group of rats receiving 1 mg niacin in addition to 10 mcg borrelidin per day. At the end of 4 weeks, the average gain per week in grams was control group 31, borrelidin group 11, borrelidin and niacin 22. In another experiment designed to test the effect of tryptophane in overcoming the toxicity, the borrelidin, niacin and tryptophane supplements were added directly to the basal ration. The average gain per week in grams at the end of 4 weeks was control group 35, group receiving 1 mg borrelidin/kg of ration 14, group receiving 1 mg borrelidin and 500 mg niacin/kg of ration 20, and group receiving 1 mg borrelidin and 5 grams DL-tryptophane/kg of ration 18.

Amylo-1,6-glucosidase GERTY T. CORI AND JOSEPH LARNER* *Dept of Biological Chemistry, Washington Univ School of Medicine, St Louis, Mo*

Phosphorolysis of branched polysaccharides by plant or animal phosphorylase stops when the enzyme approaches a branch point where one glucose residue is connected instead of with two, with three others, one of which is in 1,6-linkage. Thus only 40% of glycogen undergoes phosphorolysis and a limit dextrin is left. When a protein fraction, obtained from muscle, is added to phosphorylase, the entire glycogen molecule is digested. This fraction contains an enzyme for which the name amylo-1,6-glucosidase is proposed. A solution containing polysaccharide, phosphate, muscle phosphorylase and glucosidase was incubated (10 min) until the glycogen had disappeared. Protein, phosphorylated sugar and salt were then removed from the solution and paper chromatography for reducing sugars carried out on it. A single spot was obtained which was identified as glucose. Quantitative determinations of glucose and phosphorylated sugar revealed that the ratio, glucose/phosphorylated sugar, varied in proportion with the degree of branching of the polysaccharide used. Lowest values were obtained with amylopectins, glycogen was intermediate and limit dextrin gave the highest values. It is concluded that the glucosidase acts by hydrolyzing the 1,6-linkage in amylo polysaccharides once this linkage has been exposed by preceding phosphorylase action. Six glucose residues are left distal to each branch point after exhaustive phosphorylase action. In order to explain the formation of glucose one must assume that these residues are unevenly distributed, one branch having one and the other 5 glucose residues. The present findings furnish an explanation for the

observation that small amounts of glucose are formed in muscle during rapid breakdown of glycogen.

Subacute toxicity of radioactive phosphorus as related to the composition of the diet W. E. CORNATZER,* GEORGE T. HARRELL, JR.,* DAVID CAYER* AND CAMILLO ARTOM *Depts of Biochemistry and of Internal Medicine, Bowman Gray School of Medicine, Winston-Salem, N. C.*

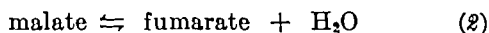
Mice were maintained on various diets for 5 days before and 21 days after the injection of a single dose of P^{32} as Na_2HPO_4 . The percentage survival, the time at which 50% deaths occurred and the average survival time were recorded. Neither the fatty infiltration of the liver (produced by a low protein, high fat diet or by poisoning with CCl_4), nor its prevention by supplementing the diet with choline affected appreciably the toxicity. Susceptibility to radiation injury by P^{32} was lowest in mice on a 10% casein, 5% fat diet, and increased distinctly when the protein, or the fat level, or both, were increased. It is suggested that these findings may be related to the specific dynamic action of dietary protein and fat. Increasing the phosphate content of the diet seemed to protect the animals, to a certain extent, presumably as the result of an increased exchange of the P^{32} deposited in the bones.

Sulfur metabolism of *E. coli* D. B. COWIE AND M. SANDS (introduced by HELEN M. DYER) *Carnegie Inst of Washington, Dept of Terrestrial Magnetism, Washington, D. C.*

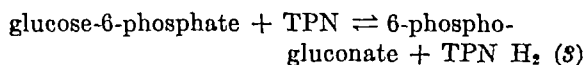
The utilization of sulfur by *E. coli* has been investigated through studies of the multiplication of cells, uptake of S^{35} by growing cells, and the biosynthesis of compounds containing S^{35} . The growth of cultures of *E. coli* is found to depend upon sulfur concentration for low levels of sulfur incorporated into media as sulfate, sulfite or sulfide, while for higher concentrations growth is independent of sulfate level and is inhibited by sulfite and sulfide. When 'water-space' sulfur is accounted for, the uptake of S^{35} may be quantitatively described by a Langmuir adsorption isotherm. Furthermore, it is demonstrated that, in the presence of adequate amounts of sulfur, S^{35} uptake is directly proportional to the number of new cells formed in a growing culture, indicating a sulfur composition of 3 to 4 mg sulfur/ml of bacterial cells. Methionine containing S^{35} is identified as one of the products of sulfur metabolism while the presence of other S^{35} compounds is indicated.

Fixation of carbon dioxide by mammalian tissues and its relation to carbohydrate metabolism ROBERT K. CRANE* AND ERIC G. BALL *Dept of Biological Chemistry, Harvard Med School, Boston, Mass.*

Studies on CO_2 fixation were carried out by incubating tissue with pyruvate in the presence of C^{14}O_2 . The residual pyruvate at the end of the experiment was isolated and purified as the 2,4-dinitrophenylhydrazone and the radioactivity of this derivative was assayed. Intact ox retina and rat diaphragm show high rates of CO_2 fixation. In the case of ox retina, the formation of radioactive pyruvate would appear to involve the following reversible reactions



The energy requirement is apparently met by coupling of reaction (1) with reaction (3)



Evidence for this is furnished by the following observations: 1) Anaerobically the rate of incorporation progressively decreases with time and is virtually zero at the end of two hours. The glycogen content of ox retina is found to average only $0.92 \mu\text{g}/\text{mg}$ wet weight. The addition of glucose anaerobically maintains the rate constant for at least 4 hours and at a level equal to the aerobic rate. 2) Neither arsenate which inhibits the production of ATP by the triosephosphate dehydrogenase reaction nor fluoride which inhibits the enolase reaction has any large inhibitory effect on anaerobic CO_2 incorporation. 3) Iodoacetate in concentrations sufficient to inhibit glycolysis by 95% actually increases the anaerobic rate by 75%.

Influence of dietary protein FRANK A. CSONKA

Bureau of Human Nutrition and Home Economics, Agricultural Research Admin., USDA, Washington, D. C.

The nitrogen, methionine and cystine contents of the hen egg will be discussed in reference to their distribution in the egg white and yolk as affected by 1) aging of the hen and 2) by the feed. Eggs of pullets in their first-year laying season were analysed. As pullets, fed a low protein diet, aged, nitrogen decreased in the egg white and increased in the yolk, with pullets fed a high protein diet the nitrogen increased in both. The quantities of sulfur amino acids in the white and yolk of their eggs generally paralleled the changes in nitrogen values. However, the cystine/methionine ratio in the egg white decreases with aging of the pullet regardless of the diets employed here. An increased ratio in the yolk resulting from aging was found to be statistically significant only during the low protein dietary regimen. This increased ratio in the yolk, characteristic for eggs laid by older pullets, may be duplicated in younger pullets' eggs by feeding them a high protein diet. Enzymatically digested casein in the hen's feed increased the cystine and methionine content of the

egg to the same extent as did casein. Previously we reported that free methionine supplementation to the hen's feed did not alter the egg sulfur amino acid content. The experiment with predigested casein indicates the importance in protein synthesis of the availability and simultaneous absorption of all the amino acids required.

Effect of high pressures on activity of proteinases

A. LAURENCE CURL* AND EUGENE F. JANSEN
Enzyme Research Division, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Admin., USDA, Albany, Calif.

The effect of high pressures on the activation of chymotrypsinogen by trypsin has been studied in connection with a general investigation of the effect of high pressures on enzymes, in particular crystalline proteinases. Chymotrypsinogen was not activated by pressure in the absence of trypsin. No significant change in the extent of activation by trypsin was found under pressures up to 700 bars. About 10% less activation than in an unpressed system was observed under a pressure of 1000 bars. The inhibition of the reaction increased with increase in pressure and practically no reaction occurred at 3000 bars. Up to this pressure the activation proceeded after release of the pressure at practically the same rate as initially in the control. Following release of systems at higher pressures than this, progressively less activity was obtained, until after pressing at 5700 bars no active enzyme was formed. The effect on the mixture of chymotrypsinogen and trypsin was much greater than on either protein alone.

Proteins of cellular nuclei R. DUNCAN DALLAM, WILLIAM R. KIRKHAM, TUNG-YUE WANG, LLOYD E. THOMAS AND DENNIS T. MAYER (introduced by SAMUEL BRODY) *Depts. of Biochemistry and Agricultural Chemistry, Univ. of Missouri, Columbia*

It was previously reported that boar spermatozoa contain alkali-soluble protein fractions which are precipitable by acid. Mechanical separation of the heads of these cells from the tails and midpieces has shown that all such proteins are present in the heads. By extracting the cells first with 0.1 N sodium hydroxide there is obtained only one protein fraction which precipitates with acetic acid at about pH 6. Subsequent extraction with 1.0 N sodium hydroxide yields a small fraction precipitating with acetic acid at about pH 10. Neither of these fractions contains appreciable quantities of nucleic acid. The residue is composed entirely of 'ghost' heads which are rich in arginine and nucleic acid. Rat liver nuclei isolated by the Dounce procedure and calf thymus 'chromosomes' isolated by the method of Mirsky and Ris contain an alkali-soluble protein fraction which precipitates at about pH 6. After extraction

of the nucleohistone and the 'pH 6 fraction' from the liver nuclei or thymus 'chromosomes' only a small residue remains, the chemical nature of which remains to be determined

Effect of exclusion of oxygen from the hydrolysis by Blau's method upon detection of thyroxine from thyroglobulin MARSHALL E. DEUTSCH (introduced by ISIDOR GREENWALD) *Dept of Chemistry, New York Univ College of Medicine, New York City*

Beef and sheep thyroglobulin containing, respectively, 0.821 and 0.784% iodine were hydrolyzed by Blau's method (*J Biol Chem* 110:351, 1935) with the exception that the hydrolyses were performed in tubes which had been sealed immediately after the air in them had been displaced by nitrogen. Under these conditions, the author could not identify thyroxine in the 'thyroxine' fraction. When the hydrolyses were performed in sealed tubes in the presence of added thyroxine equivalent to 0.237% by weight (sheep thyroglobulin) or under a reflux condenser (beef thyroglobulin), the presence of thyroxine was demonstrable. In hydrolysates of commercial egg albumin, 0.090 but not 0.045% thyroxine (added before hydrolysis) was easily detectable. The method used was to concentrate and chromatograph the material in the butanol extracts. Thyroxine and diiodotyrosine were identified by the R_f of substances giving the following reactions: a) quenching of the fluorescence of the paper under ultra-violet light, b) the colors appearing upon spraying with ninhydrin a longitudinal strip cut from the paper, c) the colors appearing on a strip sprayed with Millon's reagent, d) the colors appearing upon spraying with 1) acidulated starch-KIO₃ solution, 2) NaNO₂ solution and 3) NH₃ solution in that order.

Preliminary characterization of two biotin-containing fractions in beef liver DANIEL F. DICKEL,* A. E. AXELROD* AND KLAUS HOFMANN *Dept of Chemistry, Univ of Pittsburgh, Pittsburgh, Pa*

A large fraction of the biotin in animal tissues has been assumed to be firmly bound to the tissues and treatments known to destroy proteins have been used routinely to liberate the vitamin from these materials for microbiological assay. The present study was directed towards the isolation and characterization of biotin protein complexes from animal tissues, since it seems likely that they represent the enzymatically active forms of biotin. Beef liver was dehydrated with acetone and the resulting acetone liver powder extracted under mild conditions. The resulting extracts were assayed by the yeast growth method for their biotin content before and after hydrolysis with acid. It was found that beef liver contains at least

two distinct biotin-containing fractions. One of these is extractable with a buffer at pH 3 and loses its biotin activity on dialysis; the other is soluble in dilute sodium hydroxide and is not affected by dialysis. The pH 3 soluble substance has practically the same biotin activity before and after acid hydrolysis, in contrast to the alkali soluble compound which has very little activity initially but becomes highly active following hydrolysis with sulfuric acid. In accordance with the accepted term *flavoprotein* we propose that the term *biotoprotein* be applied to biotin-containing proteins as they occur in tissues. It is assumed that the sodium hydroxide soluble material represents such a *biotoprotein*. The possible enzymatic functions of this material are under investigation.

Studies on aconitase SHERMAN R. DICKMAN AND A. A. CLOUTIER (introduced by EMIL L. SMITH) *Dept of Biological Chemistry, University of Utah College of Medicine, Salt Lake City*

Aqueous extracts of horse heart exhibiting aconitase activity have been prepared almost free of the red pigment or pigments which usually accompany the enzyme. The pigmented material is extracted first by blending ground, frozen heart with dilute ethyl alcohol, followed by centrifugation. The supernatant solution is highly colored and contains little aconitase activity. The residue is immediately blended with water or phosphate buffer and chloroform and the emulsion centrifuged. The clear, pale pink supernatant solution exhibits appreciable aconitase activity. This activity can be augmented considerably after overnight incubation with Fe⁺⁺ and cysteine. Additional pigment can be subsequently removed from such solutions by fractionation with ammonium sulfate. The activating and stabilizing effect of ferrous ion (DICKMAN AND CLOUTIER, *Arch Biochem* In press) on crude aconitase preparations has been extended to the ammonium sulfate fractions. Many solutions which exhibit very low aconitase activity have been found to develop appreciable activity after incubation with ferrous ammonium sulfate. This activation occurs relatively slowly and often the solution does not exhibit maximum aconitase activity until after one to two days' incubation.

Neutral mucopolysaccharides from various animal tissues ZACHARIAS DISCHE AND MARTHA OSNOS* *Dept of Biochemistry, Columbia Univ College of Physicians and Surgeons, New York City*

Fractions of neutral mucopolysaccharides were prepared from brain, kidney, muscle, heart and pancreas of rat, mouse kidney, sarcoma 180 and human serum. Nucleic acids were removed from homogenized tissues by 4S^b extraction with 5% TCA at room temperature, lipids by ethanol-

ether extraction The residue, dissolved in $\frac{N}{2}$ NaOH, was precipitated with 90% ethanol, the precipitate hydrolyzed in 5 N KOH for 3^h at 100°. One polysaccharide fraction was obtained by precipitating the hydrolysate with 2, a second with 9, volumes of ethanol (fraction II) True sugars in fraction II were identified and quantitatively determined by the spectrophotometric absorption of products of their reactions with cysteine in H₂SO₄, by the characteristic speed of destruction of these products by water (*J Biol Chem* 175 595, 1948, 181 379, 1949, *Arch of Biochem* 22 169, 1949), and by fermentation Hexosamine was determined by a new reaction with indole and HCl Fraction II from 10 g brain, kidney and serum contained, 3,5 5 mg of carbohydrate, consisting of hexosamine, D-mannose, galactose and methylpentose Approximate mannose galactose ratios for brain, kidney, serum were 7,2, 1 respectively Methylpentose galactose varied in the same range as in purified blood group substances from various sources The amount of hexosamine was roughly half of the sum of mannose plus galactose Smaller amounts of the same sugars were found in fraction II of the other tissues These findings suggest the presence in most animal tissues of protein bound polysaccharides of the type found in serum and blood group substances

Effect of vitamin B₁₂ on the reduction of S-S compounds in vitro JACOB W DUBNOFF *California Inst of Technology, Pasadena*

Addition of vitamin B₁₂ concentrates to liver slices and homogenates increases the reduction of S-S compounds by preformed and added hydrogen donors The specificity of hydrogen donors and the relation of this effect to methionine synthesis from homocystine will be discussed

Synthesis of peptides containing amino acid antagonists FLOYD W DUNN AND KARL DITTMER (introduced by V DU VIGNEAUD) *Depts of Chemistry of Univ of Colorado, Boulder, and Florida State Univ, Tallahassee*

The following peptides have been prepared by the Bergmann carbobenzoxy method carbobenzoxyglycyl- β -2-thienylalanine (I), carbobenzoxyglycyl- β -3-thienylalanine (II), carbobenzoxyglycyl- β -2-thienylalanylglycine (III), carbobenzoxyglycyl-*para*-methylphenylalanine (IV), carbobenzoxyglycyl- β -1-naphthylalanine (V), and carbobenzoxyglycyl- β -2-naphthylalanine (VI) The sulfur-containing carbobenzoxy peptides were reduced with phosphonium iodide to obtain glycyl- β -2-thienylalanine (VII), glycyl- β 3-thienylalanine (VIII), and β -2-thienylalanylglycine (IX), glycyl-*para*-methylphenylalanine (X) was prepared by the usual catalytic method of hydrogenation of IV

Compounds VII, VIII and IX inhibit the growth of *Escherichia coli*, 50% inhibition being produced by 2 2 γ , 1 15 γ , and 1 25 γ , respectively, this compares with 50% inhibition obtained by 1 05 γ of free β -2-thienylalanine or by 0 6 γ of β -3-thienylalanine The growth inhibition caused by the peptides containing amino acid antagonists can be reversed by phenylalanine or *para*-methylphenylalanine, 50% reversals being obtained with 1 4 γ and 1 6 γ , respectively β -2-Thienylalanine inhibition can be reversed by phenylalanylglycine, glycylphenylalanine or glycyl-*para*-methylphenylalanine, with 50% reversals occurring at the following levels, respectively 1 9 γ , 2 3 γ and 5 4 γ When yeast is used as the test organism the peptides are much less effective than the corresponding free amino acids either as inhibitors or as reversers of inhibition The enzyme carboxypeptidase splits compounds I, II, IV, V and VI

Two-dimensional ionophoresis and electrophoresis E L DURRUM (introduced by RAY G DAGGS) *Med Dept Field Research Lab, Fort Knox, Ky*

A technique for the separation of amino acids, peptides and proteins wherein an electrical potential is applied across the ends of filter paper strips saturated with electrolyte solutions, to which have been applied at narrowly circumscribed intermediate areas the mixtures to be separated, has been extended in a manner analogous to two-dimensional paper chromatography The method appears to be widely applicable and to be much more rapid than conventional paper chromatography The technique will be described together with examples of types of separations attainable

Microbiological response to amino acid derivatives CHARLES H EADES, JR (introduced by T P NASH, JR) *Dept of Chemistry, School of Biological Sciences, Univ of Tennessee, Memphis*

The acetyl, chloroacetyl, and acetyldehydro derivatives and the keto analogs of leucine, phenylalanine, tryptophan, and valine were investigated with regard to their growth effects on 3 commonly used microorganisms, *Lactobacillus arabinosus*, *Lactobacillus casei*, and *Leuconostoc mesenteroides* The results indicate that the acetyl and chloroacetyl derivatives of DL-leucine were effective in replacing the amino acid for the growth of *L arabinosus* DL-acetyl and DL-chloroacetyl tryptophan were used in place of DL-tryptophan by *L casei* The keto analogs of leucine and valine were used by both *L arabinosus* and *L casei* to the same extent as the DL amino acids were used The acetyldehydro derivatives of the amino acids investigated were all ineffectual in supporting the

Comparison of lymphoid tumor and muscle electrolyte composition in patients treated with ACTH and cortisone acetate LEONARD P. ELIEL, OLOF H. PEARSON, BERNICE KATZ AND FRANCES W. KRAINTZ (introduced by KONRAD DOBRINER) *Division of Clinical Investigation, Sloan Kettering Inst., New York City*

Marked, progressive decrease in the size of enlarged lymph nodes and of enlarged spleens has been demonstrated in patients with chronic lymphatic leukemia and follicular lymphosarcoma who were given ACTH or cortisone acetate (*Cancer* 2:943, 1949). During treatment these patients exhibited phosphorus excretions (corrected for calcium) which were approximately twice that expected from the nitrogen loss, based on the usually accepted P/N ratio in normal protoplasm. Analyses of dry, blood-free, fat-free tissues from 4 of these patients revealed phosphorus concentrations which, per unit of nitrogen, were 3 times higher in tumors and lymphocytes than in muscle. It is believed, therefore, that the high phosphorus excretion indicates tumor destruction resulting from ACTH and cortisone administration. The ratio of P to N excreted lies midway between the ratio found in tumor tissue and that in muscle, providing evidence that both normal tissue and tumor were being destroyed. No significant changes in tissue P or N concentration were evident after therapy. The $K(mEq)/N(gm)$ ratio in untreated tumor tissue was 4.0-4.4, in muscle 2.2-3.0. Two patients demonstrated slight falls (15, 25.5 mEq/kg) in muscle potassium and large falls (194.4, 79.7 mEq/kg) in tumor potassium under therapy. The K/N ratios in the tumors fell to 3.0 and 3.7. A third patient showed no change. The significance of these findings with respect to growth and destruction of tumor tissue will be discussed.

Inhibitory effects of 2,6-diaminopurine on growth of *Lactobacillus casei* GERTRUDE B. ELION* AND GEORGE H. HITCHES *Wellcome Research Labs., Tuckahoe, N. Y.*

2,6-Diaminopurine strongly inhibits the growth of *Lactobacillus casei* in a medium containing folic acid and no purines. This antagonism of folic acid is not competitive and is only partially reversed by increasing the concentration of folic acid. At low levels of diaminopurine, reversal of the inhibition may be obtained with adenine, guanine, xanthine or hypoxanthine, whereas at high levels of the inhibitor, only adenine is effective, reversal by adenine takes place over a wide range of concentration of the inhibitor. When *Lactobacillus casei* is grown in a medium containing thymine and a purine, in place of the folic acid medium, diaminopurine appears to be less inhibitory. However, this may be due to the fact that inhibition can be

demonstrated only at suboptimal levels of adenine where growth is small. Nevertheless, the relatively greater effectiveness of adenine as a reversing agent as compared with guanine can be shown also with the thymine grown organism.

Factors affecting acetylcholine (ACh) found in rat brain K. A. C. ELLIOTT AND NORA HENDERSON* *Montreal Neurological Inst., McGill Univ., Canada*

The total ACh content of excised whole brain falls with time. Dropping the tissue into liquid air causes considerable decrease in the ACh content, apparently because sudden cold releases free ACh from the bound form and free ACh is destroyed by cholinesterase before freezing is complete. Free ACh is liberated in large amount when an eserine-containing brain suspension is poured into liquid air. The total ACh content of brain from eserinizated animals is not lowered by freezing in liquid air. Eserine administration causes a large increase, similar to that produced by anesthetics (reported in another communication), in the total ACh found in brain extracted by acid medium 3 minutes after decapitation. The bound ACh content of whole brain slices rises rapidly on incubation in Ringer-bicarbonate glucose medium to a much higher value than is found in brain extracted soon after excision. It seems that the value found for incubated slices may represent the maximal potential ACh content of the tissue. When total and free ACh are determined on halves of the same brain, by homogenizing initially in acid and neutral eserine-containing medium respectively, the free ACh found is a somewhat variable fraction, averaging 33% of the total but is not significantly dependent upon whether the total is 'normal' or elevated by Nembutal or eserine administration. Since, in the absence of eserine, brain very rapidly destroys added free ACh it is probable that the free ACh found is liberated in the process of homogenization.

Mechanism of fluoroacetate inhibition WILLARD B. ELLIOTT* AND GEORGE KALNITSKY *Dept. of Biochemistry, State Univ. of Iowa College of Medicine, Iowa City*

Citrate oxidation by a washed kidney cortex preparation was inhibited by fluoroacetate under conditions similar to those necessary for the formation of citrate from acetate. Previous workers (KALNITSKY AND BARRON *Arch. Biochem.* 19:75, 1948; KALNITSKY *Arch. Biochem.* 17:403, 1948) have shown slight inhibition of citrate in the presence of fluoroacetate. However, when the tissue preparation was incubated with 0.003 M fluoroacetate (9 μM) plus 5 μM oxalacetate for 15 minutes prior to the addition of citrate, 93% inhibition of citrate oxidation was observed. Under

the same conditions, the oxidation of *cis*-aconitate and isocitrate were not inhibited. The inhibition of citrate oxidation by fluoroacetate was shown to be competitive. The citrate formed in the presence of fluoroacetate and oxalacetate was isolated as the calcium salt after adding an excess of normal citrate as carrier. Fluoride was identified in the citrate formed, and in the pentabromacetone derived from the citrate. The inhibition of citrate oxidation and acetate oxidation is of the same magnitude. It is believed that the small amounts of fluorocitrate formed interfere with the conversion of citrate to *cis*-aconitate. The observed inhibition of citrate oxidation could account for the inhibition of acetate oxidation via the tricarboxylic acid cycle and the accumulation of citrate in systems poisoned with fluoroacetate, as observed by other workers.

Role of serine and acetate in uric acid formation

DAVID ELWYN* AND DAVID B. SPRINSON, *Dept. of Biochemistry, Columbia Univ., New York City*

L- and D-serine labeled with C^{14} in the β carbon atom and N^{15} in the amino group, and carboxyl labeled acetate were administered to pigeons. Uric acid, isolated from the excreta, was degraded and the isotope content in each position determined. It was found that the β carbon atom of L-serine is utilized for the ureide carbon atoms of uric acid to about the same extent as is the nitrogen for position 7. The incorporation of the β carbon into the ureide positions is about the same as that of formate (SONNE, BUCHANAN AND DELUVA *J. Biol. Chem.* 173: 69, 1948; KARLSSON AND BARKER *J. Biol. Chem.* 177: 597, 1949). The utilization of serine nitrogen for position 7 is close to that of the α carbon atom of glycine for position 5 (KARLSSON AND BARKER, *ibid.*). A limited incorporation of C^{14} into position 5 took place with either L- or D-serine. This may be explained by conversion of serine to pyruvate (CHARGAFF AND SPRINSON *J. Biol. Chem.* 148: 249, 1943), randomization of the isotope via the Krebs cycle and subsequent conversion through serine to glycine. This is in accord with the finding that β -labeled serine is converted rapidly to acetate in the rat. The reported utilization of the carboxyl carbon of acetate for the formation of the ureide carbon atoms of uric acid could not be confirmed. The significance of these findings to the metabolism of serine, glycine and formate, and the biosynthesis of uric acid will be discussed.

In vivo and in vitro studies with oxythiamine and neopyrithiamine. ALBERT J. EUSEBI* AND LEOPOLD R. CERECEDO, *Dept. of Biochemistry, Fordham Univ., New York City*

In mice maintained on one γ of thiamine/day, oxythiamine and neopyrithiamine have been

found to be potent antagonists of this vitamin. At molar concentrations of thiamine/oxythiamine of 1/25 mice succumb after 3-4 weeks, without manifesting the typical symptoms of polyneuritis. Neopyrithiamine was found to be more toxic, and produced typical thiamine deficiency signs at molar concentrations of thiamine/neopyrithiamine of 1/5. At this level, mice succumb after approximately 9 days. In studies on the mechanism of oxythiamine inhibition, the necessity of the alcoholic hydroxyl group on the thiazole moiety for inhibition was demonstrated in mice in which bromoxythiamine was injected at high levels. At a concentration 20 times greater than that at which oxythiamine was found to be toxic, no harmful effect of the bromoxythiamine was observed. The synthesis of oxythiamine-diphosphate afforded an excellent means of studying the mechanism of carboxylase action. With the aid of a purified enzyme system from brewer's yeast, it was found that 262 γ of oxythiamine-diphosphate produced a 77% inhibition of pyruvate decarboxylation in the presence of 4 γ of cocarboxylase. Oxythiamine-monophosphate and neopyrithiamine were inactive in this system. Further experiments showed that oxythiamine also inhibited the phosphorylation of thiamine, thereby preventing the synthesis of cocarboxylase. A similar inhibition of cocarboxylase formation by *P. pentosaceum* was observed in the presence of oxythiamine.

Analysis of the ribonucleic acid of the large cytoplasmic granules (mitochondrial fraction) of beef pancreas. JAMES L. FAIRLEY, JR.,* HARRY L. SEAGRAN* AND HUBERT S. LORING, *Dept. of Chemistry and Stanford Univ. School of Medicine, Stanford University, Calif.*

Beef pancreas was homogenized with 0.88 M sucrose in a Waring Blendor, and the large granule fraction isolated by differential centrifugation. Acid-soluble substances and lipids were removed by extraction with ice-cold, trichloroacetic acid, alcohol and hot 3:1 alcohol-ether. The ribonucleic acid was converted to monoribonucleotides with alkali, and the acid-insoluble constituents removed by precipitation with trichloroacetic acid. Acid hydrolysis of the ribonucleotide fraction and subsequent treatment with silver sulfate were employed to separate the purine bases from the pyrimidine nucleotides. After removal of silver ion from the pyrimidine fraction, cytidylic and uridylic acids were determined from UV absorption data. The purine bases were extracted from the silver-purine precipitate with 0.1 N HCl, and the concentration of guanine and adenine determined from the UV absorption of the resulting extracts. Total purine was also determined by Kjeldahl nitrogen and guanine by the colorimetric

method of Hitchings. Analyses performed in this way on several different preparations of mitochondria from beef pancreas yielded consistent values. The molar ratio of guanine to adenine averaged 1.6 as compared to values of 3 or 4 to 1 as reported previously for nucleic acid preparations from whole pancreas. The molar ratio of cytidylic acid to uridylic acid averaged about 1.3 as compared to values of 1.4 to 3.3 as reported by Kerr *et al* [*J Biol Chem* 181: 773, 1949] for different ribonucleic acid fractions obtained from beef pancreas. The present results suggest relatively large differences in the chemical composition of the various ribonucleic acid components of pancreas.

A phosphorylase in skeletal muscle which synthesizes glycogen directly IRWIN FEIGIN, JEROME FREDRICK AND ABNER WOLF (introduced by HANS T. CLARKE) *Veterans Administration Hospital, Bronx, Depts of Pathology and Neurology, Columbia Univ, and Neurological Inst, New York City*

Holophosphorylase, a phosphorylating enzyme which differs from the muscle phosphorylase previously described, has been crystallized from rabbit skeletal muscle. This enzyme synthesizes a polysaccharide which is soluble in water, is precipitated from solution by alcohol, does not reduce copper reagents until after acid hydrolysis, gives a red-brown color with iodine, and serves to prime and activate the phosphorylase reaction. In these properties, the polysaccharide resembles glycogen, rather than the amylose synthesized by muscle phosphorylase. Holophosphorylase differs also in that it is slightly activated by 0.0024 M phlorizin, while muscle phosphorylase is inhibited. These properties also characterize the enzyme in muscle homogenates. The crystals in which holophosphorylase activity is present, are hexagonal in shape, in contrast to the acicular crystals of muscle phosphorylase. It is suggested that holophosphorylase is the form of enzyme normally present in muscle, and that its molecular structure is altered in the extracting and concentrating procedures used to crystallize muscle phosphorylase. The possibility exists that similar alterations of molecular structure may occur in the concentration and crystallization of other enzymes.

Site of the influence of biotin on citrulline synthesis GLADYS FELDOTT,* PATRICIA MACLEOD* AND HENRY A. LARDY *Dept of Biochemistry, College of Agriculture, Univ of Wisconsin, Madison*

Previous studies have shown that biotin deficient animals fix less $C^{14}O_2$ into their tissue arginine, aspartic acid, guanine and citric acid than do normal rats. The washed residues of liver homogenate from biotin deficient rats synthesize citrulline from ornithine only one third to one half as fast as the same type of preparation from normal rats (*J Biol Chem* 180: 1003, 1949). Cohen and Grisolia have shown that the glutamic acid which is required for citrulline synthesis apparently functions by being converted to carbamyl glutamic acid. To trace further the specific site at which biotin influences the overall fixation of CO_2 into arginine, a study has been made of the reactions involving carbamyl glutamic acid. In the presence of glutamate, the average rate of citrulline synthesis from ornithine by washed liver residues from 14 biotin deficient rats was $0.24 \mu M/mg$ N. The corresponding rate for the pair-fed control animals was $1.38 \mu M/mg$ N which was not significantly different from that of stock rats ($1.28 \mu M/mg$ N). In the presence of carbamyl glutamate, the same liver preparations synthesized 1.34, 1.35 and 1.61 μM of citrulline/mg N respectively. It would appear that the limiting reaction in biotin deficient tissue is one involving the formation of carbamyl glutamic acid since subsequent reactions in the synthesis of citrulline proceed at the normal rate. *In vitro* additions of 'biocytin' and of another partially purified 'bound-biotin' preparation were ineffective in stimulating citrulline synthesis by liver tissue from biotin deficient rats.

Electrophoretic studies of an in vitro reaction between adenosine and rat serum MARIE A. FISCHER (introduced by W. S. McELROY) *Dept of Physiological Chemistry, Univ of Pittsburgh School of Medicine, Pittsburgh, Pa*

Incubation of adenosine in rat serum in a nitrogen atmosphere resulted in a decrease in the electrophoretic albumin area and an increase in the α_1 -globulin area of the serum. There was essentially no change in the total electrophoretic area or in the combined area of the albumin and α_1 fractions, however, the α_1 component had a slower mobility than the α_1 -globulin peak of control serum. Maximum reaction was obtained in 5 hours of incubation when 4 mg of adenosine was dissolved in 5 ml of serum. Larger amounts of adenosine did not increase the extent of the reaction. Practically no electrophoretic change occurred when the incubation was carried out under an atmosphere of 95% oxygen and 5% carbon dioxide. α -ketoglutarate reacted spontaneously with adenosine and thus prevented the reaction from taking place. Neither glutamate nor glutamine caused any inhibition but complete inhibition was obtained with glutathione. A similar change took place when ATP or adenylic acid were incubated with rat serum, however, adenine, hypoxanthine and xanthine were inactive.

Reaction of chymotrypsin with diisopropylfluorophosphate and with tetraethylpyrophosphate
J H FLEISHER,* B J JANDORF, W H SUMMERSON AND D P NORTON * *Biochemistry Section, Medical Division, Army Chemical Center, Md*

We have confirmed the findings of Jansen *et al* (*Federation Proc* 8 210, 1949) that the reaction between the enzyme chymotrypsin and diisopropylfluorophosphate (DFP) produces a crystallizable, enzymatically inert protein, containing phosphorus and isopropoxy groups, in the approximate proportion of two of the latter to one of the former, and their verbal report that treatment of this protein with periodate yields significantly less acetaldehyde than is obtained from native chymotrypsin by the same procedure. Similar results are obtained when chymotrypsin reacts with tetraethylpyrophosphate (TEPP), the enzymatically inactive protein yields less acetaldehyde after periodate treatment, and contains 2 ethoxy groups/m of bound phosphorus. Furthermore, the decrease in yield of acetaldehyde, and the amount of phosphorus and alkoxy groups bound/unit weight of enzyme, are the same when either DFP or TEPP reacts with chymotrypsin. This supports the view expressed previously (JANDORF *et al Abstracts*, Sept 1949 meeting of the American Chemical Society) based in part on manometric measurement of the rate and extent of reaction between chymotrypsin and both DFP and TEPP, that these 2 substances react with the enzyme in fundamentally the same manner, differing chiefly in rate, the combination with the enzyme in the case of DFP involving the splitting of the P-F bond whereas for TEPP the P-O-P bond is split, liberating one equivalent of acid in each instance.

Differential heat, alkali and trypsin inactivation of pancreatic lipase PAUL J FODOR (introduced by HAROLD P MORRIS) *Natl Cancer Inst, Natl Insts of Health, Bethesda, Md*

A fractionation of the pancreatic extract could be made whereby one fraction which was relatively thermostable and which acted upon the glycerides and another which acted upon the monovalent alcohol and glycol esters could be distinguished. Treatment of fractions of pancreas homogenates at various degrees of alkalinity resulted in a separation of the activities toward the glycerides on the one hand, and toward the monovalent alcohol and glycol esters on the other. Incubation of pancreas fractions with crystalline trypsin reduced the rate of enzymatic hydrolysis of the monovalent alcohol esters, the glycol ester and triacetin nearly to zero, whereas the extent of the hydrolysis of all the other glycerides was reduced to no more than 50%. This procedure was employed to isolate from pancreas homogenates a

protein exhibiting activity almost exclusively toward these glycerides. The coexistence in pancreatic extracts of at least 2 ester hydrolyzing enzymes is therefore suggested.

Brain proteolipides, a new group of protein-lipide substances soluble in organic solvents and insoluble in water J FOLCH AND MARJORIE LEES * *McLean Hospital, Waverley, Mass and Harvard Med School, Boston, Mass*

Brain and brain tumors contain compounds of a new type to which the name of proteolipides has been given. They are substances having as constituents a protein moiety and a lipide moiety combined in such a form that the resulting compounds are soluble in chloroform and/or chloroform-methyl alcohol mixtures and insoluble in water. Proteolipides are extracted quantitatively from the tissue by chloroform-methyl alcohol mixtures. From white matter, 3 different ones have been obtained (A, B, and C). A and B are soluble in chloroform-methyl alcohol mixtures and have the property of concentrating at chloroform-water interphases. C is soluble in chloroform. B is a crystalline compound which consists of 55% protein and 45% lipide. A and C are powders and do not appear yet to be pure compounds. The protein moiety from each one of them can be separated as follows. The proteolipide is dissolved in a chloroform-methyl alcohol mixture and 5% water added to the solution. The solution is taken to dryness. This destroys the bond between the constituent lipides and protein. The lipides are extracted from the residue with chloroform. The insoluble remnant is a denatured protein material which contains, in all cases, 14% N and 1.76% S. 91% of the N can be identified as amino acids after adequate hydrolysis. It is possible that the same protein moiety is present in each of the proteolipides studied.

Chemical and physical properties of tomatine and tomatidine THOMAS D FONTAINE, J S ARD,* ROBERT MA,* C L OGG* AND C O WILLITS * *Bureau of Agricultural and Industrial Chemistry Agricultural Research Center, Beltsville, Md and Eastern Regional Research Lab, Philadelphia, Pa*

Tomatine, a glycosidal alkaloid, and its aglycone, tomatidine, have previously been reported to possess *in vitro* antifungal activity (*Arch Biochem* 18 467, 1948). More recently, Wilson *et al* (*Federation Proc* 9 1950) have determined the oral toxicity of these compounds. Their report shows essentially no toxic effects when albino rats were fed diets containing up to 0.04% of these 2 compounds for 200 days, likewise, subacute and acute oral toxicity results were favorable. Crude tomatine has been prepared from dried tomato leaves on a semi pilot plant scale. A rather unique

procedure has been developed for the crystallization of tomatine—namely, the crude tomatine has been dissolved in either hot ammoniacal 70–80% dioxane or alcohol, followed by the rapid addition of 1.5–2 volumes of boiling water, with vigorous stirring. Upon addition of the hot water, a precipitate first forms but then dissolves almost immediately, and at this point crystallization of tomatine occurs in the hot solution. Tomatine, on acid hydrolysis, yields tomatidine and reducing sugars. The sugars have been identified as glucose, galactose, and xylose. Tomatidine contains a single double bond and 2 active hydrogens. Other chemical and physical data, including infrared and ultraviolet spectra, are presented for tomatidine and its acetyl derivative, and structural comparisons are made with related compounds.

Inhibition by glucose of the synthesis of proline from glutamic acid M. FORBES* AND M. G. SEVAG, *Dept. of Bacteriology, Univ. of Pennsylvania School of Medicine, Philadelphia*

In a synthetic amino acid medium free from glucose, glutamic acid (675 γ /ml) serves as precursor for the synthesis of proline associated with the growth of *Staphylococcus aureus* (strains 1A, 1E, 3A). *Staphylococci* fail to grow in the combined absence from the medium of glutamic acid and proline. Growth occurs in the presence of glucose when glutamic acid is omitted alone or together with one of the following amino acids—alanine, aspartic acid, lysine, and histidine, growth fails to occur when proline also is simultaneously omitted. Growth dependent on the synthesis of proline from glutamic acid likewise fails to occur when 5 mg of glucose or pyruvate/ml is added to the medium, showing that glucose or pyruvate blocks the reaction: glutamic acid \rightarrow proline. As little as 100 γ of glucose/ml causes 50% inhibition of this reaction. The inhibitory action of 5000 γ of glucose, or pyruvate/ml is counteracted by 1 γ of proline/ml. Ornithine or α -ketoglutaric acid were incapable of counteracting the effect of glucose. It is as yet not known whether proline bypasses the blockage, or functions as antagonist to glucose or pyruvate, or exercises a coenzyme-like effect.

The $2C_2$ condensation reaction in citric acid formation by a strain of *Aspergillus niger* J. W. FOSTER, S. F. CARSON* AND W. E. JEFFERSON*, *Biology Division, Oak Ridge Natl. Lab., Oak Ridge, Tenn.*

Previous demonstration of the $2C_2$ condensation reaction as a method of biosynthesis of C_4 dicarboxylic acids in fungi led to examination of its possible role in mold citrate formation. Radioactive citrate was produced from sucrose by *A. niger* in the presence of high specific activity methyl C^{14} -labeled acetate and $C^{13}O_2$. After purification

and isolation of the citrate as the calcium salt the citric acid was degraded. C^{14} and C^{13} specific activity were measured on each carbon atom of the citrate molecule. The data indicate that the C_4 dicarboxylic acid precursor of citrate arising from sugar can originate via the $2C_2$ condensation reaction.

Characteristics of water extractable apyrases obtained from various tissues SYLVIA FRANK AND RAKOMA LIPSHITZ (introduced by H. G. ALBAUM), *Dept. of Zoology, Columbia Univ., New York City*

Water extractable apyrases from muscle, liver, erythrocytes, plasma, and frog's eggs show characteristic and distinct pH activity curves. Optimum activity for muscle is found at pH 7.4, for liver at pH 4.5 and 9.2, for erythrocytes at 5.5 and 7.4, for plasma at pH 4.5 and 9.2, and for frog's eggs at pH 4.0 or lower and 9.2. Although some of these peaks are reminiscent of phosphatase pH optima reported in the literature, and NaF poisons the acid apyrase peak of liver as it does for acid phosphatase activity, little or no activity could be demonstrated under these experimental conditions when other substrates were substituted for ATP. The apyrase activity of erythrocytes from various sources (frog, chicken, rabbit, mouse, and human) shows a correlation between the amount of enzyme activity and the presence or absence of a cell nucleus. Attempts to separate nuclei from cytoplasm in chicken erythrocytes however show that apyrase activity is apparently associated with both cellular components. Dialysis of the water extractable apyrase from muscle results in a loss of activity which can be restored by re-introduction of the dialysate, concentrated by evaporation. The ashed dialysate fails to reactivate the enzyme. Boiled muscle extracts also activate the enzyme, and ashing destroys this ability. Crude preparations of Coenzyme A, magnesium, and glutathione and cysteine also stimulate enzyme activity. When the enzyme is saturated with magnesium or glutathione, the boiled extract continues to activate at a high rate suggesting the presence of an unknown co-factor.

Equilibrium and exchange reactions involving peptides, amino acids, and proteolytic enzymes IVAN D. FRANTZ, JR. AND ROBERT B. LOFTFIELD (introduced by LEWIS L. ENGEL), *Med. Labs. of the Collis P. Huntington Memorial Hospital of Harvard Univ., at the Massachusetts General Hospital, Boston, Mass.*

A method was recently reported (*Federation Proc.* 8:199, 1949) which makes possible the measurement of the small amount of peptide synthesized when an amino acid is incubated with a preparation of proteolytic enzymes. The first experiments with this system were carried out at

amino acid concentrations of 1 M, but more recent experiments at concentrations of 0.1 M have led to similar values for the free energy of the peptide bond. At these higher dilutions, the concentration of peptide formed amounts to only about 0.03% of the concentration of the amino acid added, and the detection of synthesis depends entirely on the use of labeled compounds. Data on the behavior of overloaded chromatographic starch columns will be presented, of interest in connection with the purification of materials present as traces mixed with large amounts of chemically similar compounds. Some evidence has been obtained, at present still somewhat equivocal, that when a peptide is allowed to split in the presence of labeled amino acids, the amino acid constituents of the peptide exchange with the free amino acids to produce, before equilibrium has been reached, a concentration of labeled peptide greater than that to be expected at equilibrium. With the enzymes which we have studied (a dipeptidase and a carboxypeptidase), this effect is slight.

Enzyme preparations from acetobacter suboxydans oxidizing inositol ROBERT E. FRANZL* AND ERWIN CHARGAFF, *Dept. of Biochemistry, Columbia Univ. College of Physicians and Surgeons, New York City*

In continuation of previous work on the oxidation of inositol stereoisomers by *Acetobacter suboxydans* (MAGASANIK AND CHARGAFF, *J. Biol. Chem.* 174: 173, 175, 929, 939, 1948) the enzyme system performing these oxidations has been studied in intact cells and in cell-free enzyme preparations. The latter were obtained by the differential centrifugation of phosphate buffer extracts of organisms crushed in a bacterial mill. Suspensions of the pellet sedimenting at 31,000 g in veronal or phosphate buffer oxidized meso-inositol with the utilization of $\frac{1}{2}$ M O_2 /M of substrate. The rate of oxidation was enhanced very strikingly by the addition of washed heat-inactivated bacteria to the enzyme solution. This effect could be partially duplicated by the addition of bacterial ash, or calcium and magnesium ions. This oxidation of meso-inositol, whose pH optimum is at pH 6.5, apparently requires an enzyme system related to the oxidases or, possibly, aerobic dehydrogenases. The oxidation of inositol stereoisomers and of other compounds by the cell-free enzyme preparations will also be discussed.

Comparison of vitamin B₁₂ and vitamin B_{12b} H. H. FRICKE,* BETTY LANIUS,* A. F. DEROSE,* M. LAPIDUS* AND D. V. FROST, *Abbott Labs., North Chicago, Ill.*

Crystalline vitamin B_{12b} was prepared from cultures of *Streptomyces griseus*. The compound showed absorption spectrum maxima at 274, 352, 411 and 526 mμ. Vitamin B_{12b} showed equivalent

activity in the assay with *L. leichmannii* and *E. coli* when compared with vitamin B₁₂. Rats maintained on a vitamin B₁₂ deficient diet gave similar growth responses upon the addition of either vitamin B₁₂ or vitamin B_{12b} to the diet. Dr. Tom Spies (personal communication) has demonstrated the clinical activity of vitamin B_{12b} for pernicious anemia, nutritional macrocytic anemia, and sprue. From clinical experience, vitamin B_{12b} appears to be equally as effective as vitamin B₁₂/γ in treating Addisonian pernicious anemia. On treating solutions of vitamin B₁₂ or vitamin B_{12b} with sodium bisulfite, both solutions exhibit identical absorption curves showing maxima at 275, 365, 418 and 515 mμ respectively. Removal of the sodium bisulfite indicates by absorption spectrum that vitamin B_{12b} is recovered from both solutions. Sodium hydrosulfite, depending on concentration, causes total or partial destruction of color and activity in solutions of vitamin B₁₂ and B_{12b}. The color is partially restored in presence of air if only small amounts of hydrosulfite are used. When crystalline vitamin B₁₂ or vitamin B_{12b} is injected intramuscularly into the rat, both can be recovered in their original form in the urine of the rat.

Distribution pattern of P³² in desoxyribonucleic acid-protein complex of intestinal tract mucosa FELIX FRIEDBERG (introduced by VERNON A. WILKERSON), *Dept. of Biochemistry, Howard Univ. College of Medicine, Washington, D. C.*

A variation in incorporation of S³⁵ of methionine into the protein of the intestinal mucosa along the length of the gastro-intestinal tract of dogs was reported recently (*J. Biol. Chem.* 173: 355, 1948). The incorporation in the stomach mucosa was low, and 3 peaks of activity were found in the intestinal mucosa. These peaks appeared in the region of the junction of the duodenum and jejunum, the junction of the jejunum and ileum, and in the large intestine. We obtained identical results when the desoxyribonucleic acid-protein complex was isolated after intravenous administration of inorganic P labeled with P³². Two female dogs (weighing 7.6 and 6.7 kg.) were injected i.v. with about 6 μc of P³² (as H₂PO₄). Food was removed 6 hours prior to the injection, and 4 hours after the injection the animals were killed. The entire gastrointestinal tract was removed and flushed with water, the mucosa (and submucosa) was obtained by pressure from the outside of the tract, and the P³² activity of the desoxyribonucleic acid-protein complex, isolated by the method of Schmidt and Thannhauser (*J. Biol. Chem.* 161: 83, 1945), suspended in acetone and dried, was determined.

Effect of amino acid antagonists on enzymes involved in nitrogen catabolism CARL FRIEDMAN

AND KARL DITTMER (introduced by R W JACKSON) *Dept of Chemistry, Florida State Univ, Tallahassee*

Since amino acid antagonists inhibit the growth of a variety of organisms, it seemed desirable to investigate their mode of action. The availability of several well defined enzyme systems involved in nitrogen catabolism permitted the study of the effect of some amino acid antagonists. The enzyme tyrosine decarboxylase (from *Strep faecalis*) acts on DL-phenylalanine at about 5% the rate observed with L-tyrosine in acetate buffer, pH 5.5, 28.5° C (conditions described by W W UMBREIT, W D BELLAMY AND I C GUNSALUS *Arch Biochem* 7 185, 1945). Phenylalanine antagonists such as β -2- and β -3-DL-thienylalanine are decarboxylated at rates somewhat less but of the same order of magnitude as phenylalanine. They do not reduce the rate at which tyrosine is decarboxylated. A preparation of 3,5-difluoro-tyrosine and 3-amino-L-tyrosine proved to be substrates with activities close to that of tyrosine. But 3,5-dibromo-L-tyrosine, 3,5-diiodo-L-tyrosine, and 3-nitro-L-tyrosine were inactive at corresponding enzyme concentrations as were other related amino acids such as p-tolyl-DL-alanine and p-hydroxyphenyl-DL-glycine. Results obtained with D-amino acid oxidase and other enzymes will also be presented.

Platinum catalyzed hydrogen-deuterium exchange with steroids DAVID K FUKUSHIMA* AND T F GALLAGHER *Sloan-Kettering Inst for Cancer Research, New York City*

We have investigated the platinum catalyzed exchange of deuterium for hydrogen with steroids in solution in acetic acid-d and D₂O at elevated temperatures. With saturated steroids containing ketone groups, an appreciable amount of stably bound deuterium was incorporated and the steroid was recovered in high yield. With increasing unsaturation or with multiple ketone groups, very much larger amounts of isotope were incorporated and in favorable instances high recovery of labeled steroid was possible. Hydroxylated steroids were less suitable for reaction since dehydrogenation and hydrogenolysis markedly diminished the yield. Acetylation led to lesser destruction without materially altering the exchange. Using Δ^4 -androsterone 3-17-dione, the effects of temperature, catalyst and substrate concentration have been investigated. Of these variables, temperature was most important since little exchange was effected below 100°. Greater incorporation of isotope was achieved when the steroid was present in relatively dilute solution. Variation in catalyst concentration gave slight differences in isotope concentration. These studies have led to the preparation of steroid hormones stably labeled with deuterium. The distribution of the isotope

has been investigated for cholesterol with 538 atoms % excess deuterium prepared by the exchange reaction according to Bloch and Rittenberg. All the isotope present in the steroid nucleus (46% of the total) was found in the vicinity of the Δ^5 -3-hydroxyl system while the isotope of the side chain (54%) was concentrated in the isopropyl group.

Comparative study on the phosphorylase content in homogenates of heart and striated muscle MARIA FULD* AND GERHARD SCHMIDT *Cardiac Lab, New England Center Hospital, Boston, Mass*

We have determined the respective amount of phosphorylase directly in homogenates of heart muscle and striated muscle of rats and rabbits. The tissues have been homogenized in the cold, dialyzed against running and distilled water until the disappearance of free phosphate and Mg⁺⁺. The dialysate has been diluted 25 times. Phosphorylase activity has been determined according to Cori, but the concentration of glucose-1-phosphate has been quadrupled. Under these conditions phosphoglucomutase and phosphatase do not interfere with the measurement. Striated muscle contains 12-20% more phosphorylase than heart muscle. These results confirm the findings of Shapiro and Wertheimer (*Biochem J* 37 397, 1943). These authors, however, worked with tissue extracts where the entire amount of enzyme present may not have been accounted for. For this reason the present study had been done on homogenates.

Conditions for determination of neutral 17-keto-steroids in urine O H GAEBLER AND WILLIAM T BEHER* *Edsel B Ford Inst for Med Research, Henry Ford Hospital, Detroit, Mich*

Continuing a study of factors affecting determinations of 17-ketosteroids (*Federation Proc* 8 182, 1949) we have sought to establish optimal conditions for the initial hydrolysis. The validity of using aqueous 5 N KOH in the Zimmermann reaction, when 2 color spectrophotometry is employed, depends upon these conditions, and was therefore investigated simultaneously, using the technique of Holtorf and Koch (*J Biol Chem* 135 377, 1940). Samples of pooled human urine were hydrolyzed for 6 different time intervals with each of 5 acids mentioned below. From ether extracts of the hydrolysates, neutral ketonic and non-ketonic fractions were prepared. After applying the Zimmermann reaction, extinction measurements were made at 515 m μ and 440 m μ . Optimal conditions of hydrolysis prevail when 1) the extinction at 515 m μ in the ketonic fraction is maximal, indicating good recovery, 2) the ratio E₄₄₀/E₅₁₅ in the non-ketonic fraction is constant over the required range of concentrations, 3)

changes produced by applying the procedure to pure solution of dehydroisoandrosterone are minimal. The best results were obtained when 150 ml aliquots of urine or solution, heated to boiling, were acidified with 22.5 ml of concentrated HCl, boiled for an additional 7 minutes, and cooled rapidly. Hydrolysis for 60 minutes with an equivalent concentration of H₂SO₄ satisfied the first 2 criteria listed above. Substituting equimolar amounts of HBr, HI, and H₃PO₄ for HCl yielded unsatisfactory results at all hydrolysis intervals. Results of analyzing 24-hour urines from 111 human subjects will also be discussed.

Carbon dioxide, inorganic phosphate and cation content of muscle in potassium deficiency

LITT I GARDNER, ELSIE A MACLACHLAN AND HELEN BERMAN (introduced by JAMES C ANDREWS) *Children's Med Service, Massachusetts General Hospital and Dept of Pediatrics, Harvard Med School, Boston, Mass*

Albino rats weighing 160-175 gm were fed a diet containing 0.003% K and 0.7% Na for 40 days. Controls were given the same diet plus added K. Measurements of skeletal muscle CO₂ and Cl concentrations showed no significant differences between the 2 groups. Muscle K was markedly reduced, whereas muscle Na, Mg, Ca and inorganic phosphate were significantly increased in the K deficient rats. Serum values for Cl were reduced and values for CO₂ were elevated in the K deficient group. When allowance was made for the Cl and CO₂ in extracellular fluid, the data indicated that in K deficiency there was a decreased concentration of CO₂ in intracellular fluid. If the latter represented a diminution in intracellular bicarbonate, partial compensation for this by increase in the inorganic phosphate as noted above seems a possibility. It is considered unlikely that Cl enters the cell, since it has previously been shown that K deficient animals are constantly in negative Cl balance. It is thought that the increased intracellular concentrations of the cations Na, Mg and Ca, which have transport rates relatively slower than K, necessitate reorganization of the ionic equilibria on both sides of the cell membrane. This may help explain the hypochloremic alkalosis of K deficiency.

Urinary excretion of thiosulfate by normal human adults JOSEPH H GAST, FRANK L ALDRICH AND KAZUO ARAI (introduced by HOWARD B LEWIS) *Dept of Biochemistry, Baylor Univ College of Medicine, Houston, Tex*

The urinary excretion of thiosulfate by adult humans has never been quantitatively established by reliable or specific methods. The reason for this is obvious even when one uses a specific precipitant for thiosulfate, such as the nickel ethylene diamine salt, because the amounts usually present

on a general mixed diet are below or at the limit of the end point error of the iodometric titration. This was established by a study of over 200 24-hour urine specimens. Application of a micro-quantitative procedure plus the use of radioactive thiosulfate enabled us to analyze both quantitative collections and random specimens for thiosulfate with the assurance of a definite recovery of the thiosulfate present. Replicate determinations indicate from 9-20 mg of thiosulfate sulfur to be present normally in 24-hour specimens, preserved with chloroform. Fresh random specimens, kept in the refrigerator indicate the presence of concentrations of the same order of magnitude ranging from 0.75-1.3 mg % thiosulfate sulfur. Values given are as determined, uncorrected for radioactive thiosulfate recovery. Isolation of nickel ethylene diamine thiosulfate from human urine with a small amount of added radioactive thiosulfate as tracer to follow losses in purification, delayed crystallization, etc will be reported together with detailed data from which the above figures were taken. Because of the ready oxidation of thiosulfate by certain tissues and organisms the data presented should suffice to require the explanation of its presence in any scheme for the oxidation of sulfur.

Influence of salt intake on chromatographic patterns of steroid excretion in essential hypertension JACQUES GENEST, GEORGE C COTZIAS, LEWIS K DAHL, WILLIAM EISENMENGER AND VINCENT P DOLE (introduced by R M ARCHIBALD) *Hospital of the Rockefeller Inst for Med Research, New York City*

Fresh urines were refluxed with 1/6 volume of concentrated HCl for 30 minutes. Their CCl₄ extracts were dried, dissolved in benzene, and aliquots chromatographed on alumina, and analyzed, according to the procedure already described for blood and urine steroids (*Federation Proc* 8:180, 1949). The analysis of such urine extracts from 3 subjects with essential hypertension maintained on high and low sodium chloride intake showed marked changes in the steroid patterns. These changes were observed in several of the fractions, but mainly in the ultraviolet absorptancy of the fraction eluted by water. An elevation noted at 268 mμ in the absorption curve of the first water fraction disappeared when the blood pressure was brought down to normal level. A subject with thalassemia minor and amoebiasis showed similar changes. Opposite, but less marked differences were observed in the cases of both a normal control and a subject with hepatic cirrhosis when placed on a high and low sodium chloride intake. The latter subject, on a 1 gm NaCl diet, showed no change in Na excretion after administration of DOCA and only slight modifications of the urinary steroid pattern.

Relation of ATP-ase and myosin JOHN GERGELY
(introduced by A SZENT-GYORGYI) *Natl Insts of Health, Bethesda, Md*

Myosin, prepared according to Szent-Gyorgyi was subjected to digestion by trypsin in order to investigate the relationship between ATP-ase activity and other characteristic properties of myosin. ATP-ase activity remained unchanged, while a decrease in the slope of the linear part of the specific viscosity-concentration curve indicated a decrease in the asymmetry of the myosin molecules. The ability of myosin to combine with actin also disappeared. By subjecting the trypsin digest to an isoelectric precipitation 2- to 3-fold increase in ATP-ase activity (Q_F) in the supernatant was achieved, with a 80-90% yield. Other procedures, including ultracentrifugation, did not bring about further purification. These experiments indicate that ATP-ase function is not essentially linked to the integrity of the myosin molecule.

Staphylocoagulase formation without plasma co-factor EARL B GERHEIM AND JULIA K GERHEIM
(introduced by ARNOLD G WARE) *Dept of Physiology and Pharmacology, Wayne Univ College of Medicine, Detroit, Mich*

Smith and Hale (*Brit J Exper Path* 25: 101, 1944) have explained coagulation by pathogenic *Staphylococcus aureus* as an activation of a bacterial component by a plasma component to form the active clotting agent. Although most investigators have accepted this thesis, actual proof of this mode of activation is lacking. Possibly, the bacteria might furnish the activator and the plasma the precursor. Miale (*Blood* 4: 1039, 1949) has stated that his preliminary observations would indicate that the precursor is found in plasma. In the experiments being reported we have been able to show repeatedly that staphylocoagulation is possible in the absence of the plasma factor. A 10% solution of the staphylococcal concentrate was placed at room temperature and over a 7 day period a portion was added to Armour's fibrinogen which has been shown to be free of what we wish to call co-factor (GERHEIM, FERGUSON AND TRAVIS, *Proc Soc Exper Biol & Med* 66: 525, 1947). Also the fibrinogen was adsorbed with barium sulfate to remove the prothrombin. While no staphylocoagulation took place initially, enough staphylocoagulase had formed at the end of 7 days to give a solid clot in less than 60 minutes. The precursor of the clotting agent (pro-staphylocoagulase), according to these results, appears to be of bacterial origin.

In vitro fatty acid metabolism and acetoacetate formation studied with C^{14} ROBERT P GEYER, MARY M CUNNINGHAM AND JOYCE PENDERGAST
(introduced by FREDRICK J STARE) *Dept of*

Nutrition, Harvard School of Public Health, and Dept of Biological Chemistry, Harvard Med School, Boston, Mass

Radioactive ($RC^{14}OOH$) pentanoic, hexanoic, heptanoic, octanoic, nonanoic and dodecanoic acids give rise to $C^{14}O_2$ and $CH_3C^{14}OCH_2C^{14}OOH$ when incubated with either liver or kidney slices.

The ratio $\frac{CH_3C^{14}OCH_2C^{14}OOH}{C^{14}O_2}$ is much higher

in the case of liver, but the total C^{14} in these products is similar for both tissues. This ratio is also much higher for the acids of an even number of carbons than for those of an odd number. This difference is mainly the result of more $CH_3C^{14}OCH_2C^{14}OOH$ being formed in the case of the even numbered acids. The ratio $\frac{CH_3C^{14}OCH_2COOH}{CH_3COCH_2C^{14}OOH}$ increases with increasing substrate chain length and is almost unity for dodecanoic acid. Studies with octanoic acids labeled in either the α - or β -position with C^{14} have also been done. Very little activity was found in the carboxyl of acetoacetic acid when the α -labeled acid was used. In the case of the β -labeled acid, both the amount and distribution of C^{14} in the respired carbon dioxide and the carbonyl and carboxyl of acetoacetic acid were the same as those observed with the carboxyl-labeled octanoic acid. The latter finding is strong evidence against the formation of acetoacetic acid by the alternate oxidation and cleavage of fatty acids. It supports the concept of C_2 -fragment formation and recondensation.

Effect of pH and ionic strength on biological activity of adrenocorticotrophic hormone (ACTH) B N GHOSH,* JOHN B RICHARDS,* MARSHAL MERKIN,* THOMAS W BURNS,* DOUGLAS M BROWN,* GEORGE SAYERS* AND EMIL L SMITH
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Solutions of ACTH (10 $\mu g/cc$) were allowed to stand at room temperature for 19-24 hours, pH and ionic strength were varied (see table). Biological activity was determined by the adrenal ascorbic acid-depletion method in comparison with a standard at pH 1.0. JACTH and BNG35 were prepared according to the method of Sayers, White and Long. La-1-A was obtained from the Armour Laboratories. ACTH is remarkably stable at low pH. Hormonal activity is completely destroyed at pH 7 and at pH 13 in the absence of salt, sodium chloride and sodium phosphate appear to have some stabilizing influence. These stability studies have stimulated a complete re-evaluation of the physical properties of this hormone. Studies of the sedimentation and electrophoretic behavior will be presented.

PREP	pH	MEDIUM	ACTIVITY % STD (1 ERROR OF ESTIMATE)
La-1-A		5 M HCl	0
JACTH	0.1	1 M HCl	72(33-155)
BNG35	1.0	0.1 M HCl	85(62-117)
BNG35	4.1	0.1 M acetate	86(67-108)
BNG35	7.0	water	0
BNG35	7.0	0.15 M NaCl	43(27-69)
BNG35	7.3	0.038 M sodium phosphate	73(53-101)
BNG35	12.9	0.1 M NaOH	0
BNG35	12.6	0.1 M NaOH	18(12-29)
		0.15 M NaCl	
La-1-A		1 M NaOH	0

Studies on 'Dietary Factor' in experimental necrotizing arteritis BLAND GIDDINGS,* PHILIP L. HARRIS AND RUSSELL L. HOLMAN* *Dept of Pathology, Louisiana State Univ School of Medicine, New Orleans and Dept of Biochemistry Distillation Products, Rochester, N. Y.*

The sequence of feeding a 'standard high fat diet' for 8 weeks or longer then producing 'standard renal insufficiency' has been shown previously to regularly induce 'typical arterial lesions' in mongrel dogs regardless of age and sex. Either factor alone has proved to be ineffective. The pathogenesis of these arterial lesions, which closely resemble those of rheumatic arteritis and periarthritis nodosa, is obscure but is being studied from the standpoints of a 'dietary factor' and of a 'renal factor'. The dietary factor is defined as that substance (or substances) contained in the standard high fat diet which when followed by production of standard renal insufficiency, results in typical arterial lesions. From the studies thus far it would appear that the dietary factor is 1) found in certain animal fats (creamery butter and certain samples of cod liver oil), 2) not present in olive oil, corn oil, mutton tallow, lard, coconut oil and oleomargarine, 3) not vitamin A or vitamin D, 4) effective in quantities roughly proportional to the amount fed, 5) effective only if fed continuously since omission of it from the diet for 4 weeks or longer eliminates the response, 6) relatively heat stable, 7) destroyed or inactivated by saponification or by acetone separation, 8) inactivated or counteracted by vitamin E, or by cholesterol, or by substituting casein for liver in the standard diet, and 9) unaffected or not influenced by choline. Conjectures on its nature and mode of action will be presented.

Question of hyaluronidase in tumor tissue DAVID GLICK, LAWRENCE B. KIRILUK*, ERICK Y. HAKANSEN* AND ARNOLD J. KREMEN* *Depts of*

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The possibility that hyaluronidase may be an agent that promotes the invasiveness of malignant cells and plays a role in the formation of metastases has led a number of investigators to examine cancer tissue for hyaluronidase activity. The reports were inconclusive and often inconsistent. A reinvestigation of this problem has been carried out on clean, non-necrotic samples of tumor tissue obtained directly from the operating room under sterile conditions. Precautions to maintain sterility were exercised throughout the preparation of extracts for the enzyme assay, and microbiological controls were run on all tissues and their extracts to detect possible contamination. In 4 different human benign tumor extracts, and 6 malignant, contamination was not found and no hyaluronidase could be detected. In most of the contaminated material from 25 other malignant tumors and as many samples of normal tissues the enzyme was present. Of 11 adenocarcinomas of the breast in mice, 5 yielded sterile extracts and no enzyme was found in them, while of the 6 contaminated extracts 4 contained hyaluronidase. The evidence indicates that the malignant cell contains no hyaluronidase unless it is contributed by microorganisms, and that previous claims for the presence of the enzyme were probably based on experiments in which contamination had occurred.

Vitamin E and hepatic necrosis MARIANNE GOETSCH, *Dept of Chemistry and Nutrition, School of Tropical Medicine, Univ of Puerto Rico, San Juan*

Preliminary tests confirm Schwarz (*Ann New York Acad Sci* 52:225, 1949) and Himsworth and Lindan (*Nature, London* 163:30, 1949) that vitamin E is a factor in hepatic necrosis of the rat on low protein diets. 21-day-old rats, with low stores of E, were given a diet containing 8.3% crude casein, 79.7% cornstarch, 6% lard, 2% cod liver oil, 4% minerals, and crystalline vitamins. 1) *Without a-tocopherol*. The rats died in 10-40 days, presenting massive hepatic necrosis. Alterations in lard content from 0-20%, without changing proportion of protein calories, were without effect. Choline and inositol apparently were not factors. The addition of 3% either methionine or cystine enabled rats to grow and survive until the present time (6 months of age) despite the onset of chronic muscular dystrophy and typical sterility. 2) *With 2 mg a-tocopherol per week*. All rats are surviving at 6 months of age. Those on the diet above grew poorly but were able to reproduce, although no young have survived the period of lactation. The young presumably died of starvation. The rats receiving in addition, .3% either methionine or cystine grew well and second generation young are

being reared. It is hoped to observe all rats until time of death.

Effect of anionic and cationic agents on urease

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Experiments have been conducted to show the effect of certain anionic and cationic surface active agents on the rate of hydrolysis of urea by urease. Purified sodium dodecyl sulfonate and commercial trimethyl dodecyl ammonium chloride (Arquad 12, Armour and Company) have been studied at different levels in pH 7 phosphate buffer at 38°C. Both commercial preparations of urease and glycerol extracts of Jack Bean meal have been used. In each case the rate of hydrolysis in the presence of one of these agents was compared with a control which was run simultaneously. The liberated ammonia was determined as a measure of the extent of hydrolysis. Sodium dodecyl sulfonate depressed the activity of the enzyme in every case, 0.016 M sulfonate inhibited the hydrolysis approximately 20% when three-fourths of the urea in the control was hydrolyzed. When equimolar quantities of the sulfonate and Arquad 12 were added to the same reaction mixture, the depression of the reaction rate was not observed. When the Arquad 12 was used alone, a slight depression was usually observed but the data are less conclusive in this series of experiments.

Ultracentrifugal stratification of human serum lipoproteins

WILLIAM H. GOLDWATER, MALCOLM L. RANDOLPH, J. R. SNAVELY, ROY H. TURNER AND WALTER G. UNGLAUB (introduced by W. B. WENDEL) *Depts. of Medicine and Physics, Tulane Univ., New Orleans, La.*

The ultracentrifuge quantity rotor has been used to study the sedimentation characteristics of human serum lipid and protein components. Pooled normal sera are spun in plastic tubes, which are then frozen, sectioned at various levels, and homologous fractions from a number of tubes combined for analyses. Total protein concentration increases in a logarithmic type of curve towards the bottom of the tube. Albumin comprises the major portion of protein in the top half of the tube, increasing towards somewhat constant values at the bottom. Globulin concentrations are low in the top of the tube, but rise rapidly towards the bottom. Lipid components show peak concentrations at various levels in the tube, these maxima corresponding in most cases to specific gravity levels characteristic for each component. Free and ester cholesterol show predominant maxima at a specific gravity of about 1.017, with a smaller cholesterol ester peak at 1.060. Phospholipid maxima are observed at about

1.020, 1.045–1.050, and either 1.085 or the bottom-most portion of the tube. Neutral fat peaks occur in the top- and bottom-most portions of the tube, as well as at specific gravity levels of 1.010–1.020, 1.035, and 1.060. These findings are interpreted in the light of the existence of human serum lipids as lipoprotein aggregates, and the positive and negative sedimentation of these aggregates in various portions of the specific gravity (total protein) gradient produced by the ultracentrifugal process.

Response of the serum level of hyaluronidase inhibitor and mucoprotein to stress

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In previous studies in these laboratories we have demonstrated that the serum levels of both the non-specific hyaluronidase inhibitor and a mucoprotein fraction are elevated in a variety of the same clinical conditions. It appeared to us that all of the situations in which the serum levels of these two entities were elevated might be interpreted as stress situations and that an adrenal mechanism might be implicated. During these studies it has become apparent that elevations in the serum non-specific hyaluronidase inhibitor level can be evoked by subjecting an animal to stress by the Arthur phenomenon, the Shwartzman phenomenon, acute or chronic chilling, sterile ferrie chloride abscess, hyperimmunization or sterile tissue destruction by electrocautery. Serum mucoprotein was elevated by chronic chilling and by hyperimmunization but not by the other stimuli mentioned. Injection of lipoadrenal extract slightly raised the hyaluronidase inhibitor but decreased the serum mucoprotein. Injection of cortate in sesame oil and of sesame oil alone caused no effect on the hyaluronidase inhibitor but the cortate decreased the serum mucoprotein. Injection of adrenalin did not change the hyaluronidase inhibitor but did raise the serum mucoprotein. Adrenalectomy per se decreased the hyaluronidase inhibitor but did not affect the serum mucoprotein. Mock operation had no effect on either hyaluronidase inhibitor or mucoprotein, nor did anesthesia by intravenous sodium nembutal. Adrenalectomized animals, when exposed to chronic chilling, exhibited no elevations of the hyaluronidase inhibitor whereas mock operated animals did.

Accumulation of 5(4)-amino-4-(5)-imidazolecarboxamide in relation to sulfonamide bacteriostasis and purine metabolism in *Escherichia coli*

JOSEPH S. GOTS (introduced by CECILIA RIEGEL) *Dept. of Bacteriology, School of Medicine, Univ. of Pennsylvania, Philadelphia*

It is known that 5(1)-amino-4(5)-imidazole-carboxamide accumulates in *Escherichia coli* cultures under sulfonamide bacteriostasis. We have found that maximal accumulation occurs in systems showing 50-60% inhibition of growth. Accumulation of the carboxamide becomes evident 2 hours after inoculation and increases at a rapid rate until maximum is reached in 24-48 hours. No conditions have yet been found which will bring about any measurable utilization of the carboxamide after it is formed or when added from without, nor will the compound support the growth of seven *E. coli* mutants which require purines for growth. *p*-aminobenzoic acid when added at intervals after inoculation will immediately relieve growth inhibition and accumulation of the carboxamide but the quantity which has already accumulated remains constant. Pteroylglutamic acid has no effect on either the growth inhibition by sulfadiazine or accumulation of the carboxamide. Methionine or vitamin B₁₂ will relieve growth inhibition by low concentrations of sulfadiazine without relieving the accumulation. Of the numerous substances tested, the most marked effect on the accumulation of this compound was found in the presence of the purines, xanthine, adenine and guanine, which in concentrations as low as 2 to 4 µg/ml will prevent accumulation almost 100% without relieving growth inhibition. The hypothesis that 5(4)-amino-4(5)-imidazole-carboxamide is a normal intermediate in the biosynthesis of purines may be valid, but the data presented here cannot support it. A more likely explanation may be that this compound is an abnormal by-product brought about by an alternate pathway after normal metabolic pathways are blocked by sulfonamides.

Effect of dietary cholesterol on hepatic cholesterol synthesis R. GORDON GOULD AND C. BRUCE TAYLOR * *Rush Depts. of Biochemistry and Pathology, Presbyterian Hospital, Chicago, Ill*

Dogs and rabbits were maintained on high cholesterol diets for 6 weeks and the relative rates of cholesterol synthesis by liver slices determined by incubation with C¹⁴ labeled acetate followed by isolation and assay of cholesterol. Comparison with control animals showed that dietary cholesterol suppressed hepatic synthesis to a few per cent of the control rate in both rabbits and dogs. *In vivo* synthetic rates were also compared by administering labeled acetate by stomach tube and isolating cholesterol from the blood 1 and 16 hours later and the results were in agreement with those from the *in vitro* studies. Comparison of the rates of hepatic synthesis between animals of different species on cholesterol-free diets, together with data on cholesterol excretion, suggests that the over all aspects of cholesterol metabolism pro-

ceed more slowly in the rabbit than in the other species studied, and it is suggested that this may account for the extreme hypercholesteremia observed in cholesterol-fed rabbits. By separation of free and esterified cholesterol, it was shown that the newly synthesized cholesterol was largely in the free form. Of the other tissues tested, only skin was found to possess cholesterol synthetic activity of the same order of magnitude as liver. Adrenal cortical tissue was found to be capable of cholesterol synthesis as previously reported by Srere, Chaikoff and Dauben, (*J. Biol. Chem.* 176: 829, 1948), but in the present experiments the amount synthesized by the adrenals was of negligible quantitative importance compared with that formed in the liver or in the skin.

Transphosphorylation in the absence of nucleotides HARRY GREEN* AND O. MEYERHOF *Dept. of Physiological Chemistry, School of Medicine, Univ. of Pennsylvania, Philadelphia*

As was shown recently (*Science* 110: 503, 1949) transphosphorylation from phosphate compounds of higher energy to those of lower energy in the absence of nucleotides occurs readily with alkaline intestinal phosphatase. It can be observed with the help of P 32 if energy rich P donors with labeled P are incubated with glycerol or sugar and inorganic phosphate together with phosphatase. This finding was extended to several more P donors like phosphopyruvate, nitrophenyl phosphate and also to acid phosphatase. Moreover, the formation of a phosphate-enzyme compound was investigated with P 32. The bearing of these facts for the mechanism of transphosphorylation and the establishment of the specificity of phosphatases will be discussed.

Mechanism of biosynthesis of purine G. ROBERT GREENBERG (introduced by HARLAND G. WOOD) *Dept. of Biochemistry, School of Medicine, Western Reserve Univ., Cleveland, Ohio*

The investigation of the hypoxanthine-synthesizing system of pigeon liver preparations (*Federation Proc.* 8: 202, 1949) has been continued. Employing C¹⁴ compounds as tracers several possible intermediates in this synthesis have now been separated by paper chromatography. Evidence will be presented that the synthesis of hypoxanthine involves the following overall reaction: NH₃, CO₂, glycine, formic acid, ribose-phosphate → intermediates → inosinic acid → hypoxanthine + ribose-phosphate. This mechanism is based in part on the following data: By specific activity versus time measurements and by balance studies it has been demonstrated that C¹⁴ formic acid is incorporated into inosinic acid prior to the formation of hypoxanthine; dilution experiments with added non-radioactive inosinic acid provide additional evidence for this observation. Addition of

ribose-phosphate or compounds forming ribose-phosphate frequently effected a threefold increase in the rate of incorporation of radioactive formic acid into inosinic acid. When carboxyl-labeled glycine was employed as a tracer, a C^{14} -containing component was separated which did not occur in simultaneous experiments with radioactive formic acid. This component apparently is not a known metabolic product of glycine. The nature of this fraction and its possible role in the reaction is being investigated. It has been shown that 4-amino-5-imidazole carboxamide per se is not involved in the synthesis of purine in this system.

Simplified serum alkaline and acid phosphatase method using phenolphthalein monophosphate substrate. ALEXANDER B. GUTMAN, *Columbia Research Service, Goldwater Memorial Hospital and Dept. of Medicine, College of Physicians and Surgeons, Columbia Univ., New York City*

The estimation of serum alkaline and acid phosphatases can be greatly simplified by use of suitable phosphoric ester substrates which on hydrolysis yield a chromogen that can be measured directly. Phenolphthalein diphosphate has been proposed for this purpose but since the phosphomonoesterases in question dephosphorylate pure diphosphoric esters slowly if at all, phenolphthalein monophosphate would seem more appropriate. The monophosphoric ester was synthesized by phosphorylating phenolphthalein in dry pyridine with 1 equivalent of freshly redistilled phosphorus oxychloride under controlled conditions. Phenolphthalein monophosphoric acid precipitated out on acidification. After dissolving with sodium bicarbonate, free phenolphthalein was removed by shaking with ethyl acetate. Reprecipitation with acid gives the monophosphoric ester in good yield and sufficiently pure for general use. The preparations are standardized by their rate of dephosphorylation by sera of known phosphatase activity and also by their optical density at 450 m μ , pH 9.0. Phenolphthalein monophosphate is rapidly hydrolyzed by both alkaline and acid serum phosphatases. With our present preparations, incubation for 14 minutes at pH 8.7 or 35 minutest at pH 4.9 gives activities for serum alkaline or acid phosphatases equivalent to those obtained in 1 hour with β -glycerophosphate or phenylphosphate respectively. The pink free phenolphthalein color is developed immediately with sodium carbonate. Units of phosphatase activity are read directly from a standard phenolphthalein transmittance-concentration curve. The method, in regular use for several years, is simple, rapid and gives results in normal and a wide variety of abnormal sera that are in good agreement with the standard methods.

Mechanism of thiamine destruction by bracken

fern J. R. HAAG, BARBARA STEARMAN*, P. H. WESWIG*, R. T. PIERCE* AND J. R. SCHUBERT* *Agricultural Exper. Station, Oregon State College, Corvallis*

Antithiamine activity in rations containing air-dried fern and the harmless nature of steamed fern have previously been noted. Confusion concerning the significance of results obtained by the thiochrome procedure was clarified by our finding (A. C. S., Sept. 1948, Portland, Oregon), that both raw and steamed fern suspensions rapidly adsorb thiamine from pH 4.5 buffers. Subsequently raw fern more slowly destroys thiamine by enzymatic activity. Studies of temperature and pH effects and inhibitors therefore yield complicated results. Thiamine removal is more rapid at 0°C than at 25°, due to adsorption. Enzyme activity is slow at 25°, increases to 60°, then decreases at 70° due to heat inactivation. Moist heat inactivation can be detected at 65°, and is complete at 100° in less than 30 minutes. Dry heat at 100° for 24 hours results in some 50% inactivation for rats. Some activity remains after 24 hrs. at 110°. Enzymatic activity increases from pH 2.0 to 5.5 or 6.5. Concentration and nature of buffers in which fern suspensions are incubated variously affect adsorption and enzymatic processes. Supercentrifuged H₂O extracts possess pronounced enzymatic activity only. Neopyrithamine inhibits both processes. AgNO₃ inhibits enzymatic activity. NaCN and NaF do not inhibit. Cysteine markedly stimulates enzymatic activity, cystine is slightly stimulating, methionine is without effect. Thiamine removed by raw fern is not available to rats, that removed by steamed fern can be recovered and is available. Raw fern does not destroy the value of thiamine for yeast fermentation.

Fluorescence scanner for evaluation of papergrams of adrenal cortical hormones. WILLIAM J. HAINES AND NORMAN A. DRAKE (introduced by M. H. KUENZING), *Research Labs. Upjohn Company, Kalamazoo, Mich.*

A rapid and convenient technique has been devised for evaluating and recording the papergrams of those adrenal steroids and related substances having strong absorption at about 2400 Å. No chemical reactions are involved, and 5 gamma or less of these compounds can be detected. The procedure involves the use of a stainless steel box (6 x 12 x 13 inches) containing an 8-watt germicidal lamp in the bottom and covered on top with a 6 x 12 inch piece of Vycor glass or a Corning #9863 filter. On top the box there is placed a special glass screen having a thin layer, on its under side, of du Pont zinc silicate phosphor #609, which emits a bright green fluorescence when exposed to 2537 Å light. A dried papergram is

placed under the fluorescing screen, thus in the path of the ultraviolet light. The locations of the steroids are seen immediately as non fluorescing spots. A permanent record of the chromatogram is made in a dark room by inserting a focal plane shutter near the top of the box, and replacing the fluorescent screen with a piece of contact printing paper. Again the papergram is in the light path when the lamps are turned on and the slit is drawn across the field. The contact paper is developed by standard photographic procedures. Since the papergram is not sacrificed by this process, it may be evaluated subsequently by other techniques or used for the actual isolation of its components.

Utilization of cystine in man W. KNOWLTON HALL, V. P. SIDENSTRICKER* AND NATHAN RIEVIS*
Depts of Biochemistry and Medicine, Univ of Georgia School of Medicine and Med Service, Oliver General Hospital, Augusta

L-cystine was administered to each of thirty fasting adult patients in amounts of 8 grams or 20 grams. In the essentially normal subjects, there was no significant change in the blood amino acid nitrogen levels or in blood urea nitrogen over a period of 4 or 5 hours. In 5 out of 6 cases of cirrhosis of the liver there was a small but consistent drop in the blood amino acid nitrogen level after administration of 8 grams of L-cystine. In 6 of 12 cases of cirrhosis or of diseases seriously involving the liver, after the administration of cystine the excretion of amino acid nitrogen in the urine was significantly higher than in the controls.

Method for the determination of proline plus hydroxyproline carboxyl nitrogen PAUL B. HAMILTON AND PRISCILLA J. ORTIZ* *Dept of Biochemistry, Alfred I. du Pont Inst Nemours Foundation, Wilmington, Del*

Proline and hydroxyproline are unaltered by nitrous acid when reaction is carried out in hot 6N HCl, while alpha amino acids are rapidly converted to alpha hydroxy derivatives. When hydrochloric acid hydrolysates of proteins are treated with nitrous acid, proline and hydroxyproline remain and can be determined by the ninhydrin method of Van Slyke, Dillon, MacFadyen and Hamilton (*J Biol Chem* 141: 623, 1941). In the method to be described, excess nitrous acid is decomposed by ammonium sulfamate and fractional amounts of N-nitroso proline and hydroxyproline, formed during the initial treatment with nitrous acid, are hydrolysed. Reaction of nitrous acid with some alpha amino acids yields products that interfere in the ninhydrin method but these are extracted by ether. Pure proline suffers a 3% loss, pure hydroxyproline a 4% loss of carboxyl nitrogen, but the losses are constant and repro-

ducible. Cystine, tryptophane, tyrosine, arginine, ornithine and citrulline give small positive errors but in most proteins positive errors contributed by these amino acids present, compensate for the loss of imino carboxyl nitrogen so that correction factors are unnecessary. The 2 mg of imino acid necessary for determination under optimal conditions is contained in 10 mg of gelatin or 50 mg of most other proteins. Several proteins have been analysed by this technique.

Uptake and conversion of radioactive iodine by thyroid gland in tourniquet shock in rats MILTON W. HAMOLSKY*, ZYGMUNT S. GIERLACH* AND H. JENSEN *Med Dept Field Research Lab Fort Knox, Ky*

A standardized method of tourniquet 'shock' in rats produced a 24-hour mortality of 84.7% (145 animals), therapy with 0.9% NaCl lowered this to 7.88% (91 animals). Tracer doses of I^{131} (5-15 μ c without carrier) were injected intravenously, thyroids analyzed for total and organic I^{131} (percentage of injected dose). In total I^{131} there was no significant difference between controls without tourniquets versus those with occlusion maintained for 3-23 hours. However, 1 hour after tourniquet release, glands contained 50% control values, 2 hours after release, 30% of control values, 16 hours after release, only 5.2% of controls. There was no significant difference in percentage organic conversion at 1 and 2 hours, only slight decrease in 16 hours. Of 27 animals treated with saline, injected with I^{131} 16 hours later, 17 had values 1.5-4 times untreated controls. Uptake by liver, spleen, kidney, gastro-intestinal tract showed no significant difference in shock versus controls, traumatized limbs contained 3-7% more I^{131} than control limbs. *In vitro* incubation of glands *in toto* did not reveal as marked changes—uptake and conversion 2 hours after tourniquet removal were equal to control values, 16 hours after removal, both were slightly, but significantly decreased.

Fixation of radioactive carbon dioxide in lysine and tyrosine by decarboxylase-enzymes of these amino acids MARTIN E. HANKE AND M. S. H. SIDDIQI* *Dept of Biochemistry, Univ of Chicago, Chicago, Ill*

When L-lysine is treated with its decarboxylase (acetone washed cells of *Bacterium cadaveris*) in the presence of $C^{14}O_2$, and the reaction is stopped when the decarboxylation is about half complete, the remaining amino acid, and also the CO_2 formed by its subsequent decarboxylation, contain radioactivity. This CO_2 fixation does not occur if the enzyme is first heated to 100°. The fraction of the lysine synthesized (or extent of CO_2 fixation) was calculated as the ratio of the radioactive counts of the remaining lysine (or the $BaCO_3$ obtained

from the CO_2 from its decarboxylation) to the radioactive counts of the BaCO_3 obtained from the residual medium CO_2 at the end of the partial decarboxylation. The extent of CO_2 fixation at pH 7.1 and an initial lysine concentration of 0.036 M is increased from 26 to 359 parts per million as the extent of decarboxylation is increased from 17 to 48%, it is greater at pH 6.2 than at 7.1, and increases with increasing initial concentration of the amino acid. The greatest extent of CO_2 fixation observed, 1 part in 200, occurred at pH 6.2, an initial concentration of 0.13 M lysine, and a degree of decarboxylation of 62%. Similar observations were made with tyrosine and its decarboxylase, but here the residual amino acid was not isolated. Added ATP had no effect on the CO_2 fixation in either lysine or tyrosine.

Enzymatic incorporation of CO_2 in oxalacetate

ISAAC HARARY,* J. B. VEIGA SALLES* AND SEVERO OCHOA. *Dept. of Pharmacology, New York Univ. College of Medicine, New York City*

Highly purified pigeon liver 'malic' enzyme (*J. Biol. Chem.* 174: 979, 1948) catalyzes reactions (1) $l\text{-malate} + \text{TPN}_{\text{ox}} \rightleftharpoons \text{pyruvate} + \text{CO}_2 + \text{TPN}_{\text{red}}$ and (2) $\text{oxalacetate} \rightarrow \text{pyruvate} + \text{CO}_2$, in presence of Mn^{++} . (1) has a sharp optimum at pH 7.4, (2) at pH 4.5. $\text{COOH}-\text{CHOH}-\text{CH}_2-\text{C}^{14}\text{OOH}$ is obtained when (1) proceeds in presence of C^{14}O_2 although little or no label is incorporated into added oxalacetate (OAA). This shows that free OAA is not an intermediate in (1). No radioactive OAA is formed when the enzyme catalyzes reaction (2) in presence of C^{14}O_2 . The same is true of bacterial preparations which catalyze (2) but not (1). This indicates that (2) is practically irreversible. Ready incorporation of C^{14}O_2 in OAA occurs, however, when Straub's malic dehydrogenase is added to 'malic' enzyme, OAA, TPN, and Mn^{++} , if a catalytic amount of malate is present. Malic dehydrogenase catalyzes reaction (3) $\text{OAA} + \text{DPN}_{\text{red}} \text{ (or } \text{TPN}_{\text{red}}) \rightleftharpoons l\text{-malate} + \text{DPN}_{\text{ox}} \text{ (or } \text{TPN}_{\text{ox}})$ and will form $\text{COOH}-\text{CH}_2-\text{C}^{14}\text{OOH}$ from C^{14} -malate. The sum of (1) and (3) is (4) $l\text{-malate} + \text{OAA} \rightleftharpoons \text{pyruvate} + \text{CO}_2 + l\text{-malate}$, i.e., $\text{OAA} \rightleftharpoons \text{pyruvate} + \text{CO}$ (Wood and Werkman reaction). Evidence for (4) was also obtained manometrically. CO_2 fixation in OAA in pigeon liver extracts with added ATP (*J. Biol. Chem.* 164: 455, 1946) may occur via reaction (4).

D_2O dilution space to measure body water—relation of body water to body size JAMES D. HARDY* AND DAVID L. DRABKIN. *Harrison Dept. of Surgical Research and Dept. of Physiological Chemistry, Grad. School of Medicine, Univ. of Pennsylvania, Philadelphia*

The D_2O dilution space, as a measure of total body water, was determined in 10 adult human subjects, ranging in size from very lean (male

of 60.9 kg mass and 91 cm height and female of 53.7 kg and 90.2 cm) to very obese (male of 121 kg and 90.2 cm and female of 86.4 kg and 76.2 cm). After equilibration of intravenously injected heavy water with the body water, the concentration of D_2O in the water distilled from plasma samples was obtained by means of accurate specific gravity determinations. The extracellular fluid and plasma spaces were also determined by means of sodium thiocyanate and the dye T-1824. The total body water and body mass were found to have an inverse relationship to each other. In percentage of body weight, body water varied from 70 (the commonly accepted value) in the abnormally lean to as low as 40 in the most obese. The volumes of extracellular fluid and plasma were more closely correlatable with total body water than with body mass. Evidence for a reciprocal relationship between water and fat is provided by our findings, and a revision of views as to the quantities of body fluids becomes necessary. An objective evaluation of overnutrition (excess fat) was derived from the nutritive index (COWGILL, G. R. AND D. L. DRABKIN. *Am. J. Physiol.* 81: 36, 1927). A nomogram has been constructed for the prediction of excess fat and total water content of subjects of widely different size.

Lactic acid oxidizing system of *Escherichia coli*

NIELS HAUGAARD (introduced by W. C. STADIE)

John Herr Musser. *Dept. of Research Medicine, Univ. of Pennsylvania, Philadelphia*

The mechanism of lactic acid oxidation by *E. coli* (strain B) has been studied. The organisms were grown in media containing DL-lactate as the source of carbon. Cell free preparations were obtained by exposing suspensions to sonic vibration. Such preparations under aerobic conditions were found to convert DL-lactic acid quantitatively into pyruvic acid. In experiments in which the reaction was allowed to go to completion an exact balance was obtained between the lactic acid added, the oxygen taken up, and the pyruvate formed. The entire metabolic activity was associated with particulate matter, the supernatant after high speed centrifugation having no activity. A complete hydrogen transferring system was apparently situated on the suspended particles since the preparations oxidized lactate in the absence of added hydrogen carrier. Addition of methylene blue did not further increase the activity. An acetone powder was prepared from the suspensions obtained by sonic vibration. A suspension of such an acetone powder was inactive in the absence of added methylene blue. When methylene blue was added it converted half of added DL-lactate to pyruvate. It was inactive in the presence of L-lactate. It is concluded that two enzyme systems are present in *E. coli*, one oxidis

mg D-lactic acid, the other L-lactic acid. Precipitation with acetone destroys the system oxidizing L lactic acid as well as part of the hydrogen transferring enzymes.

Hemoglobin, oxyhemoglobin and anhydro-hemoglobin FELIX HAUROWITZ, SAIDE TUNC* AND RADITE CINDI* *Dept of Chemistry, Indiana Univ, Bloomington, Ind*

Moist layers of oxyhemoglobin (HbO_2) exposed to low pressures are converted into hemoglobin ($\text{Hb} \cdot \text{H}_2\text{O}$), and, on evaporation of the water at low temperatures, into anhydro-hemoglobin (Hb), a hemochromogen-like substance with absorption maxima at 559 and 528 μ (v Zeynek 1926, Haurowitz, in *Hemoglobin* New York Interscience, 1949). Anhydro-hemoglobin dissolved in water in the presence of air furnishes HbO_2 which on reduction gives hemoglobin, but no hemochromogen. It is concluded that in hemoglobin one of the water molecules is bound to Fe, replacing the O_2 molecule in HbO_2 . The equilibrium of reaction (I) $\text{Hb} + \text{H}_2\text{O} \rightleftharpoons \text{Hb} \cdot \text{H}_2\text{O}$, was examined spectrophotometrically at different temperatures. The ratio $\text{Hb} \cdot \text{H}_2\text{O}/\text{Hb}$ was approximately 1.0 at 34°C and 22 mm Hg. Reaction (I) is reversible between 0° and 40°C. On the other hand, dry HbO_2 does not dissociate into Hb and O_2 , even when exposed to low pressure ($p_{\text{O}_2} = 0.06$ mm). We were not able, therefore, to determine experimentally the equilibrium constant of reaction (II) $\text{Hb} + \text{O}_2 \rightleftharpoons \text{HbO}_2$. This equation, (II), is the arithmetical sum of the above mentioned equation (I), and of equation (III) $\text{Hb} \cdot \text{H}_2\text{O} + \text{O}_2 \rightleftharpoons \text{HbO}_2 + \text{H}_2\text{O}$. Hence the equilibrium constant K_{II} is equal to $K_{\text{I}} \times K_{\text{III}}$. Using data of Roughton and Hartridge (1925, 1936) for K_{III} we find that K_{II} is approximately 10^3 . This high value of K_{II} is in accordance with the weak dissociation tendency of dry HbO_2 found in our experiments. Evidently O_2 is bound more firmly by Hb than is water. The higher affinity of Hb for oxygen is attributed to the covalent linkage of O_2 in the diamagnetic complex HbO_2 (Pauling and Coryell). The combination of hemoglobin with O_2 in aqueous solution is represented by equation (III) and not by (II).

Effect of body exposure to x-rays on rate of incorporation of C^{14} carboxyl-labeled alanine into mouse protein L. H. HEMPELMANN, S. CARR, I. D. FRANTZ, JR., R. MASTERS AND E. LANDIN (introduced by LEWIS L. ENGEL) *Med Labs of the Collis P. Huntington Memorial Hospital of Harvard Univ, at the Massachusetts General Hospital, Boston, Mass*

The influence of lethal doses of x-rays on the *in vivo* incorporation of C^{14} carboxyl-labeled alanine into the protein fraction of spleen, liver, and muscle was studied in young mice. Decarboxylation by means of the ninhydrin reaction was used

to determine the quantity of total and radioactive carbon in the free carboxyl groups of the amino acids in protein hydrolysates. The specific activity of C^{14} was unchanged in liver, spleen, and muscle protein of litter mates divided into control and irradiated groups when the alanine was given immediately after exposure and the animals killed 2 hours later. When the alanine was given 2 hours after exposure, the amount of incorporated C^{14} in the spleen protein was reduced in the irradiated groups. The total carboxyl carbon in the liver and spleen protein of irradiated animals was considerably less than that of controls 4 days after exposure. The specific activity in muscle and spleen protein was less in the irradiated than in the controls while the activity in the liver was greatly increased in the exposed animals. The distribution of C^{14} in the amino acid fractions of the liver protein of irradiated and control animals was determined by means of starch column chromatography.

The *in vivo* observations were largely confirmed by studies with spleen and liver slices, but differences from normal were less striking. The amount of C^{14} in the urea fraction of the liver slice media was greater in irradiated than in control experiments.

Effect of riboflavin and vitamin B_6 deficiencies on conversion of tryptophan and 3-hydroxyanthranilic acid to quinolinic acid L. M. HENDERSON, I. M. WEINSTOCK* AND G. B. RAMASARMA* *Division of Biochemistry, Noyes Lab of Chemistry, Univ of Illinois, Urbana*

Vitamin B_6 , thiamine, riboflavin, and folic acid deficiencies have been reported to reduce the urinary excretion of N^1 -methylnicotinamide and nicotinic acid following the administration of tryptophan to the rat. Urinary quinolinic acid accounts for a larger percentage of administered tryptophan or 3-hydroxyanthranilic acid than either nicotinic acid or N^1 -methylnicotinamide. Attempts to localize the reactions in which the vitamins are concerned have been made by studying the excretion of quinolinic acid in response to tryptophan and 3-hydroxyanthranilic acid. Control rats receiving a 9% casein diet excreted $11.0 \pm 1.1\%$ of the administered tryptophan and $12.7 \pm 2.0\%$ of the 3-hydroxyanthranilic acid as quinolinic acid. Rats acutely deficient in riboflavin excreted $1.06 \pm 0.08\%$ of the tryptophan and $7.3 \pm 1.6\%$ of the 3-hydroxyanthranilic acid as quinolinic acid, vitamin B_6 -deficient animals excreted only $0.73 \pm 0.16\%$ of the administered tryptophan and $8.8 \pm 1.1\%$ of the 3-hydroxyanthranilic acid as quinolinic acid. Results with pair-fed control groups indicated that inanition was not the cause of these changes. Thiamine-deficient animals and their pair-fed controls exhibited reduced conversion of

both tryptophan and 3-hydroxyanthranilic acid. Increasing the casein level of the diet from 9%–18% reduced the amount of administered tryptophan excreted as quinolinic acid to 3.0% for control animals and to 0.4% for vitamin B₆-deficient animals. These results suggest that riboflavin and vitamin B₆ function in the conversion of tryptophan to 3-hydroxyanthranilic acid and not in the conversion of the latter to quinolinic acid.

Specific nucleotidases LEON A. HEPPEL AND R. J. HILMOE * *Natl. Insts. of Health, Bethesda, Md.*

Studies have been carried out with enzymes from several sources that hydrolyze nucleotides containing phosphate in different positions. Preliminary experiments with potato extracts indicate the presence of a nucleotidase specific for adenosine-5-phosphate and another for adenosine-3-phosphate. Bull. seminal plasma, which rapidly dephosphorylates adenosine-5-phosphate (MANN *Biochem J* 39:451, 1945) was also investigated. A 50-fold purification of this activity was achieved by fractionation with salmine, ammonium sulfate and ethanol, and by adsorption with alumina C γ . The rate of liberation of inorganic phosphate from different compounds by seminal plasma and by the purified enzyme is given below. (The activity of both preparations is expressed on the basis of 100 for adenosine 5-phosphate.)

SUBSTRATE	ACTIVITY OF	
	SEMINAL PLASMA	PURIFIED ENZYME
Inosine-5-phosphate	115	113
Nicotinamide-ribose-5-phosphate	70	67
Ribose-5-phosphate	1.3	0.8
Adenosine-3-phosphate	2.0	<0.005
Sodium- β -glycerophosphate	1.0	0.02
Glucose-6-phosphate	0.8	<0.01

Guanosine-3-phosphate, riboflavin phosphate, fructose-1, 6-diphosphate, fructose 6-phosphate and 3-phosphoglyceric acid were hydrolyzed more slowly than glucose-6-phosphate by seminal plasma and the activity was greatly reduced on purification. These data would support the view that a single enzyme dephosphorylates the several compounds containing phosphate on position 5 of the ribose moiety. Snake venom has a nucleotidase of apparently similar substrate specificity but purification has been hampered by its instability.

Effect of cysteine and hydrogen sulfide on pteroylglutamic acid conjugase of hog kidney C. H. HILL AND M. L. SCOTT (introduced by L. C. NORRIS) *Cornell Univ., Ithaca, N. Y.*

Mims, Swendsen, and Bird (*J. Biol. Chem.* 170:367, 1947) reported that the inhibitory effect of nucleic acid upon the pteroylglutamic acid (PGA)

conjugase of hog kidney is overcome *in vitro* by the addition of cysteine or hydrogen sulfide to the enzyme system. These research workers stated that their findings suggested the presence of sulfhydryl groups in the PGA conjugase of hog kidney. In extending the studies of Mims *et al.* in this laboratory it was found that exceedingly low levels of hog kidney conjugase, which were completely inactive alone, exhibited maximum activity when cysteine was added to the enzyme system. Hydrogen sulfide also served as an activator of this system, but the rate of release of PGA was not increased with this substance to the same extent that it was with cysteine. Relatively high levels of cysteine were required to produce maximum activation of the enzyme. On the other hand, much lower levels of cysteine, which produced little activation alone, were completely effective when a low level of hydrogen sulfide was also added. The addition of sodium thioglycolate, which is known to inhibit cysteine de-sulfhydrase activity, completely inhibited PGA conjugase activation by either cysteine or hydrogen sulfide. These studies present further evidence indicating that the conjugase of hog kidney requires sulfhydryl groups. While hydrogen sulfide was shown to activate the enzyme, the results point to cysteine as the primary sulfhydryl donor. A possible explanation of the action of hydrogen sulfide is that this substance may transfer sulfhydryl groups to cysteine which in turn transfers them to the enzyme.

Inhibition of the 'oxalacetic oxidase system' in tissues adjacent to methylcholanthrene tumors ROBERT M. HILL, DORSEY E. HOLTKAMP* AND ENID K. RUTLEDGE * *Depts. of Biochemistry and Pathology, Univ. of Colorado Med. Center, Denver*

Potter, LePage, and Klug (*J. Biol. Chem.* 175:619, 1948) reported that "homogenates of several rat tumors showed negligible amounts of oxygen uptake when oxalacetate" was used as a substrate. Using a similar reaction mixture, and a transplantable methylcholanthrene induced fibrosarcoma of the thigh muscle of the rat, we were able to verify their observations. We have compared homogenates of these tumors with 1) homogenates of muscle tissue adjacent to the tumor, but not having a neoplastic appearance by histological examination and with 2) homogenates of corresponding normal muscle tissue from the opposite leg. The muscle tissue adjacent to the tumor shows some O₂ uptake but considerably less than does the corresponding normal tissue of the opposite leg. Observations have been made on 17 animals with methylcholanthrene induced tumors and on 23 animals in the first, second, and third generations of transplantation of these tumors.

Inhibitory effect of beef extract and 2-methylthio-

adenine on growth of typhoid bacillus G H HITCHINGS, G B ELION* AND M B SHERWOOD* *Wellcome Research Labs, Tuckahoe, N Y*

During routine tests of the antibacterial effects of purine and pyrimidine analogs, by the filter paper disk-agar plate technique, 2-methylthio 6-aminopurine was found to inhibit the growth of typhoid bacillus powerfully. When these tests were repeated, with substitution of a synthetic medium in the agar for the usual proteose peptone-beef extract mixture, no inhibition by the antagonist was observed, although no difference in growth on the 2 media could be detected. The addition of various substances to the synthetic medium resulted in re-appearance of the antibacterial effect of the analog. Beef extract is the most effective substance found to date, but liver extract and yeast extract have appreciable activities. The concentration of the beef extract factor was followed by preparing agar containing the synthetic medium and 2-methylthioadenine and seeding this with the typhoid bacillus. Growth on this medium is excellent, but zones of inhibition can be produced by the application of filter paper disks which have been dipped in beef extract solution. Paper chromatography of beef extract revealed the presence of at least 2 substances with activity. Fractional precipitation by alcohol and adsorption and elution from superfiltrol have resulted in a considerable concentration of the factor.

Synthesis and microbiological activity of higher aliphatic β -keto acids KLAUS HOFMANN, A E AXELROD* AND M A MITZ* *Dept of Chemistry, Univ of Pittsburgh, Pittsburgh, Pa*

In connection with studies on the relation of biotin and fatty acids, a number of higher β -keto acids were prepared and their microbiological activities determined. Although higher β -keto acids have been postulated as intermediates in the metabolism of fatty acids, little is known about their preparation and properties. Unsaturated members of the series have not been previously prepared. Highly purified fatty acids were converted into the corresponding β -keto esters containing 2 additional carbon atoms by the procedure of Stållberg-Stenhagen (*Archiv Kemi Miner Geol* 20A No 19, 1945). In view of the fact that the known procedures for the saponification of β -keto esters are highly unsatisfactory, a new procedure was developed which allows the preparation of β -keto acids in high yields. β -ketomyristic, palmitic, stearic and arachidic acids were prepared and characterized. Application of the same procedures to oleic acid leads to the synthesis of 3-keto $\Delta^{11,12}$ eicosenoic acid (oleoyl acetic acid). The β -keto acids and esters were tested for their ability to replace biotin for *Lactobacillus arabinosus*. The

saturated β -keto acids were inactive. Methyloleoyl acetate was as active as methyloleate in contrast to free oleoyl acetic acid which was practically inactive.

Alterations in tissue proteins of dogs following prolonged fasting ELMORE HOLMES* AND DEMPSEY B MORRISON *Dept of Chemistry, Univ of Tennessee, Memphis*

Proteins of heart, skeletal muscle, liver, kidney, and blood plasma were analyzed in the Tiselius-Longsworth apparatus. Dissected tissue was homogenized in a Waring blender. The homogenate was then frozen and thawed after which aliquots were taken for centrifugation (12,000 r p m), for determination of water content, total solids, and proteins (Kjeldahl), and for fractionation of the proteins by extraction. Proteins soluble in 0.12 M NaCl were first removed by repeated extraction. The residue was then similarly extracted with 1.0 M NaCl, and finally with 0.06 M NaOH. Only a small insoluble fraction remained after these successive extractions. All extractions were carried out at 3-5°. Total proteins were determined on each extract, on the final insoluble residue, and on the supernatant obtained by centrifugation. The supernatant fluids and saline extracts were also equilibrated against veronal buffer (pH 8.6) preparatory to electrophoresis. Representative electrophoretic patterns of the plasma and tissue extracts of well fed and fasted dogs will be shown. Alterations in the proteins as shown by fractional extraction and by electrophoretic analysis will be presented graphically.

Phosphogluconic acid metabolism B L HORECKER *Natl Insts of Health, Bethesda, Md*

The oxidation of 6-phosphogluconic acid was studied by Warburg and Christian and by Dickens and shown to require triphosphopyridine nucleotide (TPN). Several degradation products were obtained, none of which was positively identified. An enzyme has now been purified from yeast which quantitatively converts 6-phosphogluconate to pentose phosphate and CO_2 . The enzyme occurs in extracts of liver, kidney, brain, heart muscle and red cells, as well as in yeast maceration juice. The yeast enzyme has been purified 40-fold by a number of steps including acetone precipitation and fractionation with ammonium sulfate. With the purified enzyme pentose-phosphate accumulation was accomplished by means of the following dismutation:

- 1) 6-phosphogluconate + TPN \rightarrow
pentose phosphate + CO_2 + TPNH_2
- 2) pyruvate + $\text{TPNH}_2 \rightarrow$ lactate + TPN

In agreement with equation 1, one μ of pentose phosphate and one μ of CO_2 are produced/ μ of 6-phosphogluconate (or pyruvate) disappearing.

CO₂ production at intermediate stages in the reaction is equal to 6-phosphogluconic acid removal or pentose phosphate production, no accumulation of a 6-carbon oxidized intermediate is observed. The pentose phosphoric acid ester formed is hydrolyzed by a phosphatase purified by Dr L. A. Heppel from semen at a much slower rate than is ribose-5-phosphate. In glycylglycine buffer at pH 7.5 the rate of TPN reduction by 6-phosphogluconate is increased 5-fold by Mg⁺⁺ or Mn⁺⁺, or 2-fold by CN⁻. *Reaction 1* is used for the determination of 6-phosphogluconate.

Synthesis of rhodopsin from vitamin A₁ RUTH HUBBARD AND GEORGE WALD (introduced by J. T. EDSALL) *Biological Labs, Harvard Univ, Cambridge, Mass*

It has been believed since Kühne that the synthesis of rhodopsin from colorless precursors, now known to be vitamin A₁ and rhodopsin-protein, occurs only in the intact eye. This process has now been carried out *in vitro*. Intact retinas and retinal homogenates, incubated in the dark, regenerate about 10% as much rhodopsin from vitamin A₁ as is formed during dark adaptation *in vivo*. The addition of cozymase, or of a homogenate of the pigment layers of the eye, pigment epithelium and choroid, to a retinal homogenate approximately doubles the yield of rhodopsin. When both cozymase and pigment layer homogenate are added to a retinal homogenate, the yield is doubled again, approximating 40%. Aqueous retinal extracts to which cozymase and vitamin A₁ are added also synthesize rhodopsin in a yield of about 10%. The mechanism of these syntheses is not yet wholly understood. Retinal homogenates can oxidize vitamin A₁ slowly to retinene₁ (vitamin A₁ aldehyde) in the presence of an aldehyde-binding reagent such as hydroxylamine. In the retina, rhodopsin-protein is a specific retinene-binding reagent, condensing with retinene₁ spontaneously to form rhodopsin. One pathway for rhodopsin synthesis is therefore the oxidation of vitamin A₁ to retinene₁ by retinene reductase, coupled with the binding of retinene₁ by rhodopsin-protein to form rhodopsin. Cozymase promotes this process as the coenzyme of the retinene reductase system. Pigment layer homogenate may act upon the same system by supplying respiratory factors which drive it in the oxidative direction. Rhodopsin may also be synthesized by other mechanisms still to be explored.

Potentialiation of esterase activity of chymotrypsin by electrolytes BERNARD J. JANDORF *Biochemistry Section, Med. Division, Army Chemical Center, Md*

With commercial preparations of chymotrypsin no direct relationship was found between enzyme concentration and the rate of action on N-acetyl-

tyrosine ethyl ester as determined manometrically. The divergence from proportionality is due in part at least to a variable content of MgSO₄ in the enzyme preparations employed. When sufficient MgSO₄ is added to the reaction mixture, the esterase activity becomes proportional to the concentration of chymotrypsin. In addition to this effect, MgSO₄ increases esterase activity markedly, values exceeding the control activity by fivefold or more being obtained if sufficient salt is added. This effect of MgSO₄ can be duplicated by other salts, while no potentiation of esterase activity is found in the presence of non-electrolytes. On partial inactivation of chymotrypsin with diisopropylfluorophosphate, the enzymatic activity remaining is potentiated by salts to the same degree as that found for the completely active enzyme, this indicates that the activating effect of salts is not due to their rendering enzymatically active groups on the protein molecule more accessible to the substrate. When limiting amounts of substrate are employed, the presence of salt affects only the rate and not the extent of ester hydrolysis. No salt which was found to potentiate the esterase activity of chymotrypsin had any effect on the proteinase (casein hydrolysis) activity of this enzyme.

A crystalline, active oxidation product of α -chymotrypsin EUGENE F. JANSEN, M. D. FELLOWS NUTTING,* A. LAURENCE CURL* AND A. K. BALLS *Enzyme Research Division, Bureau of Agricultural and Industrial Chemistry, United States Department of Agriculture, Albany, Calif*

Crystalline α -chymotrypsin was found to contain an impurity which gave rise to acetaldehyde on oxidation with sodium periodate. The amount of this 'terminal threonine' varied from 0.5-1.0 M/M of enzyme, using 27,000 for the molecular weight of the enzyme. With just sufficient periodate (2.5 M/M of enzyme) to cause the maximum formation of acetaldehyde within one hour, no decrease in the activities (esterase and proteinase) occurred. Doubling this amount of periodate resulted in no more acetaldehyde, but in 60% destruction of the activities when assayed under conditions for α -chymotrypsin. Larger amounts of periodate (as much as 50 M/M of enzyme) caused no greater decrease. The oxidation product resulting from 5-10 M of periodate/M of enzyme was crystallizable from 0.5 saturated ammonium sulfate at pH 5.5, but attempts to crystallize at pH 4.0 (where g-chymotrypsin crystallizes nicely) have met with failure. After 3 recrystallizations the yield of oxidized enzyme was 35%. The proteolytic pH optimum of the recrystallized product was identical with that of α -chymotrypsin, but the optimum for tyrosine ethyl ester hydrolysis was changed by oxidation from pH 6.25 to 6.60. When compared at

the respective optima, the specific esterase activity of oxidized enzyme was 80% and the protease 45% of the original. Acetylation of α -chymotrypsin gave a product with activities very similar to the oxidized material, suggesting that the removal or blocking of an amino group may be a factor.

Competitive inhibition between certain salicylates and pantothenic and pantoic acids for *E. coli*
B. CONNOR JOHNSON AND ELLA M. COHN * *Division of Animal Nutrition, Univ. of Illinois, Urbana*

Twelve different compounds were studied as to their inhibiting effect on the growth of 4 strains of *E. coli*. Of the compounds studied, only salicylic acid and its derivatives acetyl salicylic acid and phenyl salicylate completely inhibited growth at low concentrations (approx. 5 μ M). This inhibition was completely reversed by calcium pantothenate or equally well by pantoic acid but was not reversed by β -alanine, p-aminobenzoic acid, methionine, glutamic acid, lysine or valine. Mandelic acid (in contrast to the report of PERAULT AND GRIFIN *Compt. rend. Soc. de biol.* 138: 506, 1944), cinnamic acid, benzoic acid, m- and p-hydroxy benzoic acids, anthranilic acid and gallic acid gave growth inhibition only at much higher concentrations, and this inhibition was not reversed by pantothenic acid or pantoic acid. The only diphenyl hydroxy acid tested, malic acid, was found to stimulate rather than inhibit growth of *E. coli* on the simple medium used. Salicylic acid, bismuth salicylate and phenyl salicylate have been fed to rats at 0.1 to 1% of pantothenic acid-free diets in attempts to inhibit coliform synthesis of pantothenic acid in the intestinal tract. Under some of these conditions a severe deficiency was produced which was completely counteracted by pantothenic acid.

Distribution of free amino acids between erythrocytes and plasma in normal human subjects
C. A. JOHNSON* AND OLAF BERGEIM *Dept. of Biological Chemistry, Univ. of Illinois, College of Medicine, Chicago*

The plasma and red cell levels of 12 amino acids were determined on single blood samples obtained from 24 young men in a postabsorptive state. Clotting was prevented by heparin and the cell volume measured by hematocrit. Following centrifugation the plasma was siphoned off, the white cell layer removed and discarded, and the red cells hemolysed. The plasma and red cell fractions were then deproteinized with tungstic acid. Microbiological assays of the tungstic acid filtrates were carried out. The results indicated that the concentrations (expressed in γ /ml. of plasma or red cells) of methionine, phenylalanine, threonine and tyrosine were approximately the same in both blood fractions. For the remaining amino acids, the

ratios of the concentrations in plasma to the concentrations in red cells increased in the following order: leucine, histidine, valine, lysine, isoleucine, cystine, tryptophane and arginine, the ratios ranging from 1.2 for leucine to 6.2 for arginine. If the individual amino acid concentrations were calculated on the basis of red cell water (65%) and plasma water (92%), the 12 amino acids studied might be arranged in three groups: 1) those with cell levels above plasma levels, 2) those about equally distributed, and 3) those with cell levels below plasma levels. It would appear, therefore, that red cells may show some selectivity in regulating the passage and accumulation of these amino acids.

Mode of action of the sperm 'regulator,' 2,4-dinitrophenol and usnic acid
R. BERNAL JOHNSON,* AND HENRY A. LARDY *Dept. of Biochemistry, Univ. of Wisconsin, College of Agriculture, Madison*

In studies of the function of a naturally occurring metabolic 'regulator' (*Science* 109: 365, 1949) it was found that, like dinitrophenol and usnic acid, this substance prevents the phosphorylation reactions normally associated with substrate oxidation by a washed residue of rabbit kidney or rat liver homogenate. In the absence of such 'uncoupling' agents, the oxidation of either carbohydrate or fatty acids (JOHNSON AND LARDY *J. Biol. Chem.* in press) is accompanied by a net uptake of inorganic orthophosphate. With either citrate, *cis*-aconitate, α -ketoglutarate, succinate, malate, fumarate, or pyruvate plus a trace of fumarate as the substrate, about 15 μ M of phosphate is taken up in 10 minutes and transferred to glucose by means of yeast hexokinase. In the presence of suitable concentrations of either the sperm 'regulator,' dinitrophenol or usnic acid, uptake of phosphate occurs but it accumulates in the form of an extremely acid-labile compound. This unidentified ester (not inorganic pyrophosphate or metaphosphate) is reasonably stable at pH 4. At this pH its hydrolysis is catalyzed by molybdate. Under some conditions the labile ester accumulates even in the absence of the 'uncoupling' agents indicating that it may be a naturally occurring intermediate.

Synthesis of glutathione in extracts of pigeon liver
ROBERT B. JOHNSTON* AND KONRAD BLOCH *Dept. of Biochemistry and Inst. of Radiobiology and Biophysics, Univ. of Chicago, Chicago, Ill.*

The formation of the peptide bond between cysteine and glycine in glutathione has been investigated with the aid of C^{14} -glycine. A dialyzed saline-bicarbonate extract of acetone-dried pigeon liver was the source of the enzyme. Peptide bond formation, as measured by the incorporation of labeled glycine into glutathione, occurs in such extracts at a rapid rate when glutamic acid, cysteine,

glycine, Mg^{++} , phosphate, and either adenosine triphosphate or 5-adenylic acid are added Under these conditions ATP and 5-adenylic acid are equally effective, while inosinic acid and guanylic acid show a much smaller effect The isolated glutathione contains no significant radioactivity when the adenylic acid derivatives are absent or when Mg^{++} or phosphate are omitted Under the same experimental conditions peptide synthesis can also be effected by the addition of various amides In the presence of enzyme, amino acids and salts, the following amides caused the formation of C^{14} glutathione glutamine, asparagine, pyruvamide, and acetamide In these experiments also the presence of phosphate is necessary These results suggest that the energy of the amide bond can be utilized for peptide synthesis

In vitro effect of DDT and its metabolites on rat heart succinoxidase CARTER D JOHNSTON (introduced by R LORIMER GRANT) *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D C*

DDT, its dehydrochloride, and its acetic acid derivative (DDA) were added to rat heart homogenates suspended in phosphate buffer (pH 7.4), using succinate as the substrate Oxygen consumption was measured manometrically DDT inhibited this succinoxidase system 60% at a concentration of 10^{-4} M, while DDA (an important metabolite of the insecticide) caused only 33% inhibition at 5 times this concentration The dehydrochloride, possibly a metabolic intermediate, is as effective as DDT in suppressing the oxygen uptake of these preparations

Effect of desoxycorticosterone glucoside (DCG) on glucose reabsorption in the dog kidney

EDWIN H KAUFMAN AND AGAMENON DESPOPOULOS (introduced by DONALD D VAN SLYKE) *Hospital of Rockefeller Inst for Med Research, New York City*

St Rusznyak *et al* (*Experientia* 3:420, 1947) reported that intravenous DCG (Percorten Glucoside, Ciba) in barbiturate-anesthetized dogs produced a several-fold increase in Glucose Tm The present study, in which TmG was measured before and after i.v. injection of DCG in dogs under pentobarbital anesthesia, was an attempt to confirm this remarkable observation Our results showed, on the contrary, a prompt depression of TmG following single doses of 30-100 mg in dogs weighing 13-25 kg, with TmG decreasing to zero after the 100 mg dose The inhibition persisted 30 minutes after 30 mg, and longer with larger doses Reduction of 25% of control TmG was maintained by constant i.v. infusion of 1 mg/min Marked glycosuria, with no change from normal in blood glucose concentration, following injection of 150 mg of DCG in a dog receiving no exogenous glu-

cose DCG had no consistent effect on glomerular filtration rate These results are in agreement with those recently published by Lambert and associates (*Acta Clin Belgica* 3:529, 1948) showing that DCG depresses TmG in man The failure of 30 and 60 mg i.v. doses of free desoxycorticosterone in propylene glycol to decrease TmG below levels produced by control injections of the vehicle alone, indicates that the depressant action on the glucose reabsorptive mechanism is a property of the glucoside rather than the free steroid That this action may be analogous to that of phlorizin is suggested by certain structural similarities between the two molecules

Anticoagulant polysaccharide sulfates LOUIS A KAZAL, DANIEL S SPICER* AND ROSE A BRAHINSKY* *Dept of Biochemistry, Med Research Division, Sharp and Dohme, Inc, Glenolden, Pa*

A number of polysaccharides were sulfated with pyridine chlorosulfonic acid (P.C.A.) (2.5 hours at 75-80°C), and the sulfated products screened for anticoagulant activity by a modification of the prothrombin time determination Chlorosulfonic acid and concentrated sulfuric acid also were employed as sulfating agents in some instances The sulfated products obtained with P.C.A. were more active as anticoagulants than those obtained with the other agents The most active products were derived from dextrin, karaya gum, tragacanth, chitin, sodium ammonium pectate, cellulose and oxidized cellulose Dextrin was selected for more detailed studies, it was sulfated under various conditions of time and temperature, and the resultant products tested for anticoagulant activity and for toxicity in rabbits Essentially maximum activity was reached in 2 to 1 hour periods of sulfation at 60°C and 102°C, and in 3 hours at 45°C At a constant temperature of sulfation, less toxic products were obtained with short periods of sulfation, with time a constant, less toxic products were obtained at lower temperatures of sulfation Using constant conditions for sulfation (1 hour, 60°C) the products derived from low ml wt dextrans were more active The glycolic acid ether of dextrin sulfate was slightly less active than the parent substance The dextrin sulfates have an *in vivo* activity in the rabbit approximately equivalent to $\frac{1}{2}$ of that of heparin (Connaught Standard)

Reversible changes in the L-amino acid oxidase molecule EDNA B KEARNEY* AND THOMAS P SINGER *Dept of Biochemistry, Western Reserve Univ School of Medicine, Cleveland, Ohio*

We have reported (*Federation Proc* 8 no 1) that the oxidase of many snake venoms is inactivated by low concentrations of $PO_4^{=}$ and related ions It has been found that in distilled water the enzyme is inactivated similarly because of protein-bound

PO_4^{2-} and that at higher temperatures an opposing process of reactivation occurs. An equilibrium therefore exists between the active and inactive forms of the enzyme characteristic of the temperature and pH, at 45°C (pH 7) the equilibrium is largely in favor of the active form, and at 30° , the inactive form. The temperature determines both the position of the equilibrium and the rate at which it is reached. Monovalent anions prevent the inactivation and enhance reversal, incubation with Cl^- at 38° completely reactivates the inactive enzyme. PO_4^{2-} increases the rate of inactivation and shifts the equilibrium toward the inactive form. Following inactivation by PO_4^{2-} , heating with Cl^- effects reactivation only after the excess PO_4^{2-} is removed by dialysis. While PO_4^{2-} is rapidly bound by the protein even at 0°C , the inactivation occurs only gradually on incubation at higher temperatures, indicating a secondary process with an appreciable activation energy. This process is not a polymerization or denaturation, as the inactive enzyme retains the size and shape of the active form, as judged by ultracentrifuge studies. The mechanism of the inactivation may involve an intramolecular bridge formation between PO_4^{2-} and a protein group, possibly histidine. The nature of the bond is being studied with the aid of P^{32} .

Inhibition of rat liver choline oxidase by urethane
in vitro CECILIA K. KEITH AND JAMES S. DINNING (introduced by PAUL L. DAY) *Dept. of Biochemistry, Univ. of Arkansas School of Medicine, Little Rock*

Choline oxidase was determined by standard Warburg procedure. The final volume in the flasks was 3.2 ml containing 1 ml of a 5% rat liver homogenate buffered at pH 7.8. With 5 mg of choline chloride added as substrate, 5, 10, 50, and 100 mg of urethane inhibited choline oxidase 4, 27, 78, and 84% respectively. With 50 mg of urethane the addition of 5, 10, 15, 20 and 25 mg of choline chloride resulted in the consumption of 24, 30, 38, 33, and 28 μl O_2/hr respectively. The tissue preparation with 25 mg of choline chloride as substrate and no urethane consumed 98.6 μl O_2/hr . It appears that the inhibition of choline oxidase produced by 50 mg of urethane is not readily reversible by increasing the amount of substrate. Urethane was without effect on the succinic oxidase activity of these tissue preparations. This inhibition of choline oxidase by urethane is of interest in view of the fact that the nitrogen mustards and aminopterin, also substances which reduce white cell counts in animals, inhibit choline oxidase.

Lipotropic effect of pteroylglutamic acid on rats receiving toxic levels of dietary glycine BARBARA KELLEY* AND JOHN R. TOTTER *Dept. of Biochemistry, Univ. of Arkansas School of Medicine, Little Rock*

Weanling littermate Sprague-Dawley rats were placed on an adequate purified diet containing 10% glycine with and without added pteroylglutamic acid (PGA). Another group of animals received the diet without added glycine (basal diet). Liver fats were determined on all of the animals after 54–60 days on the various diets. The addition of 10% glycine to the diets brought about a 50% increase in the fat content of the liver over that on the basal diet. When PGA was added to the high glycine diet at a level of 5 mg/kg the liver fat was reduced to a value slightly lower than that obtained on the basal diet. In one experiment the average values for liver fatty acids of 10 animals were as follows: 10% glycine diet, 5.68%, 10% glycine + PGA, 3.70%, and basal diet 3.92%. The possibility that the observed increase in liver fats on the high glycine diet is due to a metabolic deficiency of choline is suggested by an elevated kidney weight/body weight ratio, which was also reduced to normal by PGA.

An automatic multiple extraction apparatus

MARIAN W. KIES AND PAUL L. DAVIS * *Bureau of Agricultural and Industrial Chemistry, Agricultural Research Center, Beltsville, Md*

Recent work on countercurrent distribution has emphasized its usefulness in biochemical fractionations, but the available equipment is inadequate for large-scale work, and repeated extractions in separatory funnels are much too laborious. An apparatus has been devised in which large volumes of solvent can be handled with little effort. Its principle differs somewhat from that of the Craig machine, but the end result—separation of solutes having different distribution coefficients—is similar. Successive aliquots of the quiescent (heavier) phase of two immiscible solvents are subjected to continuous extraction by extremely small increments of the lighter phase. The apparatus consists of a series of side-arm test tubes, each containing a separate inner tube having a coarse fritted disk sealed to the lower end. The solute is placed in the first test tube, after which all the tubes are filled with the heavier member of a solvent pair. The lighter member is then introduced continuously and at a properly regulated rate into the first dispersion tube. Droplets of upper phase enter the lower phase through the fritted disk, rise to the top and overflow into the next dispersion tube. The number of tubes necessary, as well as the total volume of upper phase required for any degree of separation of two or more solutes can be predicted from their respective distribution coefficients. The versatility of the apparatus and the ease with which it can be assembled and used suggest a wide application for it in fractionation studies.

Hydrolysis of glutathione J R KIMMEL,* C K OLSON,* DONALD OKESON* AND FRANCIS BINKLEY *Univ of Utah College of Medicine, Salt Lake City*

The enzyme of pig kidney responsible for the hydrolysis of glutathione to glutamic acid and cysteinylglycine has been separated from the enzyme responsible for the hydrolysis of cysteinylglycine. The activity is associated with a lipoprotein of high molecular weight. Considerable activation of all preparations is obtained by the addition of glutamine, the preparations of highest purity are relatively inactive in the absence of glutamine. The hydrolysis of glutathione is inhibited in a competitive manner by various phthaleins and by penicillin G, these inhibitions may be reversed by the addition of either glutamine or glutathione. The antibacterial action of penicillin toward *S aureus* is reversed under certain conditions by glutamine and/or glutathione.

Enzymatic hydrolysis of urinary reducing lipids

RALPH A KINSELLA, JR, R J DOISY AND JOHN H GLICK, JR (introduced by EDWARD A DOISY) *Dept of Biological Chemistry, Saint Louis Univ School of Medicine, Saint Louis, Mo*

Enzymatic hydrolysis of urinary neutral reducing lipids has yielded increases in reducing activity of 3-5 times that of suitable controls. The order of increase is the same for normal male and for pregnancy urine. In terms of the reducing power of desoxycorticosterone, values ranging from 8 to 30 mg/24 hr for normal male urine and 24-41 mg/24 hr for pregnancy (9th month) urine have been obtained. The enzyme source is the culture fluid preparation of Buehler, Katzman and Doisy (*Federation Proc* 8:189, 1949) which is obtained by centrifugation (to remove cells and menthol) of a 10-day old culture of a strain of *B coli*, which was grown in the presence of menthol glucuronide, and is rich in glucuronidase. Five or 10 ml of urine is mixed with one ml of culture fluid, the pH adjusted to 6.2 ± 0.1 with 0.6 M phosphate, and the mixture incubated at 38° over chloroform for 15-18 hours (overnight). The mixture is brought to pH 1 with 12 N H₂SO₄ and extracted 4 times with 2 volumes of ether chloroform (4:1), the extract is washed with 0.1 N NaOH and water, dried with Na₂SO₄, transferred to an Erlenmeyer flask and evaporated with a water pump at a temperature not over 35°C. The reducing power is then determined directly on the residue, using 3 ml of the phosphomolybdate reagent of Heard, Sobel and Venning (*J Biol Chem* 165:699, 1946) for color development and 7 ml for dilution.

Effect of beryllium on certain enzymes FRIEDRICH W KLEMPERER *Dept of Biochemistry of Trudeau Fdn for Clinical and Experimental Study of Pulmonary Disease, Trudeau, N Y*

The effect of beryllium on tissue slices and various enzymes has been investigated. Previously it had been reported by us that alkaline phosphatase was partly inhibited by concentrations of beryllium lower than 10^{-6} M. Acid phosphatase was slightly inhibited by 10^{-3} M Be. DuBois *et al* have demonstrated an effect of 0.8×10^{-3} M Be on adenosine triphosphatase (*Science* 110:420, 1949). Respiration of rat liver slices was unaltered by 10^{-3} M Be. Succinic oxidase activity of liver-homogenate was enhanced by beryllium ions while succinic dehydrogenase was unaffected. The mechanism of this activation appears similar to that described for aluminum. Beryllium in concentrations up to 10^{-3} M had no effect on glycolysis induced by acetone powder of muscle. Aldolase, isomerase, carboxylase, and arginase were not inhibited by beryllium and the divalent metals which normally activate these enzymes could not be replaced by beryllium. Uricase and carbonic anhydrase were also unaffected by beryllium.

Electrophoretic studies of plasma proteins in children with rheumatic fever ELIZABETH L KNAPP,* ROBERT L JACKSON* AND JOSEPH I ROUTH *Depts of Pediatrics and Biochemistry, State Univ of Iowa College of Medicine, Iowa City*

Electrophoretic analysis has been made of over 200 plasma samples from 70 children with rheumatic fever in different stages of the disease. Serial studies on many children are included. Plasma samples were diluted and dialyzed with veronal buffer, pH 8.6, for 3 days in the cold. Electrophoresis was conducted at 0-8°C in the Longworth modification of the Tiselius apparatus. During the more active phase of the disease the values for plasma albumin were consistently and markedly decreased but increased with clinical improvement. Increases in the α -globulins frequently were observed in children with exudative manifestations of the disease. Marked increases in both fibrinogen and γ -globulin often were noted during the early acute phase, although a given child might not show deviations in both fractions. The γ -globulin fraction usually returns to normal more slowly than the other components, the rate of return appears to follow the rate of clinical improvement. Statistical analysis indicated a significant association between changes in sedimentation rate and changes in albumin, fibrinogen and γ -globulin. Examination of serum samples in this disease indicates that the γ -globulin component is normal. For this reason the elevation in the fibrinogen fraction would appear to be real and not an artefact. These biochemical data are being studied further in relation to the clinical and nutritional status of the child, duration of the disease, amount of residual cardiac damage, and also as a laboratory guide to determine inactivity of the disease process.

Effect of adrenal cortical steroids on arginase activity CHARLES D KOCHAKIAN AND EVANGELINE ROBERTSON * *Univ of Rochester School of Medicine and Dentistry, Rochester, N Y*

The subcutaneous injection of aqueous adrenal cortical extract (Upjohn) at 8×0.25 ml/hr and 11-dehydrocorticosterone acetate (Merck) at 8×0.25 mg/hr into fasted castrated adult mice did not alter the arginase activities of the liver or kidneys but did increase the liver glycogen and urinary nitrogen excretion. On the other hand, a subcutaneously implanted 13-15 mg pellet of 11-dehydro-17-hydroxycorticosterone acetate produced a significant increase in both the liver and kidney arginase activities after 2 days. On extending the treatment a further increase was attained after 7 and 30 days. The same increases were obtained when the enzyme was determined with CoCl_2 as the enzyme activator or by preactivation at 50°C with mm Cl_2 . The body weight of these treated mice decreased very sharply during the first 4-6 days accompanied by an increased nitrogen excretion, a nearly complete disappearance of the thymus within 2 days and a decrease in the size of the spleen. The liver increased in weight after 2 days but, thereafter, progressively decreased. The kidney showed no change after 2 days but was significantly increased after 7 and 30 days. 11-dehydrocorticosterone (Kendall) implanted as a pellet for 7 and 30 days produced effects qualitatively similar but quantitatively less than those of 11-dehydro-17-hydroxycorticosterone acetate. The injection of 11-dehydrocorticosterone acetate (Merck) in oil solution at 0.5 mg/day for 26 days was completely ineffective.

Fractionation of blood lipides in organic solvents at low temperature ALFRED E KOEHLER AND ELSIE HILL * *Sansum Clinic Research Fndn and Santa Barbara Cottage Hospital, Santa Barbara, Calif*

An apparatus has been devised consisting of two 50 cc Erlenmeyer flasks joined at their opening to a glass fritted disk. Side arm connections to the bottom of the flasks permit filling and emptying and equalization of pressure. A group of these vessels are rotated on an axis at their center so as to permit agitation and inversion for filtering. A solution containing 2-4 mg of lipides in petroleum ether is evaporated in the bottom flask and 10 cc solvent added. The vessel is stoppered to prevent evaporation or the access of moisture. After 24 hours of shaking at low temperature the apparatus is inverted for gravity filtering without any disturbance of the temperature. A useful temperature has been found to be -26° particularly so that an ordinary freezing cabinet can be adapted. In ethanol the esters of the saturated fatty acids of cholesterol are insoluble, with the exception of the butyrate but the latter has not been found in the blood lipides. Free cholesterol and neutral fats in

equivalent amounts are soluble and can be thus separated. The unsaturated fatty acid esters are soluble, particularly linoleic which constitutes about 62% of the cholesterol esters. In methanol a separation of the cholesterol esters from neutral fat can be made inasmuch as the esters are nearly completely insoluble. This permits separation of the cholesterol esters so as to enable a study of their constitution.

Failure of pterylglutamic acid (PGA) to function as growth factor for *L. arabinosus* (17-5) BERNARD W KOFT,* E STEERS* AND M G SEVAG *Dept of Bacteriology, Univ of Pennsylvania School of Medicine, Philadelphia*

It has been found that it is a decomposition product and not PGA *per se* which is a growth stimulant for *L. arabinosus*. Growth stimulation by PGA is directly related to the degree of decomposition. Aged PGA (80% decomposed, calculated as p-aminobenzoylglutamic acid (PABG)) is almost 100-fold more active than intact PGA, as are also p-aminobenzoic acid (PABA) and PABG. In systems containing 3% phosphate ($\text{pH } 6.8$) to maintain neutrality, unlike PABA, PABG and aged PGA, intact PGA fails to stimulate growth earlier than after 72 hours. The antagonism of PGA *per se* to sulfonamides here is apparent rather than real, and is controlled by the following: 1) an incubation period which is associated with the decomposition of PGA and is necessary for any antagonism to sulfonamides, 2) the concentration of PABG and/or PABA which brings about a competitive type of antagonism, PABG content of 0.01 γ of PGA, though adequate for optimal growth, is inadequate to antagonize 1 γ of sulfonamide, and 3) the rapid increment of acidity in the medium. The increased acidity suppresses the inhibitory capacity of sulfonamide and augments the anti-sulfonamide capacity of the liberated PABG. In a well-buffered medium containing 3.0 gm % of phosphate ($\text{pH } 6.8$) the complete inhibition of growth by sulfonamides persists in the presence of PGA without a detectable reversal. These data do not support the postulate (*J Biol Chem* 170:133, 1947) that sulfonamides interfere with the synthesis of PGA via PABA or PABG.

Permeability of axonal surface membranes to amino acids SAUL R KOREY (introduced by H T CLARKE) *Dept of Neurology, Columbia Univ College of Physicians and Surgeons, New York City*

The changes of permeability of axonal surface membranes are an essential element in the conduction of nerve impulses. The availability of stable and radioactive isotopes has provided the possibility of approaching the problem of the properties affecting the permeability of cell membranes. For the study of the permeability of axonal surface membranes the giant axon of Squid has proved to

be an excellent material in this connection. Studies were initiated with amino acids labeled with N^{15} . Giant axons of Squid were exposed to artificial sea water prepared according to Pantin in which 0.1 M of NaCl was replaced by the labeled amino acid in the same concentration. After 30 minutes exposure the axoplasm was extruded and the atom percentage excess N^{15} determined after the usual preparation. In the cases in which glycine containing 32 atom % excess N^{15} , alanine containing 23 atom % excess N^{15} and L-aspartic acid containing 30 atom % excess N^{15} were used, the following data may be considered typical. After 30 minutes exposure to glycine 0.949 and 1.10 atom % excess N^{15} were found in the axoplasm. This corresponds to 15.3 and 17.7 μM of N/gm. Since the outside solution contains 100 μM of N/ml, 16.5% had penetrated within the period of exposure. In the case of alanine 0.4307 atom % excess N^{15} were found, corresponding to 9.7 μM /gm. After exposure to L-aspartic acid 0.276 atom % excess N^{15} were found, corresponding to 4.7 μM /ml.

Isocitric acid breakdown in yeast ARTHUR KORNBERG *Natl Insts of Health, Bethesda, Md*

With triphosphopyridine nucleotide (TPN) as a specific coenzyme and acetone powders of animal tissues, Adler *et al* (*Biochem J* 33: 1028, 1939) demonstrated the oxidative decarboxylation of isocitric acid to α -ketoglutaric acid and CO_2 . Ochoa (*J Biol Chem* 174: 115, 1948) provided evidence for oxalosuccinic acid (OSA) as the intermediate and established the reversibility of the reaction starting either with α -ketoglutaric acid and CO_2 or with OSA. Our studies with extracts of either fresh baker's or dried brewer's yeast indicate the existence of 2 distinct and readily separable systems for isocitric acid breakdown. One is TPN-specific and the other diphosphopyridine nucleotide (DPN)-specific. The TPN system is entirely comparable with that of Ochoa. The DPN system, which has been purified over 50-fold, has certain distinctive features. Although this enzyme catalyzes the stoichiometric conversion of D-isocitric acid to α -ketoglutaric acid (identified by chemical and enzymatic means), it does not catalyze either the fixation of CO_2 in α -ketoglutaric acid or the reduction of OSA. Also, in addition to a strict specificity for DPN, it requires the presence of adenosine-5-phosphate in catalytic amounts and is inhibited by sodium azide.

Sources of bacteriophage nitrogen LLOYD M KOZLOFF*, KATHRYN KNOWLTON*, FRANK W PUTNAM AND E A EVANS, JR *Dept of Biochemistry, Univ of Chicago, Chicago, Ill*

The precursors of the nitrogenous components of *Escherichia coli* bacteriophage T r^{+} were studied using both N^{15} labeled bacterial cells and medium. While the medium is the source of about

80% of the virus N, bacterial N also appears in both phage nucleic acid and protein. The major portion of the bacterial N contribution was found in the virus nucleic acid. Sixteen-43% of the virus nucleic acid was derived from the host. The N of the purine and pyrimidine bases contained about equal proportions of host N. When the bacteria were labeled with N^{15} and P^{32} the ratio of $\frac{\% \text{ of virus nucleic acid N from host}}{\% \text{ of virus nucleic acid P from host}}$ was 1.3-1.4.

The amount of bacterial N appearing in the virus nucleic acid was found to be independent of the time of lysis or the number of virus particles produced/cell. These results indicate that bacterial desoxyribonucleic acid is utilized for the synthesis of the new virus particles. Seventy-five to 95% of phage protein N came from the medium after infection of the host while the remaining 5-25% was contributed by the host. The bacterial N contribution was distributed among the various amino acids of the virus. The actual amount of bacterial N appearing in the phage protein was inversely proportional to the number of virus particles produced/bacterial cell.

Cytosine deaminase of yeast JACOB KREAM* AND ERWIN CHARGAFF *Dept of Biochemistry, Columbia Univ, College of Physicians and Surgeons, New York City*

The discovery of an enzyme in cell-free extracts of bakers' yeast which deaminates cytosine to uracil (CHARGAFF AND KREAM *J Biol Chem* 175: 993, 1948, compare also HAHN AND LINTZEL *Ztschr Biol* 79: 179, 1923) prompted a study of its preparation and properties. A 5-fold concentration of activity in terms of protein was effected when the enzyme was precipitated from the supernatant, resulting from the centrifugation at 15,000 g of aqueous extracts of crushed cells, by adjustment to between 0.25 and 0.7 saturation with $(NH_4)_2SO_4$. Following the evaporation of the dialyzed frozen solutions in a vacuum, preparations stable for a long period when stored in the dry state at -15° were obtained. The kinetics of the enzymatic reaction were studied by means of the previously published analytical methods (VISCHER AND CHARGAFF *J Biol Chem* 176: 703, 1948). The pH optimum was found at pH 6.8-6.9, the temperature optimum lay between 34° and 37.5° (dependent upon the duration of incubation). The enzyme solutions were very sensitive to heat. Considerable inactivation at 37.5° was prevented by the substrate cytosine and to a less extent by uracil. The preparations contained neither guanase nor adenase. Conclusions as to the structural specificity of the enzyme can be drawn from the fact that it does not deaminate cytidine, cytidylic acid, thiocytosine, 2-methoxy cytosine, 5 amino uracil, or isocytosine which latter acts, in fact,

as an inhibitor 5-Methyl cytosine, however, is deaminated to thymine

Estimation of lipoproteins in serum immunologically H G KUNKEL (introduced by STANFORD MOORE) *Hospital of Rockefeller Inst for Med Research, New York City*

In order to determine whether the protein moiety of serum lipoproteins was immunologically specific from the other serum proteins and whether elevation of serum lipids causes a rise in such protein, various lipoproteins were purified from normal and pathological sera. Dilution of serum 30 times with a weak buffer solution (pH , 6.4, μ , 0.02) containing 0.005% zephiran chloride has been found to precipitate selectively the lipoproteins from serum. Analysis of such precipitates indicated a lipid content ranging from 50–70%. Further separation of this material by high speed centrifugation in solutions of high density yielded chiefly a clear lipoprotein preparation that by electrophoretic analysis showed one major β globulin component making up 90% of the total area. Injection of this material into rabbits readily produced an antiserum which did not react with purified preparations of albumin or γ globulin although it was very reactive with lipoprotein preparations or whole serum. Similar results were obtained with antisera to the protein portion of the lipoprotein after 90% of the lipid had been removed. Quantitative analysis of precipitin nitrogen indicated that normal serum contained an average of 0.03 gm of lipoprotein nitrogen/100 cc. The majority of sera containing elevated total lipid showed a high content of lipoprotein and sera containing very little lipid were very low in lipoprotein. The values showed a fourfold range of variation. Observations on sera from patients with nephrosis with high serum lipid levels indicated that this normal lipoprotein was markedly increased although the total protein, albumin and γ globulin were very low.

Effect of zinc on oxidation of citric acid cycle intermediates H O KUNKEL (introduced by JAMES B SUMNER) *Dept of Biochemistry and Nutrition, Cornell Univ, Ithaca, N Y*

Copper ions have been shown by Ackermann and Potter (*Proc Soc Exper Biol & Med*, 72, 1, 1949) to inhibit rat liver and kidney succinioxidase systems by forming an inactive non-dissociable complex with the enzyme. I find that zinc ions behave in a similar manner but show more toxicity than copper ions. Under conditions which favor oxidative phosphorylation (isotonic KCl homogenates, Mg^{++} and adenosine-5 phosphate), inhibition by zinc occurs, but this inhibition is transitory. The rate of oxidation subsequently rises to a level even higher than the control data. A later fall to a lower level of activity can be attributed

to the zinc inhibition of the oxidation of other citric acid cycle intermediates, particularly the oxidation of citrate. With the exception of succinioxidase, the oxidizing enzymes of rat kidney are more readily inhibited by zinc ions than are those of rat liver. The addition of a protein such as insulin has no protective action against zinc inhibition, but crystalline zinc insulin causes the same inhibition as does zinc sulfate. Amorphous insulin, in itself, inhibits citrate oxidation by rat kidney homogenates.

Considerations on indiscriminate use of the Waring Blendor: copper contamination and ascorbic acid loss MERTON P LAMDEN (introduced by HAROLD B PIERCE) *Dept of Biochemistry, Univ of Vermont College of Medicine, Burlington*

The Waring Blendor, a useful and popular comminuting apparatus, has been considered undesirable for use in the determination of ascorbic acid since it incorporates large amounts of oxygen into the slurry presumably thus causing oxidative loss of ascorbic acid. Also the Waring Blendor has been shown to cause oxidation of sulfhydryl groups and inactivate enzyme systems. In some instances such untoward effects could be due to the use of containers having blending assemblies with worn chrome plate and an exposed brass undersurface. That dissolved copper, probably originating in the brass, is responsible for the destruction of ascorbic acid in a blended solution was shown in the following manner: 200 ml portions of a solution of ascorbic acid (20 μ g/ml) in 5% metaphosphoric acid were blended for 3 minutes in triplicate in each of 6 Blendor containers. Copper and ascorbic acid were determined in all solutions before and after blending. The coefficient of correlation between the concentration of dissolved copper and the percentage ascorbic acid lost was 0.97. Furthermore, the extent of loss of ascorbic acid correlates rather well with the physical appearance of the blending assembly. Solutions in contact with well plated blending assemblies showed small losses in ascorbic acid, while those solutions in contact with blending assemblies having worn plating or exposed brass parts showed considerably higher losses. The Waring Blendor thus does not contribute to the oxidative loss of ascorbic acid in 5% metaphosphoric acid solution unless copper is dissolved from parts of the blending assembly having worn chrome plate.

Effect of reducing agents on the biological activities of crystalline vitamin B₁₂ CALVIN A LANG* AND BACON F CHOW *Dept of Biochemistry, School of Hygiene and Public Health of Johns Hopkins Univ, Baltimore, Md*

It was reported that reaction of crystalline vita-

min B_{12} with hydrogen in the presence of platinum catalyst with yield vitamin B_{12} , which possesses a lower microbiological activity and animal protein factor activity than vitamin B_{12} . This treatment may involve the addition of hydrogen atoms to double bonds in the molecule or reduction. Our study deals with the reaction between crystalline vitamin B_{12} and several reducing agents (ascorbic acid, cysteine HCl, thiamine HCl, and hydroquinone) in a phosphate buffer at pH 7.6. To this end, a solution of crystalline vitamin B_{12} was mixed with a solution of one of the above mentioned reducing agents, so that the ratio of B_{12} to reducing agent varied from 1:400–1:4000 by weight in several experiments. An aliquot solution of B_{12} to which no reducing agent was added served as the control. These solutions were sealed in pyrex tubes and kept at 37° C for different lengths of time, and changes in spectrophotometric transmittance at 550 m μ were followed. After incubation for a desired interval of time the tubes were opened, and the contents were assayed for excess reducing agent, for microbiological activity using *L. leichmanii*, and for animal protein activity using B_{12} deficient rats. No special precautions were used to exclude air from contact with the solutions. The results obtained demonstrated that the original vitamin B_{12} activities were greatly decreased as a result of incubation in the presence of the reducing agents listed above, whereas the vitamin B_{12} activities of the control tube treated under identical conditions were unaffected.

Radioactive tobacco mosaic virus ROBERT V LASHBROOK* AND HUBERT S LORING *Dept of Chemistry and School of Medicine, Stanford Univ, Stanford, Calif*

Several methods were studied for incorporating radioactive phosphorus (P^{32}) into tobacco mosaic virus in Turkish tobacco plants. The most highly radioactive virus was obtained when normal plants were allowed to take up the P^{32} -phosphate as completely as possible from small volumes of distilled water and were grown in nutrient medium for about two weeks after inoculation. When P^{32} -phosphate was introduced into previously infected plants, the isolated virus was only about one-half as radioactive as that from the newly infected plants. In experiments in which radioactive virus was mixed with macerated plants and re-isolated after standing at room temperature for various periods of time, 60–90% of the original virus was recovered with unaltered radioactivity. When radioactive virus was inoculated onto growing leaves, the recovered virus had the same radioactivity as that used for inoculation but the amount recovered was only 10–20% of the original when the plants were worked up 24 hours later.

In these instances over 50% of the radioactivity was found in the supernatants after ultracentrifugation. The results of the several types of experiments performed have suggested a) that there is little, if any, exchange of P^{32} between virus and host, b) that virus becomes associated with insoluble plant components such as chloroplasts after inoculation, and c) that during virus multiplication the original virus phosphate is largely converted to a non-virus form.

Trypsin inhibitor in colostrum M LASKOWSKI, JR * AND M LASKOWSKI *Dept of Biochemistry, Marquette Univ School of Medicine, Milwaukee, Wis*

Human and bovine colostrum were found to contain a compound inhibiting the proteolytic action of trypsin. The inhibitor content was determined according to Kunitz (*J Gen Physiol* 30:291, 1947). In bovine colostrum maximum content of inhibitor occurred on the 1st day after delivery. In human the peak value was reached between the 1st and 3rd day, and then gradually declined till on the 5th day it was impossible to detect. Partial purification of the inhibitor was achieved by 1) the removal of casein at pH 4.6, 2) removal of inactive proteins by 30% saturation with ammonium sulfate, 3) precipitation of inhibitor by 70% saturation at pH 3.0, 4) removal of the inactive protein by 2.5% trichloroacetic acid at 80°C and 5) precipitation with 70% ammonium sulfate. Steps 3, 4, and 5 are identical with the method of Kunitz and Northrop (*J Gen Physiol* 19:991, 1936). It seems, therefore, likely that the properties of the inhibitor from colostrum and from pancreas may be very similar, possibly possibly identical. Partially purified 'fraction 5' inhibits trypsin apparently by forming a stoichiometric compound. It also possesses a strong blood anti-coagulant action. The physiological significance is now only a matter of speculation, but it is believed that the main purpose of the inhibitor is to protect the antibodies of colostrum. Further purification and the physiological role are under investigation.

In vivo effect of toxic dose of P^{32} on phosphorus fractions of rat thymus P S LAVIK, HELEN HARRINGTON AND G W BUCKALOO (introduced by H G Wood) *Atomic Energy Med Research Project, Western Reserve Univ, Cleveland, Ohio*

In studies of the damaging effects of ionizing radiation, we have examined the over-all changes in the phosphorus fractions in the thymus of young adult rats given a single LD₅₀ dose of $Na_2HP^{32}O_4$, i.e., 4.5 μ C P^{32} /gm body weight. Comparable animals receiving 0.1 μ C P^{32} /gm body weight served as controls. Four or more animals from each group were killed at 4 hours,

1, 2, 3, 6, and 8 days after the injection of P^{32} and the thymus tissue was fractionated into the acid-soluble (AS), inorganic (INORG), phospholipid (PL), ribonucleic acid (RNA) plus 'phosphoprotein' (PP), and desoxyribonucleic acid (DNA) fractions. Specific activity measurements (% of P^{32} dose/mg P) showed a reduced uptake of P^{32} in the AS, RNA plus PP, and DNA fractions. This was particularly the case with the DNA and RNA plus PP fractions. There was histological evidence of tissue damage, and a marked loss (62%) in the gland weight occurred during the first 3 days after P^{32} injection. While the total amount of each of the phosphorus fractions decreased rapidly during this same period, the loss of DNA, RNA plus PP, and AS was greater than would be expected from the change in tissue weight. The DNA concentration (mg DNA phosphorus/gm fresh tissue) had decreased 57% by the end of 3 days and thereafter its concentration remained essentially unchanged. Similar, though less marked, changes occurred in the RNA plus PP and AS fractions, whereas the concentration of the PL and INORG fractions changed little if any.

Differential fixation of sulfate by tissues maintained in vitro LAURENCE L. LAYTON (introduced by E. V. McCOLLUM) *Dept. of Biochemistry, Johns Hopkins Univ. School of Hygiene and Public Health, Baltimore, Md.*

Tissues removed from chicks at various stages of embryonic development or after hatching, and maintained 45 hours *in vitro* in media containing S^{35} labeled inorganic sulfate, exhibit different rates of conversion of sulfate ion to insoluble tissue sulfate. It was found that sulfate fixation by heart ventricle reached a maximum of 160% / 100 mg tissue between the 13th and 15th days of incubation, and dropped to 0.37% on the day of hatching. Tissue from the aortic arch exhibited a constant rate of 2.5% / 100 mg wet tissue from the 10th day of incubation to the 44th day after hatching. Fixation by the midsection of the tibia dropped from 33% on the 8th day of incubation to 0.5% on the day of hatching. SO₄²⁻ fixation by cartilage from the proximal tip of the tibia dropped from 38% on the 9th day of incubation to 0.0% for the lateral condyle on the 6th day after hatching. Tissues from 6-day old chickens gave the following values for sulfate ion absorbed (μ g/100 mg wet tissue): tibial red marrow, 0.4; spleen, 1.0; kidney, 1.0; heart ventricle, 0.18; skeletal muscle, 0.1. Tissues from a 12-year old boy gave the following: muscle, 1.2; eosinophilic granuloma of fibula, 1.1; periosteum, 3.6; bone from fibula, 0.5; μ g/100 mg wet tissue. The indications are that sulfate is utilized in tissue formation.

Incorporation of glycine-2-C¹⁴ into proteins and nucleic acids of normal and neoplastic rat tissues G. A. LEPAGE AND CHARLES HEIDELBERGER * *McArdle Memorial Lab., Univ. of Wisconsin, Madison*

Glycine has been demonstrated to be a precursor of nucleic acids and heme in the rat (HEINRICH AND WRIGHT *Federation Proc.* 8: 205, 1949; ALTMAN, *et al.* *J. Biol. Chem.* 176: 319, 1948). No reports are available to compare the incorporation into neoplastic and normal tissues. Glycine-2-C¹⁴ was administered by stomach tube to normal rats and to rats bearing multiple 8-day Flexner-Jobling carcinoma transplants. Animals were killed at 12, 24 and 48 hours. Samples of liver, thymus and tumor were fractionated to obtain the desoxyribonucleic acid, ribonucleic acid and protein from each. Large samples of blood were taken from each animal and purified protoporphyrin-9 methyl ester isolated. There was no significant difference in specific activity of blood porphyrin, liver and thymus DNA, PNA and proteins between normal and tumor-bearing rats. The specific activities of DNA, PNA and protein of tumor were always higher than those of the liver and thymus. Specific activities of the 12-hour samples were highest. In all cases protein activities were higher than those of the nucleic acids. The purine and pyrimidine bases obtained on hydrolysis of the PNA contained C¹⁴. Further degradation studies are in progress.

Presence in wheat germ oil of factors capable of affecting endocrine metabolism I. EZRA LEVIN AND JOHN F. BURNS (introduced by B. CONNOR JOHNSON) *VioBin Labs., Monticello, Ill.*

An androgenic factor in wheat germ oil Androgenic activity has been demonstrated in wheat germ oil (solvent extracted from fresh wheat germ with ethylene dichloride) by three methods. 1) Increase in the weight of the seminal vesicles of the castrate rat. 10 cc subcutaneously every 2nd day for 3 weeks. Average seminal vesicle weight: wheat germ oil group 206.0 mg; control (injected with sesame oil) seminal vesicle 7.1. The colony was maintained on a 'synthetic' diet using glyceryl trilaurate as a fat source. 2) Increase in combs of full-grown capon. 10 cc injections daily for 5 days. Average increase (H + L) 8.7 mm. 3) Increase in weight of comb on 1- to 5-day old chicks. 0.1 cc daily injections for 15 days. Wheat germ oil group: Average comb weight 136 mg; controls (injected with sesame oil), 54.7. **A progestational factor in wheat germ oil** Progestational activity in wheat germ oil (solvent extracted from fresh wheat germ with ethylene dichloride), has been assayed by the Corner-Allen and McPhail methods, showing a 3+ proliferation, when injected with 10 cc wheat germ oil s.c.

daily for 2 days This reaction corresponds to that given by 0.75 mg of progesterone Both bioassays demonstrate the presence in wheat germ oil of a factor capable of eliciting a progestational response in the endometrium of the rabbit Activity is absent in rabbits given α -tocopherol Diminished activity was found in wheat germ oil solvent extracted from *rancid* wheat germ

Survey of serum cholinesterases in a number of mammalian species MILTON GJELHAUG LEVINE, ROBERT E HOYT* AND ANITA A SURAN* *Inst of Experimental Medicine, College of Med Evangelists, Los Angeles, Calif*

The observation published by Mendel and Rudney (*Biochem J* 37 473, 1943) that the cholinesterases found in animal tissues could be separated into 2 types (the specific and non-specific) on the basis of the hydrolysis of 3 substrates, acetyl-choline, benzoylcholine and acetyl-B-methylcholine, led them and other workers to the assumption that all mammalian species had one or the other or both of these cholinesterases They have indicated, for example, that rabbit serum has both forms (*Science* 100 499, 1944) Our work shows that only one cholinesterase is present in the serum of this species (the specific type) and in addition, an enzyme which hydrolyzes benzoylcholine but not acetylcholine A number of other mammalian species have been investigated (rat, guinea pig, dog, monkey, horse, cow, goat, swine) and indicate that there are a whole family of enzymes capable of hydrolyzing acetylcholine, rather than merely the 2 suggested by Mendel and Rudney

Serum proteins and experimental liver damage in the rabbit MILTON GJELHAUG LEVINE, ROBERT E HOYT AND ANITA A SURAN (introduced by R J WINZLER) *Inst of Experimental Medicine, College of Med Evangelists, Los Angeles, Calif*

Liver damage was produced in rabbits by the oral administration of CCl_4 in Wesson oil The animals were fed 1 cc of 20% solution twice weekly for the period of the experiment The progress of the liver damage produced was studied by biopsy and necropsy Serum proteins and serum cholinesterase were determined weekly up to the time of death Of the 23 rabbits studied, 18 developed cirrhosis and were observed for the periods up to 5 months Five of the animals showed fatty degeneration and necrosis of the liver after being killed early in the experiment (2-29 days after CCl_4 administration was started) None of the animals showed a significant decrease in either serum acetylcholine or benzoylcholine hydrolysis during the period of observation The most interesting observation made was that despite obvious liver damage, none of the animals showed a decrease in serum proteins This observation, how-

ever, coincides with that of Kwok-Kew Cheng (*J Path & Bact* 61 23, 1949) in the same animal

Influence of pH upon rates of denaturation of some proteins MILTON LEVY AND ROBERT C WARNER *Dept of Chemistry, New York Univ College of Medicine, New York City*

For many years it has been customary to distinguish between the products of acid denaturation, alkali denaturation and heat denaturation of proteins The processes leading to denaturation have been classified on the same basis Thus heat is regarded as a 'physical' denaturing agent whereas hydrogen and hydroxyl ions are regarded as 'chemical' denaturing agents Nevertheless, it is clear that the intensity factor associated with acidity and alkalinity (the pH) and with heat (the temperature) in aqueous solutions are continuous and not mutually exclusive functions We must regard the rate of denaturation of a protein in solution as a simultaneous function of temperature and pH The temperature function has been handled in terms of the Arrhenius equation or some interpretation of it but the pH function has been dealt with rationally only in limited situations Studies on the rates of denaturation of a number of proteins will be presented which demonstrate that the postulates of Steinhardt (*D Kgl Danske Videnskabers Selskab Math-fysik Med* 14 11, 1937) suitably generalized and modified lead to rational general descriptions of the relation between rate of denaturation and pH From these descriptions one can draw the conclusion that only a very limited number of the hydrogen ion donor and acceptor groups in the protein molecule are significant for its denaturation and, therefore, general salt bridges are relatively minor factors in determining the structure of native proteins

Alterations in steroids excretion after administration of ACTH in man SEYMOUR LIEBERMAN, LUCIE B HARITON* AND KONRAD DOBRINER *Sloan-Kettering Inst for Cancer Research, New York City*

The steroid excretion patterns before, during, and after the administration of ACTH to human subjects have been investigated After the liberation of the urinary steroids from the conjugates by continuous ether extraction at pH 1 without prior heating followed by hydrolysis of the 'glucuronide' fraction with calf spleen β -Glucuronidase, the free steroids were partitioned between benzene and water The constituents of the 'Water Soluble' and 'Benzene Soluble' α and β ketonic fractions have been studied Compound F and a new adrenal metabolite, pregnane $3\alpha,17\alpha,21$ -triol-11,20 dione, isolated as acetate, m p 234-238°, $[\alpha]_D^{25} + 89^\circ$, was isolated from the 'Water Soluble' fraction The latter compound was also detected

in the urine after the administration of Cortisone Acetate. At least 6 other crystalline steroids, as yet unidentified, have been isolated. Androstane-3,17-dione, etiocholan-3,17-dione, androsterone, etiocholanolone, pregnan-3 α -ol-20-one, 11-keto-etiocholanolone, and 11-hydroxyandrosterone, were present in the 'Benzene Soluble' extracts and were excreted in increased amounts during the period of ACTH administration. A number of new unidentified compounds, some of which have been observed previously only in the urine from patients with Cushing's Syndrome, have been detected in this 'Benzene Soluble' fraction. The nature of the new compounds isolated and the variations in the excretion of the individual steroids in relation to steroid metabolism during stimulation of the adrenals by ACTH will be discussed.

Specificity of xanthine oxidase DORIS C. LORZ*
AND G. H. HITCHINGS *Wellcome Research Labs, Tuckahoe, N. Y.*

Fifty purine derivatives and structural analogs of the naturally-occurring purines and 10 pyrimidines have been tested for their ability a) to serve as substrates for xanthine oxidase, b) to inhibit the action of the enzyme on xanthine. Purines in general exhibit more or less ability to combine with the enzyme as measured by the inhibition of xanthine oxidation. A number of unnatural purines and analogs serve as substrates. Thus 8-azaxanthine is oxidized to 8-azaxanthine, 2-amino-purines are oxidized to the corresponding guanines and 2-hydroxypurines are oxidized to 2,8-dihydroxypurines. 1-Methylxanthine is unique in that it is oxidized faster than xanthine. A few pyrimidines have been found to have activity. 4-Hydroxypyrimidine is oxidized to uracil and 4-hydroxy-5-methylpyrimidine is oxidized to thymine, while 4-amino-2,6-dihydroxypyrimidine is an inhibitor. From these data it is possible to determine some of the special requirements for combination of substrate and enzyme and to assess the influence of the nature of certain functional groupings on the course of the enzymatic action.

Inhibition of respiration and acceleration of aerobic glycolysis in tissue slices by certain α,β -unsaturated anions H. J. LOWE,* E. C. WEINBACH,* W. R. FRISSELL* AND LESLIE HELLMAN *Dept. of Physiological Chemistry, Johns Hopkins Univ. School of Medicine, Baltimore, Md.*

Respiration is inhibited markedly with concomitant acceleration of aerobic glycolysis, approaching the anaerobic level, in slices of various organs of the rat and guinea pig. The inhibitors include the cinnamate, benzoate, and related ions. The inhibitor ions are known to embarrass oxidation of amino acids and fatty acids in such tissues.

In our hands, they fail to slow the rate of disappearance of phosphoglycerate, lactate, and pyruvate. They do not affect significantly the rate of oxygen-uptake in the presence of key substrates for the tricarboxylic acid cycle. These agents fail to alter the rate of aerobic glycolysis in certain cultured malignant cells and tumors derived from rat fibroblasts. The phenomenon is interpreted as a 'reversal of the Pasteur effect' initiated by a denial of essential substrates through a competitive mechanism.

Use of partition chromatography for the study of porphyrin metabolism in the rat JOHN LUCAS* AND JAMES M. ORTEN *Dept. of Physiological Chemistry, Wayne Univ. College of Medicine, Detroit, Mich.*

Partition chromatography employing silica gel at a pH of 4.5 and using mixtures of chloroform and ligroin as the mobile phase has been found satisfactory for the separation of small amounts of the various porphyrins occurring in feces and urine. The urine or extracted feces are adsorbed on talc after adjusting to a pH of 4.0 and the methyl esters of the porphyrins are obtained by subsequent treatment with methyl alcohol-hydrogen chloride. The methylated products are dissolved in chloroform, washed, concentrated, and then chromatographed. The various major porphyrins separate as distinct entities on the column. By collecting fractionally and reading the fractions in a spectrophotometer or a photofluorometer previously calibrated with pure crystalline proto-, copro-(I) and uro-(I) porphyrin, respectively, small amounts of these porphyrins may be determined quantitatively. The foregoing procedure is being applied to studies of porphyrin metabolism in the rat. The influence of dietary protein and of glycine on the synthesis of proto-, copro-, and uroporphyrin as indicated by fecal excretion is now under investigation. Five groups of rats are fed the following diets: 1) a purified basal diet, 2) basal diet plus 2% glycine, 3) basal diet plus 3% sodium benzoate, 4) basal diet plus 3% sodium benzoate and 2% glycine, 5) basal diet containing only 3% protein. Feces and urine are collected quantitatively each day and body weights, food intake, and hemoglobin levels are determined weekly. The results indicate a relationship in the rat between the protein and glycine intake, and porphyrin synthesis as measured by fecal porphyrin excretion and hemoglobin formation.

Liver desoxyribonucleoprotein J. MURRAY LUCK, DONALD KUPKE,* ALBERTA RHEIN* AND A. CLARK GRIFFIN* *Dept. of Chemistry, Stanford Univ., Stanford, Calif.*

Desoxyribonucleoprotein has been prepared from the livers of normal rats. Homogenates and

nuclei were used as the source material. Differential extractions with sodium chloride and precipitation in 0.14 M sodium chloride were used in preparation. The product, dialyzed until salt-free, was lyophilized and used for determination of nucleic acid content, free and bound lipid, and protein. The purine and pyrimidine bases of the nucleic acid portion were determined as well as the amino acid distribution in the protein hydrolysate. The lyophilized nucleoprotein does not redissolve satisfactorily either in water or in molar sodium chloride. Other studies in this laboratory confirm the findings of others that dissociation proceeds in solutions of high ionic strength. The low solubility of the nucleoprotein in 0.001 M citrate or arsenate militates against the use of aqueous extraction as a preparative method.

Effect of total body x-radiation on plasma alkaline phosphatase of rats STEPHAN LUDEWIG *Biochemical Lab, Univ. of Virginia, Charlottesville*

The plasma alkaline phosphatase activity of 60-day old male rats was determined after irradiation with doses between 200 r and 600 r. The King-Armstrong method used in the determination of plasma phosphatase was slightly modified. A moderate increase in enzyme activity was noted 24 hours after irradiation. The values decreased after the 2nd day in the 400, 500 and 600 r groups. The length of time that the values remained low was dependent on the dosage. Pair-fed rats served as controls since food intake of exposed animals was influenced by x-radiation. The phosphatase activity was depressed in the plasmas of animals exposed to multiple small doses.

Liberation of amino acids in human seminal fluid FRANK LUNDQUIST (introduced by H. A. LARDY) *Inst. of Legal Medicine, Univ. of Copenhagen, Denmark*

During some attempts to determine the composition of the secretions from the accessory sex glands in man, it was accidentally observed that the amino-nitrogen of semen showed a very rapid increase on incubation. Amino-nitrogen levels of about 200 mg/100 ml were obtained in 2 or 3 hours. Experiments using the manometric ninhydrin method gave evidence that free amino acids were the substances concerned. The proteolytic enzyme responsible for this phenomenon was partly purified through salt fractionation. It has a pH optimum of about 7.5 when casein is used as a substrate. Chromatographic analyses using filter paper showed the same pattern of amino acids in deproteinized filtrates of incubated semen as in filtrates from fibrin incubated with the purified enzyme. A fibrin-like substance has been isolated from fresh semen and was found to give rise to the same amino acids as native semen. Possibly the enzyme concerned is identical with the fibrinol-

ysin observed by Huggins and Neal (*J. Exper. Med.* 76: 527, 1942) in human ejaculate.

Studies on lactalbumin heated with carbohydrate IVAN J. MADER,* LAWRENCE J. SCHROEDER* AND ARTHUR H. SMITH *Dept. of Physiological Chemistry, Wayne Univ. College of Medicine, Detroit, Mich.*

A comparison of *in vitro* and *in vivo* studies was made in an attempt to evaluate the role of heat and carbohydrate concentration in the non enzymatic browning of proteins as exhibited in the Maillard Reaction. Commercial and 'carbohydrate-free' lactalbumin, heated under various conditions, was used in *in vitro* digestion experiments. The conventional formol titration procedure, showed that the rate and amount of digestion of the protein by pancreatin is inversely proportional to the severity of heat treatment. Removal of reducing substances from lactalbumin before heating permits a greater rate and degree of enzyme hydrolysis of the heated product. Microbiological assays for valine, histidine, leucine, phenylalanine, lysine and methionine in acid hydrolysates of the various proteins showed that only lysine was partially destroyed. These observations suggest that the carbohydrate reacts with the protein to form enzyme-resistant materials. A nitrogen balance study in dogs was used to determine the relative effect of heat and of carbohydrate in the alteration of the nutritive value of heated protein. 'Carbohydrate-free' lactalbumin and extracted lactalbumin to which had been added 2 and 5% lactose served as the protein nitrogen sources. The time of autoclaving of these proteins was varied from one-half to one hour. The biological value was high in all cases. However, as either the carbohydrate concentration or the time of heating was increased, the nutritive index of the protein decreased. Lowered digestibility, shown by increased fecal nitrogen, accounted for the decrease in nutritive value.

Quick blood sugar method suitable for routine use and screening EMANUEL E. MANDEL AND RAYMOND H. OWINGS (introduced by OLAF MICKELSEN) *Clinical Pathology Section, Lab. Services, Communicable Disease Center, Public Health Service, Federal Security Agency, Atlanta, Ga.*

In the search for a blood sugar method applicable to large-scale diabetes surveys, modification of a 'bed side' procedure (*J. Lab. & Clin. Med.* 34: 720, 1949) not only lent itself to this purpose but also proved to be a useful routine method. From a mixture of blood and saturated picric acid solution in the proportion of 1:5 or higher, a filtrate is obtained which after addition of 10% sodium hydroxide, boiling and dilution with distilled water is read in a spectrophotometer at wave

length 540 The glucose color reaction is stable for one hour, readily reproducible, and follows Beer's law, it is unaffected by delay in centrifuging or filtering the initial blood-reagent mixture up to 3 days or by minor variations in the boiling time The determination includes non-fermentable reducing substances in the blood, and hence compares well with Folin-Wu's and similar methods while results are consistently higher than those obtained with 'true glucose' methods (SOMOGYI, KINGSLEY) Addition of cadmium sulfate to the picric acid reagent and alkalinizing before filtration approximates results to the true glucose levels In screening for diabetes, expediency is enhanced by 1) using automatic devices such as Cornwall syringes and pipetting machines for volumetric measurements 2) dividing the spectrophotometer scale into groups according to selected blood glucose levels and reporting group designations rather than individual values After the initial centrifugation, 5 technicians can conveniently run 2,000 determinations in less than 4 hours

A continuous indicating recording oxygen cathode

GEORGE H MANGUN AND WALTER D GRIEST *
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A recording oxygen cathode useful in studying tissue oxygen changes has been devised, based on the principle described in 1942 by Davies and Brink It differs principally in the following details 1) Semi-micro electrodes of various metals are employed, 2) Tungsten electrodes 1-20 mm long have proven very useful since they may be inserted readily without causing severe mechanical injury and give reproducible values for many hours, 3) A Ag/AgCl reference electrode is applied directly to the skin via saturated KCl Jelly, 4) A General Motors chopper amplifier of low input resistance is employed Although this amplifier interrupts the current momentarily at 60 cycles/sec, it yields current-oxygen tension curves similar to continuous direct current, 5) The amplifier output is fed into a 0-5 ma Esterline-Angus recording millimeter, 6) Resistors parallel to the cathode circuit provide for rapid calibration Since factors other than oxygen tension affect the rate of arrival of oxygen at the cathode surface, calibrations have been made by gasometric analysis of venous and arterial blood, or the results expressed arbitrarily in terms of microamperes/mm of tungsten wire Tissues rendered completely anoxic yield zero readings, furnishing one known point for calibration Studies have been made on muscle, liver, venous and arterial blood, and subcutaneous tissues, and the effects of nitrogen, oxygen, barbiturates, infra-red heat, and ice water demonstrated Comparative studies with this in-

strument and the oxymograph will be reported elsewhere by Behrmann, Griest, Mangun, Hartman

Determination of fumaric, cis-aconitic and alpha-ketoglutaric acids in rabbit kidney and liver by partition chromatography LAWRENCE M MARSHALL AND FREDERICK D DREW (introduced by VERNON A WILKERSON) *Dept of Biochemistry, Howard Univ College of Medicine, Washington, D C*

The chromatographic analysis of fumaric acid (*J Biol Chem* 179 1127, 1949) has been extended to the determination of alpha ketoglutaric, cis-aconitic and fumaric acid in rabbit kidney and liver Improved rate of flow through silica gel columns was obtained by maintaining constant pressure over the influent Cis-aconitate and alpha ketoglutarate were measured with an influent mixture of tertiary amyl alcohol and chloroform in a ratio (vol/vol) of 1:9 while for fumaric a 1:19 ratio was used Mechanically collected samples, each of 2 ml volume, showed peaks for fumaric, alpha ketoglutaric and aconitic acids at fractions 9, 33 and 56 respectively Effluent fractions were measured spectrophotometrically (λ 230) and by titration Symmetry of the chromatographic curves and a correlation between the 2 methods of measurement for each acid served to characterize the effluent zones Known metabolic acids did not interfere Average percentage recovery of each acid added to tissues exceeded 95 % The average concentrations of fumaric acid in kidney and liver in (mg/100 gm wet tissue) were 3.2 and 2.0 respectively while those of aconitic and alpha ketoglutaric acids were less than 1

Acetylation of p-amino benzoate and related compounds by the rabbit M F MASON, GUS CASTEN* AND ALLAN LINDSAY * *Parland Hospital and Southwestern Med School of Univ of Texas, Dallas*

Following intraperitoneal injection of 200-500 mg of p-amino benzoate in the rabbit, acetylation by the liver attains a maximum rate (= 'Lm acetylaminobenzoate') which is then essentially independent of the plasma concentration of p-amino benzoate achieved The acetylation rate is calculated by measuring the amount of acetylaminobenzoate appearing in the urine and accumulating in the body per unit time Calculations based on the ultimate plasma concentrations obtained after similar injections into nephrectomized rabbits indicate that the free and acetylated forms are distributed in a fluid mass approximately 30% of the body weight of the rabbit Preliminary estimates of Lm acetylsulfanilamide, and Lm acetyl p-amino hippurate, have also been made

Deamination and denaturation of crystalline egg albumin PAUL H MAURER* AND MICHAEL HEIDELBERGER *Depts of Biochemistry and Medicine, Columbia Univ College of Physicians and Surgeons, New York City*

Deamination of crystalline egg albumin (Ea) beyond 30-35% at pH 4 and 0° C leads to a deaminated denatured Ea (Fraction A), the word 'denatured' being used to indicate insolubility at the isoelectric point (*Federation Proc* 8 227, 1949) The physical, chemical and immunological properties are different from those of acid denatured Ea (DnEa) Treatment of Fraction A at 22-24° C and pH 1.5, conditions leading to acid DnEa from Ea, gives an A Dn preparation with physical, chemical and immunological properties differing little from those of the original A fraction A partly deaminated (25-30%), undenatured Ea (Fraction B) gave at pH 1.5 a B Dn preparation with properties similar to those of acid DnEa The structure arrived at by partial unfolding of the protein molecule during deamination is therefore different from that of acid DnEa It is more resistant than Ea or Fraction B to acid denaturation and is more stable than DnEa to aggregating influences, and hence has a smaller particle weight than DnEa preparations with the possible exception of heat DnEa

Hydrolysis of protamines by cathepsins MARY E MAVER AND ANTOINETTE E GRECO* *Natl Cancer Inst, Natl Insts of Health, USPHS, Bethesda, Md*

The role of cathepsins in nuclear metabolism has been studied by following the activity of these intracellular proteinases on the constituents of the nucleus The ability of calf spleen and thymus cathepsin preparations to hydrolyze thymus nucleoproteins and to depolymerize the liberated nucleic acid has been reported previously The cathepsin preparations also hydrolyzed the protamines, salmine and clupein, which are combined with desoxyribonucleic acid in the ripe sperm heads of salmon and herring The degree of hydrolysis was found by determining the increase in soluble N, amino N, arginine, serine and proline when the digests were precipitated with methyl alcohol The hydrolyses were markedly activated by cysteine and had an optimum at pH 5.0 Cathepsin preparations from calf and beef spleen, calf thymus and 2 transplantable rat tumors, a lymphosarcoma, and a hepatoma showed protaminase activities which corresponded in most cases to their proteolytic activities on hemoglobin

Relation of sulfhydryl and disulfide groups in ferritin to its vasodepressor activity ABRAHAM MAZUR AND EPHRAIM SHORR* *Dept of Medicine, Cornell Univ Medical College and The New York Hospital, New York City*

The vasodepressor activity of solutions of crystalline ferritin, as measured by its effect in the rat mesoappendix test, is shown to be related specifically to sulfhydryl groups Treatment of ferritin with reagents such as o-iodosobenzoate, p-chloromercuribenzoate, iodoacetate, iodoacetamide, or oxygen in the presence of a trace of Cu^{++} , leads to the disappearance of the SH groups as well as of the vasodepressor activity Reactivation can be obtained with cysteine, ascorbic acid or reduced glutathione When the vasodepressor activity of ferritin is inactivated by incubation with normal liver slices in oxygen, reactivation occurs after treatment with the above reducing agents, indicating a similar chemical mechanism Normal liver slices in oxygen oxidize the SH groups of ferritin, no oxidation takes place in nitrogen Saline extracts of aerobic liver slices, centrifuged free of cells, will aerobically inactivate the vasodepressor activity of ferritin as well as oxidize its SH groups Reactivation is obtained with cysteine or ascorbic acid Evidence is presented to show that the ferritin normally present in liver is predominantly in the -S-S- or inactive form but that after being subjected to anaerobic conditions the ferritin is converted to the SH or vasotropically active form Normal liver slices exposed to nitrogen and then returned to oxygen suffer an impairment in their ability to inactivate the vasodepressor activity of ferritin parallel with an impairment in their ability to oxidize the SH groups of ferritin

Enzymatic anodic phosphorylation in rat brain homogenates W K McEWEN* AND JOHN J EILER *College of Pharmacy, Univ of California Med Center, San Francisco, Calif*

A smooth platinum anode was used to accept electrons from the anaerobic oxidation of succinate in fortified rat brain homogenates A 3.3×10^{-3} M ferro/ferricyanide couple was employed as a mediator The homogenates were similar to those used in our previous work (EILER AND McEWEN *Arch Biochem* 20 163, 1949) including the use of pentobarbital to suppress the oxidation of substrates other than succinate During the course of a run the amperage was relatively constant and the coulombs produced were equivalent to the oxygen consumed by an identical homogenate under aerobic conditions The phosphate esterification was about 3 of that of the oxygen control It can be shown that both the current flow and the phosphate esterification are dependent upon active enzymes The addition of malonate depressed markedly the flow of current and abolished the phosphorylation In the absence of added succinate, or when heated brain tissue was substituted for normal brain tissue, there was neither significant current flow nor phosphoryla-

tion From the evidence available it is probable, under the conditions of anodic phosphorylation, that cytochrome oxidase is not involved in either electron transport or phosphate esterification. The general applicability of this method and its significance in the study of oxidative phosphorylation will be discussed.

Investigation of relation of insulin to lipid metabolism, with special reference to phospholipid
EVA C MCGHEE,* EVANGELINE PAPAGEORGE,*
WALTER L BLOOM* AND GEORGE T LEWIS
Biochemistry Dept, Emory Univ School of Medicine, Emory Univ, Ga

In a series of 5 experiments rabbits were used to study the effect of insulin on blood lipid components and on brain and liver lipid phosphorus. Massive doses of insulin were administered to 3 groups of animals. One was given insulin alone, the second and third were given subsequent injections of lecithin and glucose, respectively. Insulin deficiency was studied in alloxan diabetic animals, and lecithin tolerance, in normal rabbits. Normal values for tissue lipid phosphorus were obtained from a control group. A summary of the statistical appraisal of the data obtained is as follows. Heavy doses of insulin produce a significant decrease in the blood levels of total fatty acids and glucose, and a decrease in lipid phosphorus which approaches significance. These decreases appear to be a secondary insulin effect inasmuch as they do not occur when glucose is administered in quantities sufficient to maintain the blood glucose above a convulsive level. A significant increase in blood sugar follows the administration of lecithin to normal rabbits. However, the injection of lecithin does not alter the course of insulin action on blood sugar in the insulinized animal. No significant change in liver lipid phosphorus was noted under our experimental conditions. Hyperinsulinism produces a significant decrease in brain lipid phosphorus. This decrease is independent of the blood glucose level and is not alleviated by lecithin in the amount given.

Histamine release from rabbit blood cells by pure compounds—competitive inhibition phenomena
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Depts of Biochemistry and Pharmacology, Abbott Labs, North Chicago, Ill

In our investigations on the mechanism of histamine release from rabbit blood cells we have found that the histamine is released very rapidly by 2 series of pure compounds (primary aliphatic amines and alkyl β -carbomethoxypyridinium salts) and that closely related compounds will competitively inhibit this histamine release. At 37°C n-octadecylamine 8×10^{-5} M, will release all or nearly all of the histamine from the normal

rabbit blood cells. At the same concentration n-hexadecylamine is less effective and n-dodecylamine releases histamine only slightly. The histamine release by the primary amine, octadecylamine, is 50–80% inhibited by the simultaneous addition to the blood of the tertiary amine, N-dimethyloctadecylamine or N-dimethyldodecylamine, in a ratio of 3 molecules/molecule of octadecylamine. A maximum histamine release results also when n-hexadecyl- β -carbomethoxypyridinium bromide is used at a concentration of 8×10^{-5} M. The histamine release by this compound is almost completely blocked by the simultaneous addition of 1) aliphatic quaternaries, e.g. N-trimethyloctadecylammonium chloride, or 2) alkyl pyridinium quaternaries where the β -carbomethoxy is replaced by a carbamyl group or halogen, e.g. n-hexadecyl- β -carbamylpyridinium bromide or n-hexadecyl- β -bromopyridinium bromide.

At 37°C the histamine release by these compounds is 90% complete in one minute. The fact that pure compounds will release histamine and the rapidity of the reaction cast doubt upon the theory that the release of histamine requires a protease activation as a preliminary step. 1,10-Diaminodecane and 1,6-diguanidylhexane (MacINTOSH AND PATON *J Physiol* 109:190, 1949), 5×10^{-4} M, released little or no histamine from rabbit blood cells.

Chemical components and metabolic activities of relapsing fever spirochete (*Borrelia novyi*).

RALPH W MCKEE AND QUENTIN M GEIMAN*
Dept of Biological Chemistry, Harvard Med School, and Dept of Tropical Public Health, Harvard School of Public Health, Boston, Mass

Studies of *B. novyi* have been conducted to determine the biological nature of the spirochetes and to indicate a logical approach to the formulation of a nutrient culture medium which will produce *in vitro* growth and multiplication. The organisms were isolated from the blood of infected white rats by differential flotation and centrifugation. A partition of the phosphorus (P) components of the isolated spirochetes was conducted according to the method of Schmidt and Thannhauser (*J Biol Chem* 161:83, 1945). The trichloroacetic acid insoluble material was extracted with fat solvents and the extract analyzed for total lipid P (2.01 mg/10⁸ organisms), lipid (382 mg) and cholesterol (73 mg). The remainder of the trichloroacetic acid insoluble fraction was partitioned and analyzed for desoxyribonucleic acid P (0.694 mg), ribonucleic acid P (1.565 mg) and phosphoprotein P (0.606 mg). The trichloroacetic acid soluble fraction was analyzed for inorganic P (0.352 mg), 15-minute hydrolyzable P (0.691 mg) and residual organic P (0.352 mg). The total nitrogen of the isolated spirochetes was 99 mg.

The organisms are being analyzed for amino N and amino acids. Glycolytic studies show that *B. novyi* utilize large quantities of glucose (0.4 mM) but only 0.1 mM of oxygen/hr. About 65% of the glucose was accounted for as accumulated lactic acid. Thus no more than 10% of the consumed glucose could have been oxidized completely to carbon dioxide and water. Use of the accumulated data has been made in preliminary cultivation studies. Growth and prolonged motility of the spirochetes *in vitro* have been obtained but without multiplication.

Plasma growth and inhibitory factors for malarial parasite (*Plasmodium knowlesi*) RALPH W. MCKEE AND QUENTIN M. GEIMAN* *Dept. of Biological Chemistry, Harvard Med. School, and Dept. of Tropical Public Health, Harvard School of Public Health, Boston, Mass.*

Although *in vitro* multiplication of *P. knowlesi* can be obtained consistently in cultures using a synthetic medium, the addition of normal monkey plasma enhances growth and multiplication. The effective components of plasma are only slowly dialyzable but they can be separated by prolonged dialysis through cellophane membranes. In a study to determine the animal species yielding the highest plasma concentration of the stimulatory agents, blood from cattle, man and monkey were studied. Bovine plasma inhibited growth while human plasma stimulated growth to only a small extent. Monkey plasma produced the best results with marked enhancement of growth (multiplication approximately doubled when 20% of the culture was plasma). Simultaneous perfusion cultures (*J. Exper. Med.* 84: 583, 1946) were carried out in an effort to separate by dialysis any growth stimulatory material. Very striking results were obtained indicating that bovine plasma contains stimulatory materials but their effects were masked by the inhibitory substances which dialyzed away faster than did the active components. Tests of the dialysate showed definite inhibition of growth while the impermeate produced stimulatory effects with *P. knowlesi*. The implications of these findings are two-fold. Not only is the stimulatory effect of importance for a complete evaluation of the nutrient requirements and for prolonged cultivation but the chemical differences among various animal species, particularly the presence of inhibitory substances, may help to explain host-parasite specificity.

Effect of acute choline deficiency on tissue lipids of young puppies J. M. MCKIBBIN *Dept. of Biochemistry, Syracuse Univ. College of Medicine, Syracuse, N. Y.*

Total lipid nitrogen, phosphorus, choline and sphingosine were determined on purified extracts of 10 tissues of choline deficient puppies and their

littermate controls. Total liver lipid phosphorus and nitrogen were not decreased in the deficient animals although considerable variation was observed in both groups. Lipid choline/gm of dry lipid free tissue was not significantly decreased but the percentage of lipid nitrogen represented by choline was very significantly decreased. Sphingolipids were significantly increased in the deficient dog livers on both the per gram and percentage of lipid nitrogen basis. These are interpreted as sphingomyelins. Lecithins were therefore significantly decreased on both a per gram and percentage basis in the deficient livers. The pattern of lipid nitrogen is altered more than the absolute amounts of the various lipids. Blood plasma total lipid phosphorus and nitrogen were reduced to an average of about one-third that of the controls. The choline to phosphorus ratio was not reduced but the sphingosine to phosphorus ratio was increased in the deficient plasmas. It is concluded that sphingomyelins are replacing lecithins in the plasma. The intestine phospholipids were decreased about 15% in the deficient animals and this decrease was not confined to the choline phospholipids. Skeletal muscle phospholipids were slightly lowered due principally to an 18% reduction in the choline phospholipids. The lipids of lung, cerebrum, spleen, pancreas, kidney and heart were rigidly maintained in this severe deficiency.

Factors affecting synthesis of acetylcholine (ACh) by rat brain cortex slices HUGH McLENNAN* AND K. A. C. ELLIOTT *Montreal Neurological Inst., McGill Univ., Montreal, Canada*

In confirmation of work of Mann, Tennenbaum and Quastel, the synthesis of free ACh by brain cortex slices in the presence of glucose and oxygen was found to be greatly stimulated by the presence of 0.027 M K^+ , this effect occurred in bicarbonate but not in phosphate buffered medium and was inhibited by high Ca^{++} concentration. Contrary to the report of the previous authors, no equilibrium concentration of free ACh was found but synthesis continued for several hours, the rate tended to increase during the first 2 hours and addition of ACh often accelerated, never inhibited, the synthesis. No consistent change in the bound ACh was produced by added K^+ . The synthesis was very sensitive to the concentration of Ca^{++} . Synthesis was low without Ca^{++} maximal at 1.3 mM Ca^{++} , and fell off sharply with higher concentrations. Similar but less marked dependence on Mg^{++} was observed. Citrate inhibited synthesis in Ca^{++} -containing medium. Synthesis was greatly reduced in unbuffered medium containing 1.3 mM Ca^{++} or in debicarbonated serum. The presence of bicarbonate- CO_2 was evidently essential and experiments indicated an optimal

bicarbonate concentration (at nearly constant pH) around 25 mM. Bicarbonate could not be replaced by oxaloacetate, when glucose and bicarbonate were present, oxaloacetate and pyruvate caused some inhibition. Under otherwise optimal conditions sodium pentobarbital (Nembutal) inhibited synthesis by about 50% at a concentration, 0.5 mM, which inhibited respiration by only about 15%. Metrazol, 5 mM increased synthesis, higher concentrations were inhibitory, with little effect on respiration.

Mechanism of carbon dioxide fixation in *M. lysodeikticus* ROSABELLE McMANUS (introduced by M. F. UTTER) *Dept. of Biochemistry, Western Reserve Univ. School of Medicine, Cleveland, Ohio*

The oxalacetic carboxylase reaction was first demonstrated in *M. lysodeikticus* and its apparent reversibility shown by incorporation of $C^{14}O_2$ in the β -COOH of oxalacetate during its breakdown to pyruvic acid and CO_2 in the presence of $C^{14}O_2$. We have continued the study of this reaction in *M. lysodeikticus* using an aqueous extract from disintegrated cells as enzyme source and have demonstrated certain properties of this system which differ from the system occurring in pigeon liver. In contrast to the stimulatory effect of ATP on the fixation reaction catalyzed by pigeon liver, ATP fails to enhance the fixation of CO_2 by *M. lysodeikticus*. That muscle ATP is active under the conditions of this study was established by demonstrating its participation in the hexokinase reaction and in the phosphopyruvate transphosphorylase reaction using the same bacterial preparation. After purification of oxalacetate from the incubation mixture by means of chromatographic techniques, there was no indication that substances other than oxalacetate contained radioactivity. Under our experimental conditions, fixation of CO_2 in oxalacetate formed enzymatically from malate appears to be comparable to fixation in synthetic oxalacetate. Possible operation of Ochoa's 'malic' enzyme system in the bacteria prompted study of fixation in the presence of malate. In contrast to results with pigeon liver where there is some fixation of CO_2 in malate, the bacterial system fails to fix CO_2 into malate even in the presence of TPN although good fixation is occurring in oxalacetate, precluding malate as an intermediate in this system.

Composition and densities of β -lactoglobulin crystals in sucrose and in serum albumin solutions T. L. McMEEKIN, M. L. GROVES* AND N. J. HIPP* *Eastern Regional Research Laboratory, Philadelphia, Pa.*

The fact that the density of a virus as determined by ultracentrifugal studies varies in different suspending media (SMADL, PICKELS AND SHEDLOVSKY *J. Exper. Med.* 68: 607, 1938) sug-

gested that a study of the densities and composition of protein crystals suspended in similar solutions would be of value in interpreting the behavior of the virus. The composition and densities of β -lactoglobulin crystals have been determined in sucrose and in serum albumin solutions. Several relations were found between the composition of the crystal and the suspending medium which are consistent with the variations in the density of the virus in similar solutions as determined by ultracentrifugal studies. It was found that sucrose penetrates the protein crystal, and that the concentration of sucrose in the water of the crystal varies with the concentration of sucrose in the suspending medium. In contrast, serum albumin does not penetrate the β -lactoglobulin crystal. It was also found that protein crystals suspended in sucrose solutions lose water and that the loss in water is directly proportional to the difference between the osmotic pressure of the sucrose in the water of the protein crystal and the osmotic pressure of the suspending sucrose solution. These findings indicate that the value for density of a virus as determined by ultracentrifugal studies in serum albumin solutions is less likely to be in error than the value obtained in sucrose solutions. Also, the calculated nonsolvent water or 'bound' water of a protein crystal is shown to vary with the environment.

Lipids of peripheral nerve after nerve crush (axonotmesis) A. R. McNABB* AND R. J. ROSSITER *Dept. of Biochemistry, Univ. of Western Ontario, London, Canada*

Previously it has been shown (*Biochem. J.* 45: 500, 1949) that when a cat sciatic nerve undergoes Wallerian degeneration after nerve section (neurotmesis) there is at first an increase in the content of water and a temporary decrease in the content of neutral fat. After 8 days there is a steady decrease in the concentration of both total lipid and myelin lipid, i.e. cerebroside, free cholesterol and sphingomyelin, the three substances which we have suggested are the principal lipid components of the myelin sheath of a mammalian nerve. There is also a remarkable esterification of cholesterol, greatest at 16 days. For the first 32 days after nerve crush (axonotmesis) the changes in the concentration of water and lipid were essentially the same as those following nerve section. Then from 32 to 64 days the concentration of the various lipids decreased less rapidly in the crushed nerve, and by 64 days the concentration of lipids in the crushed nerve had commenced to increase. The concentration of ester cholesterol reached a maximum between 16 and 32 days, and thereafter decreased progressively, none remaining at 144 days. The slower decrease in the concentration of the lipids between 32 and

64 days and the actual increase in the concentration of these lipids after 64 days undoubtedly represent the formation of new myelin sheaths, and other lipid-containing structures, following upon the regenerative peripheral outgrowth of new axons. The individual myelin lipids, i.e. cerebroside, free cholesterol and sphingomyelin, returned at similar rates.

Histochemical distribution of indophenol oxidase and acid phosphatase in rat cortex J A MEATH AND A POPE (introduced by J FOLCH) *McLean Hospital, Waverley, Mass., and Harvard Med School, Boston, Mass.*

The cytological distribution of indophenol oxidase (cytochrome oxidase) and of acid phosphatase was determined histochemically in the motor somatosensory and entorhinal areas of rat cortex. Coronal sections of unfixed tissue were cut at -10°C and used alternately for histochemical and histological study. Indophenol oxidase activity, determined by a modification of Graf's method, was greater in isocortex (motor and somatosensory) than in allocortex (entorhinal), the increase being the outer lamina (layers I-IV). In motor cortex the greatest activity was in layers I, II and IIIA, in somatosensory cortex in layers I, III and IV. Intensity of oxidase reaction was similar to degree of vascularization, both possibly reflecting the local metabolic rate. Acid phosphatase activity, determined by a modification of Gomori's method, was greatest in axones and in the larger pyramidal cells irrespective of location, their cell body and apical dendrites being most intensely stained. Smaller neurones were less intensely stained. Glial elements were unstained with the exception of subpial astrocytes. With unfixed frozen cortex, the phosphatase reaction differed quantitatively and qualitatively from that observed when acetone fixed and paraffin-embedded tissue was used. Optimal incubation time was 3 hours for the former as compared to 72-96 hours for the latter. In the former, the cytoplasm of the neurones was maximally stained. In the latter, staining was usually more intense in the nucleolus and nucleus than in the cytoplasm.

Effect of keto acids on the in vitro metabolism of glutamine and glutamic acid ALTON MEISTER (introduced by JESSE P GREENSTEIN) *National Cancer Inst., Bethesda, Md.*

The effect of a number of acids possessing α , β , γ , or δ -keto groups on the desamidation of glutamine by liver homogenates and partially purified liver fractions has been investigated. Only α -keto acids produced significant acceleration of glutamine desamidation. Although α , γ -diketo acids accelerated this reaction this was due to pyruvate formed by hydrolysis of the diketo acids. Protein fractions were obtained from rat

liver homogenates which exhibited a 30-fold increase in desamidase activity and which also exhibited considerable transaminase activity. With these preparations no desamidation of glutamine occurred in the absence of pyruvate, while in the presence of pyruvate the products were alanine, α -ketoglutarate and ammonia. Similar results were obtained with α -ketoisocaproate and phenylpyruvate, although desamidation of glutamine in the presence of these keto acids occurred more rapidly than did transamination. The amino acids were identified and quantitated by microbiological and chromatographic methods, α -ketoglutarate was identified as the 2,4 dinitrophenylhydrazone. In experiments with glutamine labelled with N^{15} in the amide group and with added pyruvate, all of the isotope was recovered as inorganic ammonia. Pyruvamide did not substitute for pyruvate nor was this compound desamidated in this system, while the γ -methylamide of glutamic acid was considerably less active than glutamine or glutamic acid.

Studies in the metabolism of L-threonine HERBERT L MELTZER* AND DAVID B SPRINSON *Dept. of Biochemistry, Columbia Univ., New York City*

L-threonine, containing C^{14} in the γ -carbon atom and N^{15} in the amino group, has been prepared and fed to rats. Urinary acetyl-L- α amino- γ -phenylbutyric acid and hippuric acid revealed that at least 25% of the administered threonine was converted to acetate while conversion to glycine occurred to a smaller extent. Almost all of the activity of the excreted acetate was found in the methyl carbon. The ratio of activities in the internal organ cholesterol and fatty acids suggests that the acetylating agent formed from threonine is acetate. The N^{15} concentration of the internal organ serine was higher than that of the glutamic acid, indicating a direct conversion of threonine to glycine followed by formation of serine. Isotopic threonine was isolated from the internal organ proteins by large-scale starch chromatography (MOORE AND STEIN *J Biol Chem* 178: 53, 1949). By the analysis of the internal organ threonine isolated after feeding N^{15} -L-leucine it was demonstrated that the carbon chain of threonine, like that of lysine, does not accept nitrogen from the metabolic pool (Cf. ELLIOTT AND NEUBERGER *Biochem J* 45, no 2 viii, 1949). When the labeled threonine was added to duck blood *in vitro* the isolated hemin had C^{14} and N^{15} in agreement with the known utilization of acetate and glycine for heme synthesis (RADIN, RITTENBERG AND SHEMIN *Federation Proc* 8: 240, 1949).

Chemical and biological properties of biotin sulfide DONALD B MELVILLE, DOROTHY S GENGHOF* AND JOHANNA M LEE* *Dept. of Bio-*

chemistry, Cornell Univ Med College, New York City

A crystalline compound highly active as a yeast growth factor was isolated from esterified biotin concentrates from milk, and was shown by analysis and by synthesis from biotin to be the methyl ester of biotin sulfoxide. The free acid was prepared by treatment of biotin with the theoretical amount of hydrogen peroxide in acetic acid solution. Under these conditions two diastereoisomeric forms of biotin sulfoxide are produced, by virtue of the asymmetric nature of the sulfoxide grouping. The isomer produced in largest amount is dextrorotatory and possesses essentially the same growth-promoting activity for *Saccharomyces cerevisiae* as biotin, conversion of this isomer to the methyl ester yields a compound identical with that isolated from esterified biotin concentrates. The other isomer is levorotatory and is not more than one-thousandth as active as biotin toward *Saccharomyces cerevisiae*. Both isomers on further oxidation with hydrogen peroxide yield biotin sulfone. The dextrorotatory sulfoxide shows the same growth activity as biotin toward *Lactobacillus arabinosus*, but negligible activity, compared to biotin, for *Lactobacillus casei*. The levorotatory sulfoxide shows approximately 5% of the activity of biotin toward both these microorganisms. The injection of either the dextro or levo sulfoxide into biotin-deficient rats, at a level of 10 γ daily, did not alleviate the deficiency symptoms.

Nitrate and ammonia assimilation in plants JULIUS L MENDEL AND DONALD W VISSER (introduced by R J WINZLER) *Dept of Biochemistry and Nutrition, Univ of Southern California School of Medicine, Los Angeles*

A comparison of the assimilation of nitrate and ammonia in nitrogen deficient tomato plants was investigated by measuring the incorporation of N^{15} after administration of N^{15} labelled nitrate or N^{15} labelled ammonia. The plants were divided into root, leaf, and stem tissues which were fractionated into various nitrogenous constituents (ammonia, amides, and amino acids). For a given tissue, the N^{15} distribution followed the same general pattern whether nitrate or ammonia were the nitrogen source, the highest atom percentage excess of N^{15} in both cases was found in the ammonia and amide fractions. In the plants given ammonia, there was a higher atom percentage excess of N^{15} for each nitrogenous constituent in the roots than in the leaves, while in the plants given nitrate, there was a higher atom percentage excess of N^{15} in the leaves than in the roots.

Nature of the intrinsic factor CURTIS E MEYER, SAMUEL H EPPSTEIN*, FRANK H BETHELL* AND BYRON E HALL * *Research Labs, Upjohn Company, Kalamazoo, Mich, Thomas Henry Simp-*

son Memorial Inst, Ann Arbor, Mich, and Mayo Clinic, Rochester, Minn

During the course of concentrating the intrinsic factor from hog intestines, certain similarities between this factor and lysozyme became apparent. It has been observed that lysozyme derived from egg white has the property of combining with vitamin B-12, thus rendering it unavailable to *Lactobacillus lactis* Dorner and *Escherichia coli*. Oral administration of 9 μ g of vitamin B-12 in the form of the complex, daily, to patients with pernicious anemia results in remission, with characteristic reticulocyte response and erythropoiesis.

Hydrolysis of sulfated mucopolysaccharides by testicular hyaluronidase KARL MEYER AND MAURICE M RAPPORT* *Dept of Medicine, Columbia Univ, College of Physicians and Surgeons, New York City*

Chondroitin sulfate of hyaline cartilage has been shown to be hydrolyzed by testicular, but not by pneumococcal (MEYER, K, E CHAFFEE, G L HOBBY AND M H DAWSON *J Exper Med* 73 309, 1941) or streptococcal (HUMPHREY, J H *Biochem J* 40 442, 1946) extracts active as hyaluronidases. The hydrolysis of chondroitin sulfate was believed to be due to an enzyme distinct from hyaluronidase (MEYER, K *Physiol Rev* 27 335, 1947). Comparison of the activity of 10 testicular hyaluronidases of greatly varying potency and mode of preparation toward hyaluronate and 3 different samples of chondroitin sulfate showed a constant ratio of the activities on the 2 substrates. The depolymerization was measured by turbidimetric and reductimetric assay methods for both activities. These experiments indicate that testicular hyaluronidase hydrolyzes chondroitin sulfate. Other as yet unidentified sulfated mucopolysaccharides isolated from heart valves, tendon and other connective tissues are hydrolyzed by testicular hyaluronidase at rates similar to that obtained with hyaluronate as substrate.

Pasteur effect in dead yeast O MEYERHOF AND SILVIO FIALA * *Dept of Physiological Chemistry, Univ of Pennsylvania School of Medicine, Philadelphia*

The quickly dried yeast which was recently described (O MEYERHOF *J Biol Chem* 180 575, 1949) has been used for the study of the Pasteur effect. This yeast preparation which has preserved its ATP-ase and which ferments sugar with constant speed shows a respiration similar to living yeast. Quickly dried brewers' yeast has a very low uptake of oxygen but quickly dried bakers' yeast shows an appreciable respiration in the presence of sugar. The Pasteur quotient, moles carbon dioxide disappeared/moles oxygen consumed, is around two like that in the living

yeast With such a yeast, in which phosphate does not accumulate anaerobically, accumulation of phosphate occurs in the presence of oxygen coupled with respiration Substances which inhibit the oxidative phosphorylation at least partially without inhibiting the respiration as nitrophenol and dinitrophenol inhibit also the Pasteur effect The same substances do not inhibit anaerobic phosphorylation The meaning of these observations for the theory of the Pasteur effect will be discussed

Micro-method for determining carbohydrate components of gastric mucin MORITZ MICHAELIS*, FRANKLIN HOLLANDER AND CARYL TARR* *Gastro-Enterology Research Lab, Mount Sinai Hospital, New York City*

Methods in current use for determining gastric mucin are based on estimations of either tyrosine for the protein moiety, or total reducing matter or glucuronic acid for the prosthetic group These methods all assume that the component being determined is an indicator of the total amount of mucin, even for mixed stomach contents which contains two or more varieties of mucoprotein This assumption still remains to be validated The present report describes a relatively simple procedure for the simultaneous determination of glucuronic acid, glucosamine and reducing substance Total reducing power is measured by the arseno-molybdate methods (Nelson), and glucosamine by Ehrlich's reaction with p-dimethylaminobenzaldehyde according to Elson and Morgan, as modified by Blix A third aliquot is treated with $\text{Ba}(\text{OH})_2$ and ZnSO_4 under critical conditions to precipitate glucuronic acid quantitatively, as checked with Dische's carbazole reaction, without removing any of the other components Total reducing power is measured in the supernatant fluid, the difference between this and the initial reducing power gives glucuronic acid This procedure, when tested on mixtures of the pure carbohydrates in question, in quantities of the order of 35-55 γ/ml , has been found reliable to about 5% The entire determination involves a single precipitation and two series of colorimetric readings This procedure is now being adapted to the study of gastric mucin following acid hydrolysis, with a view to its ultimate use in accurately determining mucin content of various gastrointestinal secretions

Studies on the orcinol method for determining pentoses GAIL LORENZ MILLER, ELIZABETH ESHELMAN MILLER*, AND RICHARD H GOLDER* *Inst for Cancer Research and the Lankenau Hospital Research Inst, Philadelphia, Pa*

Bial's color test for pentoses has evolved from a qualitative to a quantitative status, yet the effects of the principal variables have been but partially

investigated Studies were, therefore, made of the effects of the concentrations of orcinol, iron and HCl , and of the time of heating, on the development of color Ribose nucleic acid was used as the test substance Optimum results were obtained with a reagent containing 0.4 gm of ferric alum and 2 gm of orcinol in 100 ml of concentrated HCl , added in equal volume to the aqueous sample to be tested, and heated for 45 minutes in a constant boiling water bath The rate of color development and the absorption spectrum of the final color, obtained with ribose nucleic acid, were then compared with corresponding rates and spectra obtained with adenosine-3-phosphate, adenosine-5-phosphate and free ribose The different test substances developed colors at decreasing rates in the order adenosine-5-phosphate, ribose, adenosine-3-phosphate, and ribose nucleic acid The total color intensities were, however, the same per unit ribose content, except for that produced by the ribose nucleic acid which was only 63% of the others The absorption spectra were essentially identical The findings extended and, in part, confirmed those of previous investigators

Plasma protein synthesis observed in direct study of the liver with aid of lysine- $\epsilon\text{-C}^{14}$ L L MILLER, C G BLY*, M L WATSON* AND W F BALE *Depts of Radiation Biology and Pathology, Univ of Rochester School of Medicine and Dentistry, Rochester, N Y*

A direct study of the isolated surviving rat liver *in vitro* yields evidence that the liver synthesizes all plasma albumin and fibrinogen and at least 80% of the plasma globulin fraction Livers are obtained from adult Wistar strain rats and rapidly incorporated in a simple system for the continuous perfusion of isolated intact organs with heparinized oxygenated blood at known temperature and pressure With the aid of lysine $\epsilon\text{-C}^{14}$ the liver is found to respond to variations in amino acid substrate in a fashion qualitatively and quantitatively analogous to that of the intact animal Very little plasma protein is synthesized when the liver is offered only lysine $\epsilon\text{-C}^{14}$ in the perfusing blood The addition of the essential amino acids enhances plasma protein synthesis tenfold Protein synthesis is further increased by the simultaneous addition of the non essential amino acids The addition of extra glucose to the blood slows the disappearance of the free amino acids, but enhances not only the plasma protein production but also the apparent deposition of new liver tissue protein When under optimal conditions for plasma protein synthesis DL lysine- $\epsilon\text{-C}^{14}$ is replaced by D lysine- $\epsilon\text{-C}^{14}$, protein synthesis is less than 10% of that seen with the DL lysine- $\epsilon\text{-C}^{14}$ A comparison of the rat liver with

the surviving posterior half of the rat carcass including gonads and kidneys (BLX, C G, L L MILLER, AND W F BALE *Federation Proc*, this issue) demonstrates the dominant role of the liver in plasma protein synthesis

Factors affecting pyruvate and lactate metabolism in cardiac muscle slices O NEAL MILLER AND ROBERT E OLSON (introduced by HALVOR N CHRISTENSEN) *Dept of Nutrition, Harvard School of Public Health, and Dept of Biological Chemistry, Harvard Med School, Boston, Mass*

Previous experiments with radioactive carboxyl-labeled pyruvate in this laboratory (*Federation Proc* 8 391, 1949) have shown that only part of the pyruvate which disappears in cardiac muscle slices undergoes oxidation to carbon dioxide. In the present investigation the effect of varying initial concentrations of pyruvate and lactate upon oxygen consumption and disappearance of these substrates, the effect of varying concentrations of carboxyl- and carbonyl-labeled pyruvate upon $C^{14}O_2$ production, and the effect of certain inhibitors of glycolysis upon pyruvate and lactate utilization have been studied. Slices of cardiac muscle from healthy ducklings fed *ad libitum* were incubated with pyruvate or lactate at concentrations of 2-40 mM/l in Warburg vessels at 37° for one hour. Changes in the amounts of pyruvate and lactate in these vessels were determined chemically and the rate of oxidation of radioactive substrates, when present, was determined by counting the $C^{14}O_2$ trapped in the center well. It was found that both the disappearance and oxidation of lactate and pyruvate increased with increases in initial concentration of substrate. At 2 mM/l of pyruvate the $C^{14}O_2$ from labeled pyruvate amounted to 30% of the total, or an amount equivalent to the stimulation of respiration caused by addition of substrate, while at 40 mM/l it amounted to 90% of the total. At all concentrations of pyruvate and lactate, the amount of disappearance was greater than could be accounted for by interconversion or oxidation. At 10^{-4} M iodoacetate and 10^{-3} M arsenate, pyruvate and lactate utilization were reduced without change in the respiration. Fluoride at 10^{-4} M reduced pyruvate but not lactate utilization.

A study of the scattering of light in myosin solutions W F H M MOMMAERTS (introduced by GEORGE W SCHWERT) *Dept of Biochemistry, Duke Univ School of Medicine, Durham, N C*

Myosin was obtained in crystalline form, and the homogeneity of the preparations was controlled by ultracentrifugation. The scattering properties of myosin solutions were studied after ultracentrifugal clarification by measuring the absolute scattering intensity and the dissymmetry of the forward and backward scattered light

at various concentrations and at two different wavelengths. The dissymmetry studies, interpreted with the aid of Debye's theory, indicate a particle length of about 2000 Å. The molecular weight is computed from the absolute turbidity is between 1 and 1.6 million. These results are in good agreement with ultracentrifugal and other data. Actomyosin solutions show a much higher turbidity. The dissymmetry is, even at high dilution, considerably higher than the limited value for very long rods, so that no quantitative deductions are permissible. Upon addition of ATP to the actomyosin, the turbidity decreases considerably, indicating dissociation of the actomyosin. At high concentration of the protein, the dissymmetry increases under influence of ATP, but this is not the case for dilute solutions. No contraction of dissolved particles is indicated.

Determination of total serum bilirubin FERRIN B MORELAND, WILLIAM W O'DONNELL AND JOSEPH H GAST, with the technical assistance of MARIE MCGINN (introduced by J M JOHLIN) *Dept of Biochemistry, Baylor Univ College of Medicine, and Veterans Admin Hospital, Houston, Texas*

The use of sulfanilamide instead of sulfanilic acid in the Malloy-Evelyn diazotization of bilirubin gives a much more rapid color development (maximum within 5 min instead of 30-40 min), the intensity is about the same. Sulfanilamide can also be used as an artificial standard for the bilirubin determination, developing a color by the usual coupling with N-(1-naphthyl)-ethylene-diamine dihydrochloride after diazotization. With the Klett-Summerson photoelectric colorimeter, using the green filter #54 (maximum transmission at 525-530 mμ), it has been found that 1 mg sulfanilamide is equivalent in color produced to 2.95 mg bilirubin. Several samples of sulfanilamide, including one which we recrystallized, all gave the same factor, thus avoiding the variability of commercial bilirubin and giving the clinical laboratory a simpler, more stable, more reliable standard. In the determination of bilirubin, more color developed when methyl alcohol was used than with ethyl alcohol. For this reason and because of the easier availability of methanol to physicians' and commercial laboratories, the use of methyl alcohol is recommended. The alcohol should be redistilled, otherwise some samples yield less color than others. To avoid errors inherent in low optical densities, undiluted serum should be used when the bilirubin concentration is low. If the consequent slight turbidity is the same in blank and sample, it is of no importance, if it is not the same, it can be corrected for by reading the tubes also with the red filter #66 and calculating a corrected blank $B_c = B_s \times \frac{X_c}{B_{66}}$

Tracer studies on the biosynthesis of glucuronic acid E H MOSBACH* AND C G KING *Dept of Chemistry, Columbia Univ, New York City*

The utilization of glucose and carbon dioxide for glucuronic acid synthesis in the guinea pig has been investigated using uniformly labeled C^{14} -D-glucose and $NaHC^{14}O_3$. Borneol was administered orally to induce stimulation of glucuronic acid synthesis, and urinary borneol glucuronide was isolated as the zinc salt. After 24 hours, 2 to 4% of the radioactivity of uniformly labeled C^{14} -D-glucose (administered by intraperitoneal injection) was recovered in the urinary glucuronic acid, while only 0.2 to 0.3% of the injected dose was found in the glucuronic acid when $NaHC^{14}O_3$ was used as the source of radio-carbon. Partial degradation of the isolated glucuronic acid indicates that the carbon chain of glucose is available directly for conversion to glucuronic acid. $C^{14}O_2$ appears to enter the glucuronic acid molecule via fixation into glucose and liver glycogen. Injection of 1- C^{14} -L-ascorbic acid into borneol-treated guinea pigs showed that this compound is unavailable for conversion to borneol glucuronide.

Pituitary preparations more potent than electrophoretically homogeneous adrenocorticotrophic hormone (ACTH) PAUL L MUNSON, FRANCES L NAYLOR AND WILLIAM D GRAY (introduced by C N H LONG) *Dept of Pharmacology, Yale Univ, New Haven, Conn*

Because of its high potency, stability, thorough biological evaluation and availability, 'La-1-A', a hog pituitary fraction prepared by Munson in 1944, is accepted as the practical working bioassay standard for adrenocorticotrophic hormone (ACTH). Although La-1-A contains two electrophoretically distinct components it is equal or superior in potency to the homogeneous protein isolated previously by others. Two additional pituitary fractions have been prepared which are approximately twice as potent as pure ACTH, yet neither is electrophoretically homogeneous. Both were obtained from acid acetone extracts of hog pituitaries, the first by the usual methods of salt and pH precipitation. The second was prepared by the application of what is believed to be a new approach in protein fractionation. The ACTH in the crude extract was concentrated by extraction with 0.1 M Na_2HPO_4 , precipitation with $(NH_4)_2SO_4$, and removal of fractions insoluble between pH 9 and pH 4.7. The remaining solution at pH 4.7 contained 5% protein and 5% $(NH_4)_2SO_4$. When two-thirds volume of acetone was added and the mixture chilled below 0° C, the solution separated into two liquid phases, and the bulk of the ACTH activity was found in the lower. In other applications of this procedure,

however, the distribution of the ACTH activity between the two phases has been found to vary with the pH, temperature and relative concentrations of water, acetone, protein and inorganic salt.

Blood phosphorus, calcium, citric acid and total protein after oral administration of calcium hexose phosphate SAMUEL NATELSON,* MARGARET KLEIN* AND BENJAMIN KRAMER *Pediatric Research Lab, Jewish Hospital of Brooklyn, Brooklyn, N Y*

Calcium fructose diphosphate was administered orally (6 gm) in pill form to 10 normal adults. Blood levels of calcium, protein, citric acid, inorganic phosphorus and organic phosphorus not precipitable by trichloroacetic acid were determined on fasting, and at the end of one, 2 and 3 hours. An average rise of approximately 16% of inorganic phosphorus was noted, of 5% in calcium levels, 6.5% in citric acid levels and 1% in protein levels. The most dramatic rise was observed in the levels of non-protein-organic phosphorus, which rose approximately 200% above the fasting levels. Approximately from 8-35% of the organic phosphorus taken orally may be accounted for in the extracellular fluid in combined form as summing uniform distribution. The rise in calcium levels was less than might be expected after the administration of 6 gm of calcium hexose diphosphate. This probably signifies the dissociation of the compound in the intestines with absorption of the fructose-diphosphate radicle independently of the calcium. Similar results were observed with calcium glycerophosphate in one instance and direct absorption of combined phosphate is probably a general characteristic of the phosphoric acid esters of polyhydroxy compounds.

Effects of anions on the activity of carboxypeptidase HANS NEURATH AND GLENN DE MARIA* *Dept of Biochemistry, Duke Univ School of Medicine, Durham, N C*

It was recently reported that the hydrolysis of carbobenzoxyglycyl-L-leucine by carboxypeptidase is inhibited by orthophosphate, pyrophosphate, citrate, oxalate and cyanide (SMITH AND HANSON *J Biol Chem* 179: 803, 1949). From the nature of the inhibitory anions it was concluded that carboxypeptidase is a metal-protein and that magnesium is the essential activating metal ion. Because of the general significance attached to these findings (SMITH *Federation Proc* 8: 581, 1949), the effect of these anions on the enzymatic hydrolysis of the typical substrate carbobenzoxyglycyl-L-phenylalanine was investigated and evaluated in terms of the kinetic constants k_2 and K_m , determined from initial reaction velocities, and also in relation to the reaction course. Ortho-

phosphate, up to 0.2 M, has no effect on k_2 and K_m , nor on the inhibition by D-phenylalanine (K_i). However, beyond the initial phase, the reaction was retarded in 0.2 M orthophosphate, owing to inhibition by L-phenylalanine, as confirmed also by measurements in which L-phenylalanine was added in stoichiometric excess. The orthophosphate effect was instantaneous and reversible. Inhibition by L-phenylalanine was negligible as compared to D-phenylalanine. Similar, though accentuated, effects were obtained with 0.01 M pyrophosphate. Citrate (0.1 M), oxalate (0.1 M) and cyanide (0.002 M) had no measurable effects whatsoever. It is concluded that combination of carboxypeptidase with orthophosphate or pyrophosphate, in itself, does not impair catalytic activity but merely retards the rate of desorption of one of the end products. A specific contribution of magnesium to the catalytic activity of carboxypeptidase is not evident from these findings.

Effect of methionine on toxicity of crystalline 'agene factor' against *Leuconostoc mesenteroides* G. W. NEWELL AND W. W. CARMAN (introduced by L. REINER) *Research Labs of Wallace and Tiernan Products, Inc., Belleville, N. J.*

It has been reported that the crystalline toxic factor obtained from nitrogen trichloride treated zein inhibits the growth of *Leuconostoc mesenteroides*, P-60 (HEATHCOTE *Nature* 161:43, 1949). This has been confirmed with material isolated in our laboratories. The relationship between concentration of toxic factor and percentage inhibition of growth was studied under various conditions. Fifty % inhibition of growth was obtained with 4-6 $\mu\text{g}/\text{cc}$. Methionine reversed the growth inhibition almost completely when its concentration was 12 times that of the toxic material and a detectable reversing effect of methionine still occurred at concentrations equal to that of the toxic material. A number of compounds have been tested to determine whether the effect of methionine is specific. Among sulfur-containing compounds methionine sulfoxide and glutathione showed about 1/10 the activity of methionine, while methionine sulfone produced little reversal of the inhibition. Cystine, cysteine, cysteic acid and homocystine were without any effect. Methyl donors, such as choline, gave no protection.

Preparation, bioassay and metabolism of radioactive 17-methylestradiol H. J. NICHOLAS*, S. A. THAYER, E. A. DOISY, JR.*, WILLIAM H. ELLIOTT*, B. BOCKLAGE* AND EDWARD A. DOISY *Depts. of Biochemistry and Internal Medicine, St. Louis Univ. School of Medicine, St. Louis, Mo.*

A preliminary study has been made of the metabolism of 17-methylestradiol, labeled with car-

bon 14 at the C-17 methyl group. The non-radioactive compound, reported as the 3-methyl ether in crude form by Cohen and Cook (*J. Chem. Soc.* 445, 1935), was prepared from estrone acetate by the reaction with methyl magnesium iodide. The 17-methylestradiol and a derivative were characterized by the customary procedures. A similar procedure using 1 millicurie of $\text{C}^{14}\text{H}_3\text{I}$ produced 17-methylestradiol with activity of 9662 c/m/mg. The free phenol was approximately equal in estrogenic activity to α -estradiol when assayed by 4 different methods. The radioactive compound was injected into normal adult female rats at 10 mg levels. Most of the radioactivity was recovered from the feces over a 72-hour period, with a small amount in the urine. In rats with bile fistulas, the major portion of the radioactivity was recovered from the bile.

Enzymatic degradation of coenzyme A G. DAVID NOVELLI*, NATHAN O. KAPLAN AND FRITZ LIPMANN *Biochemical Research Lab., Massachusetts General Hospital, Dept. of Biological Chemistry, Harvard Med. School, Boston, Mass.*

It was reported previously that intestinal phosphatase inactivates coenzyme A (Co A) and liberates all bound phosphate, with prostate phosphatase, however, which acts exclusively on monoesters, no inactivation of Co A occurs. More recently, the action of a potato dinucleotidase on Co A was studied. Lowry and Colowick observed (unpublished) that between 1.2 and 1.8 M $(\text{NH}_4)_2\text{SO}_4$ concentrations, a fraction was obtained from potato extract which splits diphosphopyridine nucleotide and flavinadenine dinucleotide into the respective mononucleotides. If Co A was incubated with this potato enzyme in conjunction with prostate phosphatase, complete inactivation and liberation of inorganic phosphate occurred. In absence of prostate phosphatase, the action of the dinucleotidase was obscured by the fact that in crude liver extracts, in the presence of ATP, a resynthesis of Co A occurred from the split products. To show inactivation of Co A by dinucleotidase, a purified liver fraction, obtained between 40 and 70% $(\text{NH}_4)_2\text{SO}_4$ saturation, had to be used for the tests on sulfanilamide acetylation. With this assay system, activity disappeared after incubation of Co A with potato enzyme. These experiments seem to suggest a structure for Co A which is similar to that of pyridine and flavinadenine dinucleotide. It is of interest that in Cheldelin's *Acetobacter* growth test, after incubation with dinucleotidase presumably liberating pantothenyl phosphate, Co A shows a 2- to 2.4-fold increase in activity. This seems to support our earlier suggestion that Cheldelin's factors were a degradation product of Co A.

Intracellular distribution of phosphatase activity in rat liver ALEX B. NOVIKOFF, ESTELLE PODBER AND JEAN RYAN (introduced by ELVIN A. KABAT) *Depts. of Pathology and Biochemistry, Univ. of Vermont College of Medicine, Burlington*

Rat liver homogenates, prepared in distilled water and 0.88 M sucrose, were separated by differential centrifugation into 4 fractions: nuclei, mitochondria, sub-microscopic particles and 'supernatant fluid'. Homogenate, fractions, and a mixture of the fractions reconstituting the original homogenate were tested for phosphatase activities with disodium phenyl phosphate (OP) at pH 4.5 and 9.1, adenosine-5-phosphate (A5P) at pH 7.4, and adenosine triphosphate (ATP) at pH 7.4, under conditions in which enzyme activity (micrograms P split from substrate) bears a linear relation both to tissue concentration and to time of incubation. A chart shows the distribution of phosphatase activities in the fractions obtained from water homogenates, expressed in percentage of original homogenate activity.

	P, pH 4.5	P, pH 9.1 $10^{-2}M$ MgCl ₂	A5P, pH 7.4 $3 \times 10^{-2}M$ MgCl ₂	ATP, pH 7.4 $10^{-2}M$ MgCl ₂
Nuclei ¹	5-10%	10-18%	40-45%	10-20%
Mitochondria	35-40	17-20	40-45	70-75
Sub-microscopic particles	5-10	0-10	5-10	2-4
'Supernatant fluid'	35-50	55-70	10-15	0-1
Mixture	90-100	85-105	85-105	85-105

¹ Contaminated with unbroken cells and mitochondria.

The intracellular distribution of activities is the same in 0.88 M sucrose homogenates as in water homogenates in the case of OP at pH 4.5 and 9.1, but significant differences occur with A5P and ATP. Other data to be presented for each fraction include 1) phosphatase activities with A5P and ATP in the presence of CaCl₂, 2) nucleic acid (DNA, PNA) and nitrogen content and 3) succinoxidase activities.

Nucleic acids in development and division of plant cells MAURICE OGUR AND RALPH O. ERICKSON (introduced by WAYNE UMBREIT) *Botanical Labs, Univ. of Pennsylvania, Philadelphia*

A quantitative study of the nucleic acids during development and mitotic division has been made based upon further extensions of assay methods previously reported (OGUR AND ROSEN *Federation Proc.* 8:234, 1949; *Arch. Biochem.* In press). Suspensions of microsporocytes, microspores and

pollen grains from anthers of *Lilium longiflorum* have been prepared. Cell numbers have been counted and cell homogenates have been analyzed for pentose nucleic acid (PNA) and desoxypentose nucleic acid (DNA). Analytical results have been calculated to the nucleic acid content per cell. These results have been related to the logarithm of flower bud length, which serves as an external time index of the developmental and cytological status of the cells (ERICKSON *Am. J. Bot.* 35:729, 1948). This report covers the developmental period from microsporocyte meiosis to pollen release. A sharp drop in DNA content per cell is observed at meiosis followed by a gradual linear increase during the interphase of microspore development. Just prior to microspore mitosis DNA content doubles rapidly. The resulting binucleate pollen grain shows a further increase in DNA content with time before the pollen is normally released from the flower. The PNA content follows the same pattern up to microspore mitosis at a level about twice that of DNA, increases sharply at mitosis, and continues to increase rapidly at a rate nine times that for DNA in the maturing pollen grain.

New coenzymes of cell-free fermentation PAUL OHLMEYER (introduced by ARTHUR KORNBERG) *National Insts. of Health, Bethesda, Md.*

Previous investigations showed that the glucose fermentation in dialyzed *Lebedewsaft* is stimulated by a thermostable fraction prepared from fresh yeast (*Ztschr. f. physiol. Chem.* 267:264, 1941). It contained 2 factors and could not be replaced by known coenzymes including adenosinetriphosphate. Further studies have now led to the observation that the fraction maintains a high rate of fermentation even after the inorganic phosphate is exhausted, contrary to the Harden and Young equation. The velocity is constant and near maximal as long as free sugar is available, thus, the addition of inorganic phosphate or arsenate does not even double the rate and phosphopyruvic acid does not influence it at all. This effect which appears related to the transfer or liberation of phosphate is not due to activation of adenosinetriphosphatase; the fermentation of hexosediphosphate or phosphopyruvic acid in the absence of glucose is not accelerated by the thermostable yeast fraction. Purification of the fraction showed that one of the factors (A) is very similar to or identical with the adeninedinucleotide of Meyerhof and Kiessling (*Biochem. Ztschr.* 296:410, 1938). While factor A alone acts as a transphosphorylating coenzyme it does not give the fermentation effect described above in the absence of the second factor. The nature of the coenzyme factors and the mechanism of their action are under investigation.

Comparative metabolism of radioactive glucose in heart, brain, kidney and liver slices ROBERT E OLSON, JAMES S ROBSON, HEIDI RICHARDS AND EMILY G HIRSCH (introduced by HALVOR N CHRISTENSEN) *Dept of Nutrition, Harvard School of Public Health and Dept of Biological Chemistry, Harvard Med School, Boston, Mass*

Glucose utilization by slices of heart, brain, kidney, and liver from representative mammalian (rat, rabbit, guinea pig, cat) and avian (duck, pigeon) organisms was studied with the aid of radioactive glucose prepared photosynthetically. Tissue slices were incubated in Warburg vessels at 37°C with C^{14} -labeled glucose at concentrations ranging from 1.25 to 20 mM/l for one hour. Glucose disappearance or appearance and aerobic glycolysis were determined chemically, and the rate of oxidation of added glucose determined by counting the $C^{14}O_2$ captured in the center well. Oxidation of glucose to CO_2 occurred in all tissues but at vastly different rates. $C^{14}O_2$ production from added glucose in mammalian liver was of a very low order ($QC^{14}O_2 = ca\ 0.1$) as indicated previously in a comparison with hepatoma (*Proc Am Chem Soc* 116:61C, 1949). Oxidation rates were 15–20 times as rapid in heart, avian liver, and kidney slices, and 60–100 times as rapid in brain slices. Increasing initial glucose concentration uniformly resulted in increased oxidation rates. In all cases, except with livers rich in glycogen, disappearance of glucose exceeded oxidation rate by several-fold. Neither glycogenolysis nor glycogenesis in liver slices appeared to affect the rate of oxidation of added glucose. Aerobic glycolysis accounted for part of the glucose which disappeared and bore a positive relationship to the rate of $C^{14}O_2$ production. Studies of anaerobic glycolysis in homogenates of duck and rat liver with various physiological hexose phosphates as substrate suggest that the activity of phosphohexokinase may be the limiting factor in breakdown of glucose by mammalian liver.

Preparation of thymus nucleohistone in native form GERALD ORGEL* AND KURT G STERN
Dept of Chemistry, Polytechnic Inst of Brooklyn, N Y

The isolation of a desoxyribonucleoprotein from calf thymus with the aid of desoxyribonuclease inhibitors and aqueous solvents of low ionic strength has been briefly reported (*Federation Proc* 6:296, 1947). It has since been found that this material exhibits significant ATP-ase activity (114th Meeting, A C S, Wash, D C, 1948). The isolation procedure has been modified to improve its reproducibility and the uniformity of the nucleoprotein preparations. The new method involves the following steps: removal of cytoplasmic proteins from thymus homogenates

by exhaustive extraction with 0.14 M NaCl at 0°C, extraction of the desoxyribonucleoprotein from the nuclear residue with 20% ethanol at -5°C, precipitation of the nucleoprotein with acetate buffer, pH 4.6, at 0°C, dissolving the precipitate in phosphate buffer, pH 7.4, of low ionic strength, reprecipitation of the nucleoprotein with 0.14 M NaCl at 0°C, and redissolving the nucleoprotein in distilled water or phosphate buffer, pH 7.4, of low ionic strength to form colorless, opalescent sols. The final product may be preserved as a white, fluffy, soluble material by freeze-drying. The DNA-content (Dische Reaction) approximates 20%. The nucleoprotein contains arginine (Sakaguchi Reaction) and forms a stable, insoluble complex with lanthanum salts at pH 7. The sedimentation and electrophoretic behavior of the nucleohistone preparations, as well as their viscosity, flow birefringence, infrared and ultraviolet light absorption, and enzymatic activity towards energy-rich phosphate compounds are described.

Application of partition chromatography to study of absorption of amino acids from the intestine

ALINE UNDERHILL ORTEN, C J FRANCE AND K KIOZUMI (introduced by ARTHUR H SMITH)
Depts of Surgery and Physiological Chemistry, Wayne Univ College of Medicine, Detroit, Mich
Employing the starch column chromatographic technic (STEIN AND MOORE *J Biol Chem* 176:337, 1948; MOORE AND STEIN *J Biol Chem* 176:367, 1948), the first 4 emerging amino acids, leucine, isoleucine, phenylalanine and methionine, have been studied. The results of Stein and Moore in which approximately 0.1 mg quantities of each of the 4 amino acids are separated and recovered quantitatively from pure solution have been confirmed. The identical mixture of these amino acids in the same amounts have been separated quantitatively from intestinal loop contents with the same degree of accuracy. A method for removing protein and other contaminants which may interfere in the determination of amino acids by the ninhydrin reaction has been developed. Thiry jejunal loops of dogs were prepared by the method of Johnston (*Proc Soc Exper Biol & Med* 30:193, 1932). Water was placed in the loop, the loop contents withdrawn after an hour, and the samples prepared for chromatographic analyses in such a way that a flat base line chromatogram showing no effluent peaks was obtained. Leucine placed in such loops and immediately withdrawn and determined by the above procedure is consistently recovered. Studies of the comparative absorption rates of leucine, isoleucine and phenylalanine from Thiry loops are reported.

Ergosterol synthesis in neurospora R C OTTKE*, E L TATUM, I ZABIN* AND K. BLOCH *Dept of Chemistry, Yale Univ, New Haven, Conn, Dept of Biology, Stanford Univ, Calif, and Dept of Biochemistry, Univ of Chicago, Chicago, Ill*

The synthesis of ergosterol has been studied in a *Neurospora* mutant which requires acetate for growth. The mold was grown on a medium which contained sucrose, 15 gm and doubly labeled acetate ($C^{14}H_3C^{18}OOH$), 5 gm. Calculated on the basis of a C^{13} concentration of 100 and a specific activity for C^{14} of 100 in the added acetate, the isotope concentrations were 20 in the mold mycelium and 60-70 in the ergosterol isolated from the cells. Acetate is therefore a preferential carbon source for the mold steroid as previously demonstrated with deuterioacetate (OTTKE, TATUM AND SIMMONS, *Federation Proc* 8 235, 1949). In a second experiment the acetate-less strain of *Neurospora* was grown on a medium containing sucrose, 15 gm, acetate, 5 gm, and a small quantity of isotopic isovaleric acid. The labeled substrate contained C^{13} in the end methyl groups and C^{14} in the carboxyl group. The isotope concentrations in the mycelium and in ergosterol were less than 1% of those in the isovalerate, indicating either that isovalerate was not appreciably metabolized or that isovalerate carbon was greatly diluted by carbon from other sources. It was found however that the C^{13} concentration was 5 times greater in ergosterol than in the mycelium, while the C^{14} concentrations of both mycelium and of the sterol were negligible. Therefore, in the synthesis of ergosterol by *Neurospora* as well as of cholesterol by the rat (ZABIN AND BLOCH, *Federation Proc* 8 267, 1949) the isopropyl moiety of isovalerate is a more efficient source of carbon than the carboxyl portion of the molecule.

Diamine oxidase in streptomycin sensitive and resistant strains of *Mycobacterium smegmatis*

C A OWEN AND E A ZELLER (introduced by M H POWER) *Division of Biochemistry, Mayo Foundation, Rochester, Minn*

Previous studies indicated that the diamine oxidase (DO) of *Mycobacterium tuberculosis* var *hominis* is sensitive to less than 10^{-5} M dihydrostreptomycin. The DO of sensitive strains of *Mycobacterium smegmatis* is nearly completely blocked by 10^{-4} M dihydrostreptomycin, while the same concentration does not influence the same enzyme of the corresponding streptomycin resistant strain (ZELLER, E A, C A OWEN AND A G KARLSON, *Proc Staff Meet, Mayo Clin* 24 490, 1949). The question arises as to whether this loss of enzymic sensitivity toward dihydrostreptomycin is due to a change in the physicochemical structure of the DO or to factors apart from the enzyme. The observation that the DO of sensitive

and resistant bacteria is equally sensitive toward pyocyanin, chloramphenicol and streptothricin, points more toward the second alternative. The DO of resistant smegma bacteria, after their brief treatment with acetone, is much more sensitive toward dihydrostreptomycin than before treatment. On the other hand, when sensitive bacteria are incubated with putrescine (which was used in all these studies as the DO substrate), and then washed, they become resistant, even 10^{-3} M dihydrostreptomycin is no longer able to inhibit completely the DO of these pretreated bacteria. Thus the sensitivity of DO of smegma bacteria can be altered by various procedures. A tentative hypothesis to explain these phenomena will be presented.

Studies on the metabolism of pyrimidine ribosides L M PAEGE* AND F SCHLENK *Dept of Bacteriology, Iowa State College, Ames*

Cytosine riboside and uracil riboside are rapidly metabolized by bacterial enzymes. Cytidine is deaminated, nucleoside phosphorylase splits off the base, and rapid disintegration of the carbohydrate occurs as described earlier for purine ribosides (SCHLENK, F *Adv Enzymol* 9 455, 1949). No indication was found that the action of bacterial pyrimidine nucleoside phosphorylase is preceded by opening of the pyrimidine ring as suggested by Mitchell and Houlahan for *Neurospora* (*Federation Proc* 6 506, 1947). For determination of pyrimidine-bound ribose the ordinary orcinol technique is not suitable. A modification has been elaborated which permits distinction between pyrimidine nucleosides and other pentose derivatives.

Effect of growth hormone on the glucose uptake and glycogen synthesis by the rat diaphragm

C R PARK AND WILLIAM H DAUGHADAY (introduced by M E KRAHL) *Dept of Biological Chemistry, Washington Univ School of Medicine, St Louis, Mo*

Glucose uptake by rat diaphragm *in vitro* and the effect of added insulin diminish rapidly during 2 hours of incubation. Using pooled sections of diaphragms from 6 rats suspended in Krebs-Henseleit medium containing 140 mg % glucose, the rates of glucose uptake (mg/gm wet tissue/hour for the first 30-min period) were as follows: normal rats 3.2, hypophysectomized rats 5.0, hypophysectomized - adrenalectomized rats 4.7, with insulin added *in vitro* normal 6.7, hypophysectomized 6.5, hypophysectomized - adrenalectomized 6.9. Crystalline growth hormone of high growth potency injected intraperitoneally into hypophysectomized rats reduced glucose uptake to normal. This effect was obtained 24 hours after one injection of 20 γ per 100 gm of rat or after repeated injections of 5 γ daily. Adrenal-

cortical extract or ACTH did not depress glucose uptake under the conditions tested. In hypophysectomized - adrenalectomized rats growth hormone led to less depression of glucose uptake than in hypophysectomized rats unless adrenal cortical extract was injected concurrently. The initial glycogen content of diaphragm from hypophysectomized rats fasted 24 hours was not altered significantly by growth hormone or adrenal cortical extract injection. Initial glycogen in diaphragm of hypophysectomized - adrenalectomized rats was very much higher than in diaphragm of hypophysectomized or normal rats. Glycogen synthesis was proportional to glucose uptake in all these cases and corresponded to about half of the glucose utilized.

Isolation of three labile phosphate compounds containing uracil from penicillin-treated *Staphylococcus aureus* cells JAMES T. PARK (introduced by MARVIN J. JOHNSON) *Camp Detrick, Frederick, Md.*

The accumulation of acid-soluble, labile phosphate in *S. aureus* cells during growth in the presence of 0.5 unit of penicillin per ml. has been reported (PARK, J. T. AND M. J. JOHNSON, *J. Biol. Chem.* 179: 585, 1949). Purified preparations rich in labile phosphate (10-min. hydrolysis in 1 N HCl at 100° C.) were obtained by alcohol precipitation of the barium salts and use of the cation exchange resin, IR100. The material was resolved into 3 components by use of partition chromatography (mobile phase: phenol, non-mobile phase: 0.1 M sulfate buffer, pH 1.7). Each of the separated components contained, in approximately equimolar proportions, uracil, labile phosphate, stable phosphate, pentose (orcinol test), and an unknown sugar. Preparations of the component most soluble in phenol gave positive biuret tests. This component contains approximately 3 moles of alanine per mole of labile phosphate, and it may contain 1 mole of glutamic acid. The second component, which is believed to be a mixture of 2 labile phosphate compounds, contains alanine. The third component contains no amino acids. The amino acids have been tentatively identified by paper chromatography employing 82% phenol as the solvent. It is suggested that this series of complex molecules may be normal intermediates of the cell and that the inhibition of a specific synthetic reaction by penicillin causes them to accumulate in abnormal amounts.

Interaction of quinoline and acridine derivatives with nucleoproteins and nucleic acids FRANK S. PARKER,* J. LOGAN IRVIN AND ELINOR MOORE IRVIN * *Dept. of Physiological Chemistry, Johns Hopkins Univ. School of Medicine, Baltimore, Md.*

A recently reported study (*Science* 110: 426,

1949) of the reversible interaction of antimalarials of the 4-aminoquinoline and 9-aminoacridine series with nucleic acids has been extended to nucleoproteins. The cationic species of quinacrine, of 7-chloro-4-(1'-methyl-4'-diethylaminobutylamino)-quinoline (SN-7618) and of 2-methoxy-6-chloro-9-(1'-methoxyl-8'-diethylamino octylamino)-acridine (SN-12868) interact strongly with a highly polymerized, asymmetric desoxy-pentose-nucleoprotein of spleen. For the interaction with this nucleoprotein the values of log *k* (the association exponent, formulated as described previously for the case of interactions with nucleic acids) are 3.7 for SN-7618 and 4.3 for SN-12868 at pH 6 and ionic strength 0.02. At ionic strength 1.0 the value of log *k* is 2.8 for SN-12868, thus demonstrating that the effect of ionic strength on the interaction with nucleoprotein is comparable with the large effect previously reported in the study of nucleic acids. Each of these antimalarials interacts with the nucleoprotein more strongly than with a less highly polymerized preparation of pentose-nucleic acid from yeast. All interactions were evaluated spectrophotometrically at concentrations of the nucleoprotein (0.5 to 10 µg. of nucleoprotein-phosphorus per ml.) and of the antimalarial compound (5×10^{-5} M) at which the complex remains in solution. At higher concentrations (5×10^{-4} M, and greater) of the antimalarial compounds precipitation of the complex occurs. The compound, 2-methoxy-6-chloro-9-aminoacridine, also interacts strongly with nucleoprotein, which suggests that the side-chains of quinacrine and SN-12868 are not essential for the interactions although the side-chains modify other properties, such as solubility, to an important degree.

Electrophoretic analyses of plasma and serum proteins in rheumatoid arthritis and rheumatoid spondylitis W. D. PAUL* AND JOSEPH I. ROUTH *Dept. of Medicine (Div. of Physical Medicine) and Biochemistry, College of Medicine, State Univ. of Iowa, Iowa City*

One hundred and twenty-two plasma and serum samples were obtained from 70 patients with arthritis. Included in this series were plasma-serum pairs from 34 patients. These samples were diluted with 3 volumes of barbiturate buffer (pH 8.6), dialyzed 3 days at 5° C. and subjected to electrophoresis in the Tiselius apparatus. The analytical cell was used. The plasma proteins of 57 patients with rheumatoid arthritis showed a marked decrease in albumin, a marked increase in fibrinogen and γ globulin, and a moderate increase in α_1 and α_2 globulin components when compared to plasma from normal individuals. Ten children, under 15 years of age, with this disease, showed a greater decrease in plasma albumin and increase in γ globulin than the adults. An

analysis of plasma-serum pairs from 27 adults in this group gave values similar to those obtained with plasma. The average γ' globulin content of the serum was 3.0%, which is a normal value. A group of 7 patients with rheumatoid spondylitis showed strikingly different plasma and serum patterns from rheumatoid arthritis. Average values for all components fell within the range for normal individuals. The γ' globulin component in the sera averaged 1.9%. Other types of arthritis including osteo or degenerative arthritis are being studied.

Isolation of cell nuclei in neutral medium MARY L. PETERMANN AND ROSE M. SCHNEIDER *Sloan-Kettering Inst. New York City*

The great elevation in total pentose nucleic acid (PNA) found in the spleen of the leukemic mouse is caused by increases in the PNA content of both the cytoplasm and crude 'nuclear fraction' (*Cancer* 2:510, 1948). In order to determine the PNA content of the nuclei alone an isolation procedure which would avoid contamination with cytoplasmic nucleoprotein was required. This has been accomplished in a neutral medium containing 0.88 M sucrose and approximately 0.002 M CaCl_2 . The entire procedure was carried out at 4° C. One gram of fresh mouse spleen was crushed in a glass homogenizer in 15 cc. of sucrose. It was then strained successively through surgical gauze, single-napped flannelette, and double-napped flannelette, into a 50 cc. centrifuge tube. The contents were homogenized by means of a stirrer consisting of stainless steel blades rotating at 16,000 r.p.m. The homogenate was centrifuged for 10 minutes at $600 \times g$ and the sediment washed three times. The final sediment was resuspended to give approximately 10^8 nuclei/cc. and counted in a haemocytometer. Wet smears stained with methyl green-pyronin or orcein-fast green (KURNICK AND RIS, *Stain Technol.* 23:17, 1948) showed well dispersed and morphologically intact nuclei, free from microscopically evident cytoplasmic material. Their content of nitrogen and of both types of nucleic acid has been determined.

Net production of serum albumin in vitro THEODORE PETERS, JR. AND CHRISTIAN B. ANFINSEN *Biophysical Lab and Dept. of Biological Chemistry, Harvard Med. School, Boston, Mass.*

Serum albumin content of a chicken liver slice system was found to increase from about 0.3 mg/gm. liver to 0.6-1.0 mg/gm. in a 5-hour incubation in O_2 , but only to 0.35-0.4 mg/gm. in N_2 . These results lend direct confirmation to previous conclusions (PETERS AND ANFINSEN *J. Biol. Chem.* 182: No. 1, In press) regarding serum albumin synthesis based on measurements of the *in vitro* incorporation of radioactive CO_2 . Serum albumin was determined by a quantitative immunochem-

ical precipitin technique, using rabbit antiserum against serum albumin of over 99% electrophoretic purity prepared from chicken serum. A standard curve relating the amount of precipitate formed to the amount of antigen added was determined. Aliquots of the combined homogenized slices and medium were analyzed for serum albumin content after centrifugation. The initial serum albumin level was lowered by perfusion of the intact liver and by washing the slices in medium for an hour prior to the experiment. The bulk of the serum albumin present after 5 hours was in the medium. Studies were also made on the effects of ions, substrates, and inhibitors. Calculations based on the observed rates of production indicate that the liver could replace half the circulating serum albumin of the intact animal in 5-8 days.

Characteristics of the amino acid incorporation system of liver homogenates ELBERT A. PETERSON,* DAVID M. GREENBERG AND THEODORE WINNICK *Div. of Biochemistry, Univ. of California Med. School, San Francisco*

The incorporation of carboxyl and α -labeled glycine, phenylalanine- $\beta\text{-C}^{14}$, serine- $\beta\text{-C}^{14}$ and methionine- $\text{CH}_3\text{-C}^{14}$ into protein has been studied, using a suspension of cytoplasmic granules obtained by fractional centrifugation in a $\text{KCl} - \text{KHCO}_3$ medium. The incorporation activity of the cytoplasmic granules, which was relatively low alone, was increased as much as seven-fold by mixing it with the supernatant from a concentrated (1:1) homogenate centrifuged at 2700 g or with a medium containing ATP, Mg^{++} , citrate, and a mixture of a number of L-amino acids, except with methionine. When ATP was omitted, the incorporation was even lower than with no additions. With methionine, only ATP was activating. In the presence of the above-mentioned activators, plus phosphoglycerate, a particle free supernatant (20,000 g) can incorporate the label of glycine aerobically, but not anaerobically. The radioactive protein obtained with radio-glycine loses a considerable portion of its label when treated with mercaptoethanol or with performic acid evidently as a result of the cleavage of disulfide bonds. This is true for whole liver homogenates also but not for protein from embryo homogenates or tissue slices. The removed radio material may possibly be glutathione but this has not yet been positively identified. When the label is methionine- $\text{CH}_3\text{-C}^{14}$, most of the radioactivity is removed by mercaptoethanol and protein labeled with radio serine loses a small amount of activity on such treatment. However, the radio activity of protein incubated with phenylalanine $\beta\text{-C}^{14}$ is completely resistant to both reagents.

Pyrimidine content of certain pentose and desoxy-pentose nucleic acids ANTHONY PIRCIO* AND

LEOPOLD R. CERRERO *Dept of Biochemistry, Fordham Univ., New York City*

A colorimetric method for the determination of cytosine and uracil, developed in this laboratory (*J Biol Chem* 181 713, 1949) has been applied to a study of the distribution of the free pyrimidines, the pyrimidine nucleosides and nucleotides of the pentose nucleic acids of yeast, dog liver, and wheat germ. The separation of cytosine and uracil was carried out by means of the zeolite, Decalco, while cytidine and uridine could be separated with the aid of the resin, Zeo-Rex. The pyrimidine composition of both the yeast and wheat germ pentose nucleic acids was found to be closely similar, but that of the dog liver differed in that approximately twice as much cytosine as uracil was found. Although the yeast and wheat germ pentose nucleic acids contained nearly equal amounts of the free pyrimidines and of the pyrimidine nucleosides, the quantities found were not as high as would be expected from the tetranucleotide hypothesis. For the determination of thymine, a method based on Hunter's qualitative test (*Biochem J* 30 745, 1936) has been developed. While this work was in progress, Woodhouse (*Biochem J* 44 185, 1949) published a method which is based on the same principle. Using this procedure in conjunction with the aforementioned method for the estimation of cytosine, thymine and cytosine were determined in the hydrolysates of the desoxy-pentose nucleic acids of fish sperm and calf thymus. The molar ratios of thymine to cytosine found were for fish sperm DNA, 1.2:1, for calf thymus DNA, 1.6:1.

Quantitative histochemical distribution of enzymes in cytoarchitectural layers of cerebral cortex. ALFRED POPE, JAMES R. WARE, AND RUBY H. THOMSON (introduced by J. FOLCH) *McLean Hospital, Waverley, Mass. and Harvard Med School, Boston, Mass.*

In the nervous system the mutual interdependence of structure and function makes the correlation of chemical composition with histological structure of especial importance. Because of its laminated architecture the cerebral cortex is suitable for such correlative study by the methods of quantitative histochemistry (LINDERSTRÖM-LANG, *K. Harvey Lect* 34 214, 1939). Accordingly, techniques for alternate frozen section sampling, histological control, and ultra-micro enzyme assay are being used to establish the quantitative distribution of certain enzymes within the cytoarchitectural layers of rat somatosensory isocortex. Enzymes of probable importance in the general or special metabolism of the nervous system are thus being studied, and attempts made to correlate their distribution patterns with details of the histological fine structure of this region. Thus,

diphosphopyridine nucleotide and alanyl-glycine dipeptidase have been found most abundant in layers II, IIIB-IV, and VC-VIA (designations of Brodmann) which are levels numerically rich in ganglion cells, thereby suggesting that these substances are mainly located within nerve cell bodies together, perhaps, with their protoplasmic expansions. Similarly, acetylcholinesterase and adenosinetriphosphatase (preliminary experiments) have been found most active in layers I, IIIA, IIIB, and VA-VB. These are levels within or just superficial to zones containing many horizontal myelinated fibres and are probably regions of extensive fine arborization of axons and hence of maximal axonal surface. It is suggested, therefore, that in cerebral cortex as in peripheral nerves these enzymes may be principally located at axonal surfaces.

Effect of fluoroacetate on reactions in the Krebs oxidative cycle. VAN R. POTTER AND HARRIS BUSCH * *McArdle Memorial Lab., Med School, Univ of Wisconsin, Madison.*

Buffa and Peters reported in 1949 that injections of lethal doses of fluoroacetate produced striking increases in the citrate content of brain, heart and kidney and questioned earlier reports that fluoroacetate interfered with acetate metabolism. We have confirmed their findings using 5 mg of fluoroacetate per kg body weight in rats. After one hour the citrate content of these organs was 212, and 1029 $\mu\text{g/gm}$ of wet weight, respectively, while in uninjected animals the citrate content was only about 50 $\mu\text{g/gm}$. The citrate content increased over 2 successive 30-minute periods but ceased thereafter. In addition to the three organs previously studied, increases in all of the other normal organs of the body have been found with the exception of blood and liver. Studies with homogenates showed that liver is capable of producing large amounts of citrate *in vitro*. However, fluoroacetate appeared to divert pyruvate metabolism into acetoacetate formation rather than to cause an increase in citrate in contrast to the results with kidney, in which the alternative pathway is weak or absent, and in which citrate accumulates both *in vivo* and *in vitro*. The results with liver *in vitro* thus seem to explain the lack of citrate production *in vivo*.

Alternative metabolic pathways in rat liver homogenates. VAN R. POTTER AND RICHARD O. RECKNAGEL * *McArdle Memorial Lab., Med School, Univ of Wisconsin, Madison.*

Lehninger previously demonstrated 2 co existing alternative metabolic pathways open to pyruvate in washed residues of liver, one leading to acetoacetate, the other to citrate. In the present study an assay system was devised to determine the relative activities of the two systems in whole

homogenates of rat and mouse liver. Measurements are based on determinations of pyruvate removal and acetoacetate formation. Under optimum conditions for the citrate pathway, i.e., in the presence of adequate quantities of oxalacetate, the pyruvate accounted for as acetoacetate was found to be only 10% of the total pyruvate oxidized, thus demonstrating the high priority of the citrate pathway. However, with no available oxalacetate, pyruvate is quantitatively converted to acetoacetate. Acetoacetate production is therefore a measure of the availability of oxalacetate in this system. This fact forms the basis for studying a related pair of alternative pathways. When ammonium chloride was added to the pyruvate system in the presence of catalytic levels of C_4 acids their regeneration via the Krebs cycle was prevented and acetoacetate production was decreased by an amount equivalent to the C_4 acid originally added. Furthermore, in the ammonium chloride system, pyruvate preferentially forms citrate so long as C_4 acids are present, but is diverted to acetoacetate production as soon as the supply of C_4 acid is exhausted. The ammonium chloride was effective at physiologically significant levels and is believed to divert α -ketoglutarate to glutamate. Ammonium chloride is more effective in blocking regeneration of added C_4 acid than is malonate.

Enzymatic resolution of racemic histidine, cystine, and alanine VINCENT E. PRICE,* LEON LEVINTOW* AND JESSE P. GREENSTEIN *Natl. Cancer Inst., Natl. Inst. of Health, Bethesda, Md.*

Although most of the racemic amino acids can be easily resolved by asymmetric enzymatic hydrolysis of their N-acylated derivatives, histidine, arginine, cystine, and proline are not resolvable by this method. Since one of the reasons for acylation of the amino acids is to remove their dipolar character, it was considered that this could be accomplished as well by amide formation. Consequently, DL-histidine amide, S-benzyl-DL-cysteine amide, and DL-alanine amide were prepared and subjected to the action of hog kidney or liver concentrates at pH 8.8-9.2. The concentrates were prepared by low temperature-alcohol fractionation of the tissue homogenates. Mn^{++} in a final concentration of 0.01 M was used in the digestion of the former two substrates. The racemates were hydrolyzed asymmetrically to a mixture of L-amino acid, ammonia, and D-amino acid amide. After removal of the L-amino acid from the deproteinized digest, the mixture was concentrated, brought to pH 11, the D-amide extracted into acetone, the solvent removed, the amide hydrolyzed with dilute HBr, and the free D-amino acid isolated after neutralization. The isomers were obtained in good yield and possessed rotation

values which compared favorably with those in the literature. DL-Proline amide was almost completely resistant to the animal enzymes studied, but both isomers were readily hydrolyzed by mushroom preparations.

Sedimentation and diffusion of *E. coli* bacteriophage T₆ FRANK W. PUTNAM AND EUGENE GOLDWASSER* *Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.*

The sedimentation and diffusion behavior of *E. coli* bacteriophage T₆ was investigated in the analytical ultracentrifuge and in a refractive index apparatus in a study of the molecular homogeneity, physical constants, and apparent size of this tadpole shaped virus. Although the phage sediments with a single sharp boundary ($S_{20} = 1050S$) at pH 4.9 to about 6.5, a second more slowly sedimenting boundary ($S_{20} =$ about 850 S) is observed at higher pH. The proportion of the two components depends on the age and history of the preparation but is independent of the gravitational field and concentration and is unrelated to the infectivity. Since S_{20} is constant within experimental error from 715g to 18,900g, the particles are unoriented in sedimentation. The virus obeys the laws of normal diffusion, and, at pH 5.5, D_{20} is 0.45×10^{-7} cm²sec⁻¹ for phage which is homogeneous in electrophoresis and in the ultracentrifuge. Equivalent spherical diameters calculated from diffusion (94 μ), from biological infectivity (98 μ), and from sedimentation (60 μ) are in fair agreement with the dimensions previously obtained directly from electron micrographs (head = 60×80 m μ , tail = 100×20 m μ). The particle weight calculated from S_{20} and D_{20} is 1.7×10^8 gms per mole. The results appear to exclude motility for this tailed bacteriophage. Estimates of shape based on S_{20} and D_{20} suggest that the slowly sedimenting form is the monomer and the fast form is a dimer with particles aligned end-to-end.

Effect of surface on coagulation reactions

ARMAND J. QUICK *Dept. of Biochemistry, Marquette Univ. School of Medicine, Milwaukee, Wis.*

A glass surface influences coagulation in a two-fold manner: it causes platelets to adhere and to undergo lysis, and it plays an essential role in the conversion of prothrombinogen to active prothrombin. No adequate explanation for the action of surface on platelets has been offered. Paraffin, silicone and certain plastics having non-wettable surfaces preserve platelets, but collodion which has a wettable surface likewise is equally efficient as a preserver. Since there is good evidence that thrombin is the specific agent responsible for the lysis of platelets, a probable explanation for the paradoxical behavior of collodion can be offered.

A rough surface such as glass has no effect on platelets *per se*. It acquires this property under circumstances which permit the formation of thrombin. It can be postulated that thrombin is adsorbed to a wettable surface and that this layer is responsible for the disintegrating action on platelets. Collodion which not only adsorbs but also absorbs thrombin fails to become coated with a continuous film, therefore platelets coming in contact with its surface do not suffer destruction. The absorption of thrombin by collodion can readily be demonstrated by testing the potency of a standard thrombin before and after contact with collodion. A distinct diminution of activity occurs. The activation of prothrombinogen which is mediated through a rough surface such as glass requires neither thrombin, thromboplastin, calcium nor platelets. The action occurs slowly in ovalated or citrated plasma and rapidly in native plasma, irrespective of the number of platelets.

Glyoxalases E. RACKER, *Dept. of Microbiology, New York Univ. College of Medicine and College of Dentistry, New York City*

In the presence of reduced glutathione, tissue extracts convert methyl glyoxal to lactic acid. Yamazoye using rabbit liver extracts presented evidence for a biologically formed addition compound between reduced glutathione and methyl glyoxal which is not identical with the chemically formed addition compound. This finding has been confirmed in the present study and an enzyme obtained from baker's yeast which catalyzes the formation of the 'biological' addition compound. This enzyme, referred to as glyoxalase I, has been purified by acetone and ammonium sulfate fractionation. When glyoxalase I is added to methyl glyoxal and glutathione, an increase in absorption in the ultraviolet at λ 240 m μ can be observed, which is proportional to the concentration of enzyme added. This is a convenient method for the assay of glyoxalase I. The enzymatic formation of the intermediate compound is proportional to the concentration of both glutathione and methyl glyoxal. Glyoxalase I is used for the preparation of the addition compound which is isolated as an alcohol insoluble barium salt. A second enzyme which catalyzes the breakdown of the intermediate addition compound to lactic acid and glutathione has been purified from beef liver. The activity of this enzyme is measured spectrophotometrically by the disappearance of the absorption of the intermediate. The initial rates are proportional to the enzyme concentration. This enzyme is referred to as glyoxalase II. When purified glyoxalase I and II are combined, a very active glyoxalase preparation is obtained which catalyzes the conversion of methyl glyoxal to lactic acid. This preparation is a sensitive system for the assay of

glutathione since as little as 5 μ g are readily determined.

Analgesic action of 3-hydroxy-N-methyl morphinan hydrobromide (Nu-2206) LOWELL O. RANDALL AND G. LEHMANN, *Pharmacology Dept., Hoffmann-La Roche Inc., Nutley, N. J.*

Nu-2206 is a synthetic substitute for morphine. Structurally, it lacks only the ether-oxygen bridge of dihydrodesoxy morphine-D. The analgesic activity was measured as the percentage increase in reaction time to a radiant heat stimulus applied to the back of rats. By the subcutaneous route, Nu-2206 was found to be 4 times as strong as morphine and about twice as long in duration. By the oral route, Nu-2206 was slightly stronger than Methadon and longer in duration. Tolerance to the analgesic effects developed at about the same rate as with morphine. The response to a constant daily *s.c.* dose decreased about half in a period of 8 weeks and to the limit of error of the method in 11 weeks. Respiration of unanesthetized rabbits was decreased by doses of Nu-2206 which were about $\frac{1}{4}$ those of morphine and the duration was also longer. The compound was spasmogenic to the dog intestine as measured in a Thiry Vella loop but was nearly inactive on isolated rabbit intestine. Nu-2206 is 5 to 6 times as toxic as morphine in several species but about half as toxic as Methadon. Toxic doses produced characteristic morphine effects in animals including tail erection in mice, excitement in cats, depression in dogs and catatonia in rats. The safety factor is slightly less than that for morphine but at least twice that of Methadon. The L-form of the racemic mixture, Nu-2206, has twice the analgesic activity and respiratory depressant action of the DL-mixture and equal toxicity.

Correlation of reductimetric and turbidimetric methods of hyaluronidase assay MAURICE M. RAPPORT,* KARL MEYER AND ALFRED LINKER,* *Dept. of Medicine, Columbia Univ. College of Physicians and Surgeons, New York City*

The mechanism of action of hyaluronidase on hyaluronic acid is not well defined. Of the changes measured to provide estimations of hyaluronidase activity, only the increase in reducing sugar is unambiguously interpretable in a mechanistic sense. The reducing sugar results from the breaking of glucosidic bonds between N-acetyl-hexosamine and glucuronic acid residues. It is further agreed from observations of the color development with dimethylaminobenzaldehyde that the first bond broken is that which liberates the reducing group of the N-acetylglucosamine. It has been suspected that the decreased turbidity formation as well as the decrease in viscosity of the low viscosity

* Deceased

preparations employed in viscosimetric assays is the result of hydrolysis of glucosidic bonds. However, the absence of exact information on this point has, in general, prevented correlation of information obtained by the 3 different methods. By application of the submicro determination of glucose of Park and Johnson (*J Biol Chem* 181: 149, 1949), it has been possible to show that under the conditions employed for turbidimetric assay of hyaluronidase (MEYER, *K Physiol Rev* 27: 335, 1947), namely, the action of one turbidimetric unit of enzyme on 200 μ g of hyaluronic acid in 1 ml of acetate buffer at pH 6 for 30 minutes, reducing sugar of the order of 2-3 μ g glucose equivalent is liberated, representing 1-2% hydrolysis of the substrate. The reaction responsible for the decreased turbidity formation is thus the same as that measured by the reductimetric method. The suitability of this submicro reducing sugar method for assay of hyaluronidase will be discussed.

Enzymatic conversion of homogentisic acid to fumarylacetoacetate ROBERT G. RAYDIN AND DANA I. CRANDALL (introduced by SAMUEL GURIN) *Dept of Physiological Chemistry, Univ of Pennsylvania, School of Medicine, Philadelphia*

A concentration of 30% alcohol in a water clear centrifugate of homogenized rat liver at 0°C precipitates a protein fraction (I) which converts homogentisic acid into a beta-keto-acid which is very slowly decarboxylated by aniline citrate at 38°C. Another precipitable protein fraction (II) obtained by increasing the alcohol concentration to 50%, discarding the precipitate, and increasing the alcohol to 70%, converts the slowly decarboxylating beta-keto-acid into acetoacetate. The beta-keto-acid was precipitated as a silver salt from a trichloroacetic acid deproteinized incubation mixture of homogentisic acid and protein fraction I. The acid was obtained from the silver salt by acidification and ether extraction and purified by repeated precipitation from ether-petroleum ether. The free acid (M.P. 158°C) titrates as a dicarboxylic acid with an apparent molecular weight of 200. It appears to be a diketone for the following reasons: a) reactions with o-phenylene diamine yields a lavender color similar to that exhibited by beta-diketones (WITTER, SNYDER AND STOTZ *J Biol Chem* 176: 493, 1948); b) two additional titratable groups appear at alkaline pH's, and c) hydrolysis to fumarate and acetoacetate is brought about by a purified protein fraction known to hydrolyze triacetic acid (CONNORS AND STOTZ *J Biol Chem*, 178: 881, 1949). These observations suggest that the acid is fumarylacetoacetate.

Properties of a red cell metabolism regulator

ALLEN F. REID AND ELLSIE B. RYAN (intro-

duced by M. F. MASON) *Southwestern Med School of Univ of Texas, Dallas*

A component of human blood serum has been found to have an inhibiting effect on the rate of phosphate metabolism of human erythrocytes. This component is dialyzable, but not ionically removed by strong anion or cation ion exchange resins. A similar factor is found in the serum of cattle, dogs and mice. None was found in human urine. Physical and chemical properties of this material were similar to those of simple carbohydrates. A much less pronounced but similar effect on red-cell phosphate metabolism was shown by some carbohydrates but not by others, providing a lead to the functioning structural units of the molecule.

Nitrogen trichloride-treated prolamines VII. Further characterization of toxic factor L REINER, F. MISANI,* M. G. CORDASCO* AND T. W. FAIR* *Research Labs, Wallace and Tiernan Products, Inc., Belleville, N. J.*

It has been postulated on the basis of analytical data that the toxic factor is a derivative of methionine (REINER *et al J Am Chem Soc* In press). It differs in composition from methionine only by one additional atom of each hydrogen, nitrogen and oxygen. Furthermore the development of convulsions caused by the toxic factor was found to be suppressed by the administration of large doses of methionine (REINER *et al Arch Biochem* In press). Evidence is now being presented indicating that the methionine skeleton is maintained in this derivative. Hydrolytic degradation with aqueous hydrochloric or sulfuric acid yielded some homocysteic acid identified chromatographically, refluxing with hydriodic acid yielded methyl iodide and a residue which gave a positive nitroprusside test and contained the thiolactone of homocysteine, the latter was converted into homocystine, hydrogenolysis with Raney nickel yielded alpha-amino-n-butyric acid. The toxic derivative was found to be transparent to ultraviolet rays. It seems that only the sulfur atom of methionine is involved in the formation of the toxic derivative.

Metabolism of polyunsaturated fatty acids in chickens RAYMOND REISER (introduced by CARL M. LYMAN) *Texas Agricultural Experiment Station, College Station*

A group of 12 White Leghorn Cockerels were fed a low fat synthetic ration, and 3 similar groups the same basal ration in which 1% bayberry tallow, cottonseed oil or cod liver oil were substituted for 2% sucrose. At the end of 5 weeks the chicks were killed and the polyunsaturated fatty acids of the phospholipids and triglycerides of the carcasses and pooled organs determined by alkaline isomerization. Fatty acids with 5 and 6 double bonds were universally present only in those chicks fed cod liver oil. Five double bond

acids were also found in the organ glycerides and 5 and 6 double bond acids in the organ phospholipids of cottonseed oil fed birds. No evidence of 5 or 6 double bond acids was found in the tissues of the chicks on the fat free or bayberry tallow rations. Arachidonic acid was present in significant amounts in all cases. Linolenic acid was completely absent or present in traces only. Linoleic acid composed about 5% of the triglycerides and 10% of the phospholipids of the tissues of the fat free and bayberry tallow fed chicks, but 10% of the triglycerides and 15% of the phospholipids of the cottonseed oil and cod liver oil groups. It appears that the growing chick cannot convert linoleic acid to 3, 5, or 6 double bond acids but probably does convert it to arachidonic acid. However, when 8-week old chicks were fed conjugated linoleic acid, this acid was not found in the tissues but apparently was converted to a conjugated 3 double bond acid with maximum absorption at 270 m μ .

Purine metabolism in rat liver homogenates

DAN A. RICHERT* AND W. W. WESTERFELD
Dept. of Biochemistry, Syracuse Univ. College of Medicine, Syracuse, N. Y.

The increased oxygen uptake due to the addition of various purines, nucleosides, and nucleotides to rat liver homogenates of varying xanthine oxidase activities was determined. Decreases in liver xanthine oxidase were obtained by feeding a purified 8% casein diet. Normal rat liver homogenates gave a theoretical increase in the oxygen uptake from the addition of guanine, guanosine, xanthosine, adenosine, and adenosine-5-phosphate, due to the oxidation of xanthine and hypoxanthine formed from these substrates by enzymatic hydrolytic reactions. The 3-phosphate derivatives of guanosine and adenosine were hydrolyzed slowly. Adenine gave no oxygen uptake due to the absence of adenase. When the xanthine oxidase activity was reduced to a zero oxygen uptake with xanthine, the homogenate failed to oxidize any of the above substrates. *In vitro* addition of purified milk xanthine oxidase to such a liver preparation restored its normal capacity to oxidize all of the substrates. It was concluded that 1) no unidentified nucleoside or nucleotide oxidative enzymes were present in rat liver, 2) the hydrolytic enzymes involved in nucleotide metabolism were not destroyed by feeding a low protein diet, and 3) inosine was a key intermediate in the formation of uric acid from adenylic acid in rat liver.

γ -Aminobutyric acid in brain EUGENE ROBERTS AND SAM FRANKEL (introduced by C. CARTHERS) *Division of Cancer Research, Washington Univ., St. Louis, Mo.*

Relatively large quantities of an unidentified ninhydrin-reactive material were found in numer-

ous two dimensional paper chromatograms of protein-free extracts of fresh mouse, rabbit, and frog brains. At most, only traces of this material were found in a large number of many other normal and neoplastic tissues and in urine and blood. The eluate from suitably chosen strips of one-dimensional phenol chromatograms of mouse brain extract contained the unknown substance and only traces of valine and an unidentified peptide material. A comparison of the properties of the unknown substance in the eluate with those of known compounds by chromatography in different solvent systems showed it to be identical with γ -aminobutyric acid. This conclusion was independently confirmed by the isotopic derivative method using the I-131- and S-35-labeled p-iodophenyl sulfonyl derivatives (S. Udenfriend). Experiments with brain homogenates showed a formation of γ -aminobutyric acid which appeared to be accelerated when glutamic acid was added. γ -Aminobutyric acid was also formed in homogenates of liver and muscle. Experiments are under way to characterize the precursors of γ -aminobutyric acid and the enzymes involved in its formation.

Role of potassium in cellular metabolism I. Z. ROBERTS (introduced by L. B. FLEXNER), *Carnegie Inst. of Washington, Dept. of Terrestrial Magnetism, Washington, D. C.*

Various methods have been used to investigate the effects of potassium deficiency on cells of *E. coli*. In cells partially depleted of potassium the rates of growth and glucose utilization were reduced. Radioactive tracers gave a more sensitive indication of changes in the metabolic activity of the cells. With glucose as a substrate, cells deficient in potassium showed less incorporation of radioactive sulfate into proteins and less incorporation of radioactive phosphate into phospholipids and nucleic acids. The most marked reduction (factor of 8) was found in the incorporation of phosphate into desoxyribose nucleic acid. With other substrates such as lactate, pyruvate, glutamate, and hexose phosphates, a smaller effect from potassium depletion was found. These observations are in accord with the hypothesis that potassium is involved in one of the early steps in the glucose cycle.

Biological activity of some uridine derivatives MARTIN ROBERTS, T. KAY FUKUHARA AND DONALD W. VISSER (introduced by RICHARD J. WINZLER) *Dept. of Biochemistry and Nutrition, Univ. of Southern California School of Medicine, Los Angeles*

There is evidence that nucleosides are the building stones used by organisms to synthesize the nucleic acid portion of nucleoproteins. It was therefore of interest to synthesize some uridine derivatives and investigate the ability of these

derivatives to replace or antagonize the utilization of naturally occurring nucleosides. Bromouridine (1 D-ribofuranosyl-2,4-dioxy-5-bromopyrimidine) and chlorouridine (1 D-ribofuranosyl-2,4-dioxy-5-chloropyrimidine) were synthesized by bromination and chlorination of natural uridine. Ribosylthymine (1 D-ribofuranosyl-2,4-dioxy-5-methylpyrimidine) was synthesized from thymine and D-ribose. Chlorouridine competitively inhibited the growth of the uracil-requiring mutant of *Neurospora* (1298). The molar ratio of chlorouridine to uridine to produce 50% growth was approximately 4. Ribosylthymine neither replaced nor inhibited uridine under the same conditions. The growth of the wild strain of *Neurospora* was not inhibited by either chlorouridine or ribosylthymine in the concentrations tested. The growth of *Mycobacterium tuberculosis* was not inhibited by bromouridine or chlorouridine but was inhibited by chlorouracil at a concentration of 0.0001 gm in 10 cc medium. Studies are in progress testing these uridine derivatives with thymidine-requiring organisms. The propagation of mouse encephalomyelitis virus, Theiler's GD VII, in cultures of mouse brain is also being investigated.

Influence of adrenal cortex on antibody production in vitro SIDNEY ROBERTS* AND ABRAHAM WHITE, *Dept of Physiological Chemistry, School of Medicine, Univ of California at Los Angeles*

Previous studies have demonstrated that splenic tissue, obtained from rats given a single intravenous injection of sheep erythrocytes 5 days earlier, released large amounts of hemolysin against this antigen on incubation under 95% oxygen, 5% carbon dioxide in serum obtained from non-immunized animals. This release of antibody did not occur when the incubation was carried out under an atmosphere of 100% nitrogen. Similar experiments have been conducted with animals which had been either adrenalectomized 2-4 days earlier, or injected intraperitoneally 2 hours earlier with 2 ml of Wilson's aqueous adrenal cortical extract (ACE). It was found that the amount of antibody released by splenic tissue from adrenalectomized rats was much smaller than that seen with splenic tissue from unoperated animals, and that there was no evidence for new formation of antibody. The level of antibody in the serum of the adrenalectomized animals was slightly lower than that of the control, and definitely lower in extracts of spleen and mesenteric lymph nodes of the adrenalectomized rats. In contrast, splenic tissue from immunized rats injected with ACE produced very large amounts of antibody *in vitro*. In addition, evidence was obtained that the injection of ACE resulted in the release of antibody from spleen

and lymphoid tissue in the intact immunized animals, and in a prolongation of the period of maximal antibody production.

Chemical composition of some bacterial pyrogens.

GERTRUDE RODNEY AND H. B. DEVLIN (introduced by J. J. PIFFNER), *Research Labs., Parke, Davis & Co., Detroit, Mich.*

Pyrogenic material was isolated from strains of *B. prodigiosus*, *E. typhi*, *B. subtilis* and *Ps. aeruginosa* grown on synthetic media. The pyrogens were purified by fractionation with organic solvents after treatment by various procedures to remove protein and nucleic acid. Pyrogen from different sources prepared by the same procedures differed in activity. The most active, from *B. prodigiosus*, reacted in concentrations of 0.01-0.005 µg/kg in rabbits. The pyrogens were analyzed for N and P, and for reducing substances and glucosamine after hydrolysis. Quantitative filter paper partition chromatography was applied to the determination of the sugars present in the hydrolysates, and the composition of the 4 pyrogens was found to differ qualitatively and quantitatively.

The α -glucosaminidase activity of partially purified testicular extract SAUL ROSEMAN* AND ALBERT DORFMAN, *Dept of Pediatrics, Univ of Chicago, Chicago, Ill.*

Partially purified testicular extract was found to hydrolyze N-acetyl-phenyl- α -D-glucosaminide (prepared by way of its tetracetate). This hydrolysis does not parallel hyaluronidase activity as evidenced by the fact that the glycosidase activity was markedly decreased in a more highly purified hyaluronidase preparation. The β -glucosaminidase discovered by Helferich and Ploff (*Z. physiol. Chem.* 221:252, 1933) in emulsin and snail extract (NEUBERGER AND PITT-RIVERS, *Biochem. J.* 33:1580, 1939) had been previously demonstrated in testicular extract (EAST ET AL. *Biochem. J.* 35:872, 1941). This fact has been confirmed thus indicating that crude testicular extract contains both α - and β -glucosaminidases. That the apparent hydrolysis of the α -sugar is not due to β contamination is indicated by the failure of emulsin to act on the α -glucosaminide. These data suggest the presence in testicular extract of a previously undescribed α -glucosaminidase.

Effect of protein starvation on enzyme activity of normal and regenerating rat liver OTTO ROSENTHAL, JAMES C. FAHL*, GORDON M. KARN* AND CHARLES S. ROGERS*, *Harrison Dept of Surgical Research, Univ of Pennsylvania Schools of Medicine, Philadelphia*

The liver enzyme study previously reported (*Federation Proc.* 8:244, 1949) has been extended to include additional enzymes and prolonged

periods of protein-starvation Male 250-gm Wistar rats were used Expressed in terms of mass of protein or of enzyme activity per total liver per 100 gm initial body weight, the following percentages of normal values (obtained with protein-fed rats) were found after 2 and 6 weeks of protein depletion respectively: protein (57%, 50%), arginase (32%, 20%), rhodanese (32%, 29%), nonspecific cholinesterase (43%, 42%), adenosine pyrophosphatase (50%, 46%), alkaline phosphatase (142%, 107%) With the exception of alkaline phosphatase which increased, all enzymes exhibited sharp initial drops which exceeded the protein loss to a varying degree Prolonged protein-starvation produced an additional significant decrease of arginase and a reduction of alkaline phosphatase to normal levels Partial hepatectomy in depleted animals caused a sharp rise in alkaline phosphatase content of the liver remnant The rise which greatly exceeded the protein increase resembled that of arginase previously reported No other enzyme displayed this phenomenon Differential changes of enzymes with gain or loss of liver protein may be explained to a large extent by location differences With arginase and alkaline phosphatase, however, the metabolic state of the animal appears to be an additional factor which controls either quantity or activity of the enzyme

Sulfur balance of rats fed excess DL-methionine plus glycine or DL-alanine JAY S. ROTH*, JAMES B. ALLISON AND LAWRENCE J. MILCH* *Bureau of Biological Research, Rutgers Univ., New Brunswick, N. J.*

Four groups of mature, male Sherman strain rats were fed a basic 12% casein diet and in addition to this, *Group II* was fed 4.8% DL-methionine, *Group III*, 4.8% DL-methionine plus 4.8% glycine, and *Group IV*, 4.8% DL-methionine plus 5% DL-alanine All the groups were fed the same weight of diet consumed by *Group II* over 20-day period Nitrogen and sulfur balances were determined on the 4 groups and in addition, liver and kidney weights, liver fats and plasma pseudocholinesterase Urinary sulfur partitions were performed also and it was found that in *Group I*, two-thirds of the urinary sulfur was excreted as sulfate and one-third as organic sulfur This proportion was maintained in *Groups II* and *IV*, but in *Group III*, excess glycine favored the excretion of sulfur in the organic form The animals in *Group III* were in sulfur balance while those in *Groups II* and *IV* maintained a positive sulfur balance equivalent to 10% of the ingested sulfur Excess glycine counteracted in part the kidney hypertrophy and weight loss observed when excess methionine was fed but an equivalent quantity DL-alanine had no significant effect in this way

showing that the action of glycine was quite specific High liver fat values were observed in rats fed excess methionine plus alanine

Enzymatic formation of citric acid studied with C¹⁴-labeled oxalacetate HARRY RUDNEY*, VICTOR LORBER, M. F. UTTER AND MARGARET COOK* *Dept. of Biochemistry, Western Reserve Univ. School of Medicine, Cleveland, Ohio*

The concept that citrate may behave as an asymmetrical molecule in enzymatic reactions, proposed by Ogston (*Nature* 162:963, 1948) and supported by the work of Potter and Heidelberger (*Nature* 164:180, 1949), removes prior objections to the inclusion of citrate in the main path of the Krebs cycle In addition, Stern and Ochoa (*J. Biol. Chem.* 179:491, 1949) have demonstrated citrate formation from oxalacetate and acetate in an enzyme preparation containing negligible aconitase activity, indicating that citrate may be the primary reaction product This implies condensation of the methyl carbon of acetate on the carbonyl group of oxalacetate This reaction was studied in an enzyme preparation similar to that of Stern and Ochoa using non-isotopic acetate and C¹⁴- β -carboxyl labeled oxalacetate, prepared enzymatically and isolated by partition chromatography The resulting C¹⁴-citrate (plus carrier) was isolated by partition chromatography and the quinidine derivative prepared Degradation to yield the non-carboxyl carbons, the tertiary carboxyl, and primary carboxyls, as three separate fractions, and enzymatic conversion to α -ketoglutarate followed by α -decarboxylation revealed significant activity only in the primary carboxyl corresponding to the α -carboxyl of α -ketoglutarate These results are in accord with the reaction mechanism postulated for citrate formation from acetate and oxalacetate, and confirm the observations of Potter and Heidelberger offered in support of Ogston's proposal Oxalacetate, recovered after incubation of C¹⁴-citrate with the citrate-forming enzyme in the presence of non-isotopic acetate and oxalacetate, contained no activity, suggesting though not establishing the irreversibility of the condensation reaction

Inheritance of rate of protein synthesis in the liver of inbred strains of rats ROBERT RUTMAN (introduced by HAROLD TARVER) *Univ. of California, Berkeley*

Two inbred strains of rats, differing in body weight, were compared as to *in vitro* incorporation of S³⁵ labeled DL-methionine into the protein of liver slices The heavier F strain (Wistar Albino) showed 50-57% greater uptake than did the lighter J strain (Fisher #344), for the 3-4 generations tested Mature FJ and JF and young FJ hybrids showed essentially the same *in vitro* activity as the F parent Young JF hybrids were not signifi-

cantly different from the J parent. Average activities for backcrosses showed no significant differences from the higher inbred strain. However, the distribution of individual values showed that 45% of the offspring of J males and hybrid females resembled the J strain, whereas 95% of the comparable F offspring equalled or exceeded the F strain. Foster nursing of J males on inbred F mothers raised the liver activity to the level of the foster parent. Fostering of F males had no effect. Thus, the characteristic liver activities are determined by both the individual and the maternal genotypes. Backcross segregation suggests that a single genetic factor contributes a major portion of the strain difference. The superior potency of this factor in the F strain is shown by the equivalence of the hybrids. Maternal influence is shown in the depressed activity of young hybrid liver tissue and is confirmed by the foster nursing studies.

Acetone metabolism in the intact rat WARWICK

SAKAMI *Dept of Biochemistry, Western Reserve Univ School of Medicine, Cleveland, Ohio*

Borek and Rittenberg (*J Biol Chem* 179 843, 1949) have indicated that acetone is metabolized at least in part via acetate in surviving rat liver. We have found that it also contributes to the pool of 'formate' or formate derivative for which the β -carbon of serine is used as an indicator. Acetone is also converted to the methyl groups of choline and methionine. When C^{14} -methyl-labeled acetone was administered to rats, the β -carbon of the isolated serine and the methyl carbons of the choline and methionine contained appreciable activity. The formation of the β -carbon of serine from formate did not occur via acetate. When C^{14} -methyl-labeled acetate replaced the acetone in a parallel experiment, the β -carbon of serine contained negligible activity. Comparison of the incorporation of C^{14} into the β -carbon of serine from methyl labeled acetone and formate (Sakami *J Biol Chem* 176 995, 1948) indicate that formate formation is a major mechanism of acetone metabolism under these conditions. This suggests that the principal pathway of acetone utilization involves cleavage of its carbon chain into acetate and formate or substances derived from these compounds. The finding of C^{14} in the methyl groups of choline and methionine afforded the possibility that the synthesis of labile methyl groups occurred via formate.

Studies on the biosynthesis of chlorophyll KURT

SALOMON, KURT I. ALTMAN AND ROCCO DELLA ROSA (introduced by W. R. BLOOR) *Dept of Radiation Biology, Univ of Rochester, Rochester, N. Y.*

The utilization of the alpha-carbon atoms of glycine and acetic acid in the biosynthesis of

chlorophyll by *Chlorella vulgaris* has been investigated. *Chlorella* cells were grown in a synthetic sterile medium containing mineral supplements and 1% glucose. To this medium were also added either sodium acetate or glycine labeled with C^{14} in their respective alpha-carbon atoms and having a C^{14} -activity of 2.57×10^3 and 2.55×10^3 disintegrations/min/ μ M carbon respectively. The final concentration of glycine and acetate in the medium was 0.01M. The cells were grown for one week under stationary conditions in fluorescent light at pH 5.8, and were then harvested by centrifugation. The harvested cells were washed with water and then with dilute solutions of either inert glycine or inert acetate. The *Chlorella* cells were then extracted exhaustively with dioxane and the chlorophyll thus prepared was precipitated with a concentrated aqueous NaCl solution. The chlorophyll preparation was purified by several reprecipitations with NaCl and converted to methylpheophorbide, which was recrystallized several times from chloroform-ligroin. The contribution of the alpha-carbon atom of acetate to methylpheophorbide was 3.5 times greater than that of the methylene-carbon atom of glycine. In the case of *Chlorella* grown in the presence of acetate, the isotope dilution of methylpheophorbide isolated was 20 as compared with 60 in the case of *Chlorella* grown in the presence of glycine. The data indicate that *Chlorella vulgaris* is able to utilize directly the alpha-carbon atoms of glycine and acetate for the biosynthesis of chlorophyll.

Physiopathology of intermediary carotene metabolism in animal organism STELIOS C.

SAMARAS, WILLIAM REALS AND DANIEL HINGERTY (introduced by VICTOR E. LEVINE) *Depts of Biological Chemistry and Nutrition, and Pathology, Creighton Univ School of Medicine, Omaha, Nebr.*

Forty male white rats, 4 weeks old and divided into groups, were put on a vitamin A-deficient diet. When the classical symptoms appeared, the animals were given orally 400 U.S.P. units of carotene in oil. Control animals were able to build up great reserves of vitamin A without accumulation of carotene (carotene liver 2.3 μ g/gm, intestine (small) 3.5 μ g/gm, lung 3.1 μ g/gm, vitamin A liver 85.1 μ g/gm, intestine 1.5 μ g/gm, lung 1.8 μ g/gm). Rats, 4 weeks deficient, with liver vitamin A less than 1 μ g/gm, without ocular symptoms and with considerable weight loss, showed immediate gains in weight after carotene administration. Killed one week later they had carotene liver 3.8 μ g/gm, intestine 2.1 μ g/gm, lung 2.0 μ g/gm, vitamin A liver 9.1 μ g/gm, intestine 1.2 μ g/gm, lung 0.9 μ g/gm. Rats with more advanced deficiencies also showed striking amelioration, but lasting only 2-5 weeks. No

measurable quantity of vitamin A was found in their organs. Very severely deficient rats with marked hemorrhagic conjunctivitis were unable to convert given carotene, dying despite the presence of appreciable carotene (carotene: liver 8.1 $\mu\text{g/gm}$, intestine 12.6 $\mu\text{g/gm}$, lung 7.3 $\mu\text{g/gm}$). Histological examinations showed that deficient animals first develop reversible changes. In advanced stages the alterations are irreversible, impairing carotene absorption and conversion. Especially in gastrointestinal tracts of animals with advanced symptoms we found a marked desquamation of the columnar epithelium. This was especially prominent in animals unable to use the administered carotene. Evidently in vitamin A deficiency vitamin A, not carotene, should be administered.

A simple densimetric determination of heavy water LEO A. SAPIRSTEIN (introduced by WALTER MARX) *Dept. of Physiology, Univ. of Southern California Med. School, Los Angeles*

Density determinations can be performed on deuterium oxide-water mixtures with an accuracy of 3 parts in a million by the present method, which requires only standard laboratory equipment and a Beckman differential thermometer. A small hollow glass bead is prepared and its specific gravity is adjusted by trial and error until it just sinks in redistilled water at 20–25°C. The temperature at which this bead just begins to float is determined to 0.1°C for standard D_2O - H_2O mixtures ranging from 0–2%. With pyrex beads the end-point for 2% D_2O is 7°C higher than for distilled water and the plot of temperature vs. D_2O concentration is linear. By substituting a redistilled sample of the unknown D_2O - H_2O mixture, and determining the end-point temperature, the D_2O concentration may be read directly from the calibration curve. The method differentiates D_2O concentrations of 3×10^{-5} with no difficulty. Samples containing approximately 1% D_2O in water have been determined with an accuracy of 0.3% by this method.

Concentration of the factor required for the growth of *Leuconostoc citrovorum* 8081 H. E. SAUBERLICH (introduced by W. D. SALMON) *Lab. of Animal Nutrition, Alabama Polytechnic Inst., Auburn*

Further studies were made on the concentration of the factor from Wilson's liver 'L'. This was dissolved in water, adjusted to pH 3.0 with sulfuric acid and treated repeatedly with charcoal (Norite A) at room temperature. The factor was eluted from the charcoal with ethyl alcohol-water-ammonium hydroxide solution at 65°C. The eluate was evaporated to dryness and the residue taken up in water. The solution was adjusted to pH 3.0 with sulfuric acid and the precipitate filtered off. The

filtrate was extracted repeatedly with n-butyl alcohol at a pH of 3.0. The butyl alcohol fractions were then extracted with water at pH 8.5. The water extracts were adjusted to pH 7.0 and passed over amberlite columns to remove anions and cations. The filtrate was evaporated partially and then extracted at a pH of 3.0 with butyl alcohol. The butyl alcohol extract was further purified by partition chromatography. This was accomplished by placing the solution on potato starch columns and developing with butyl alcohol saturated with water and made acidic with acetic acid. Various bands and fractions were collected and analyzed microbiologically for activity. Active fractions were further purified by partition chromatography. In this manner concentrates of the unknown factor were obtained which were exceedingly active and of very low solids content. Details of the procedure employed and the preparations obtained will be presented.

Effect of adrenal mince and cell-free filtrate on desoxycorticosterone KENNETH SAVARD*, LENA A. LEWIS* AND ARDA ALDEN GREEN *Research Division, Cleveland Clinic Foundation, Cleveland, Ohio*

Desoxycorticosterone has been incubated with minced or filtered extracts of beef adrenal tissue. The neutral steroid fraction extracted from these incubation mixtures exhibits glycogenic activity in fasting adrenalectomized mice (Venning test). This activity is substantially greater than that of the controls incubated without added desoxycorticosterone. Studies on possible coenzymes, on different substrates, and on the chemical characteristics of the products will be reported.

Ketoglutaric acid carboxylase in higher plants

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Extracts from a variety of higher plants were found to decarboxylate pyruvic acid (PA) and α -ketoglutaric acid (KGA). The addition of phenol (2 mg/ml) to the incubation mixtures completely inhibited the decarboxylation of PA, but interfered less strikingly with the decarboxylation of KGA, suggesting that the two keto acids were attacked by different enzymes. Extracts from different plants varied widely in their activity against PA and KGA. Cucumber extract, for example, decarboxylated PA, but had no significant effect on KGA. Various types of potatoes yielded extracts with considerably higher activity against KGA than against PA. Squash, corn and cantaloupe were found to be particularly rich in KGA carboxylase. The new enzyme (from squash) showed optimal activity at pH 6.0–6.1. It lost 95%

of its activity after 17 hours dialysis against 30-40 volumes of distilled water, while PA carboxylase was only slightly inactivated by this treatment. Both carboxylases were reactivated on addition of concentrated dialysate to the dialyzed material. PA carboxylase but not KGA carboxylase was also activated on addition of diphosphothiamin (cocarboxylase) plus Mg or Mn ions to extracts which had been dialyzed for several days. Yeast extract (Difco) activated dialyzed KGA carboxylase but did not activate dialyzed PA carboxylase. The easily removable prosthetic group of KGA carboxylase is not identical with diphosphothiamin.

Intracellular localization of uricase activity

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Nuclei, mitochondria, sub-microscopic particles and 'supernatant fluid' were separated from rat liver homogenate by differential centrifugation. The uricase activity of each fraction, of the original homogenate and of a mixture prepared by combining the separated fractions in the proportions found in the homogenate were measured. Three dilutions of tissue were incubated with a known amount of uric acid in borate buffer pH 9.3, at 45°C in a Dubnoff Metabolic Shaking Apparatus. Under the conditions employed, uricase activity (μg uric acid destroyed/30 min) was in each case proportional to the quantity of tissue used. Seventy to 80% of the uricase activity was found in the mitochondrial fraction, 10 to 15% in the nuclear fraction. Little or no activity could be demonstrated for the sub-microscopic particles and the supernatant fluid. The uricase activity of the mixture closely approximated that of the original homogenate, indicating the absence of interaction among the materials of the 4 fractions. As a check on the effective separation of the fractions, nucleic acids (DNA, PNA) and succinoxidase determinations were made. The fractions were also examined microscopically (phase contrast). The mitochondrial contamination (as free particles or in unbroken cells) may account for the uricase activity of the nuclear fraction. The mitochondrial fraction was completely free of nuclei.

Nature of rabbit liver glycogen

MAX SCHLAMOWITZ (introduced by DAVID M. GREENBERG)
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Studies have been carried out on the influence which type of carbohydrate and the mode of its administration has in determining the structure of glycogen deposited in rabbit livers. Measurements of degree of branching and iodine adsorbing and light scattering properties have been used to es-

tablish differences in the structures of the several glycogens isolated. The end-group assay as determined by periodate oxidation was found to correspond to an average branch-length of 19 to 25 glucose residues, depending on the type carbohydrate administered and the mode of its administration. Under no condition thus far tested has it been possible to produce the more highly branched 12 unit glycogen, generally considered to be the 'normal' glycogen. Glycogens with low-degrees of branching such as those obtained in the present investigations, have in the past been considered 'abnormal'.

Action of ribonuclease and its bearing on structure of yeast ribonucleic acid

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Prostate phosphatase releases from yeast RNA 7 to 8% of its total P as inorganic phosphate without formation of nucleosides. After precipitation of the total P with uranium acetate the supernatants contained negligible amounts of nitrogen, phosphatase action did not lead to an increased consumption of periodate. After digestion with ribonuclease, approximately one half of the total phosphorus of RNA was hydrolysed by phosphatase. The pyrimidine nucleotide groups were practically completely dephosphorylated, the purine nucleotide groups were practically completely preserved as polynucleotides. The uranium supernatant contained no purines. The periodate test showed that 70 to 80% of the pyrimidine groups were liberated as free nucleosides, the rest remained in linkage with the purine polynucleotides. Yeast ribonucleic acid consists of long chains of purine nucleotides linked to long chains of pyrimidine nucleotides. Titration experiments showed that a large part of the alkali uptake of yeast RNA between pH 5 and pH 8.5 originates from groups other than secondary phosphoryl groups. Crystallized ribonuclease does not hydrolyse pancreas RNA. Pancreas contains a second ribonuclease (B) which hydrolyses yeast and pancreas RNA. Its action differs from that of Kunitz's ribonuclease because ribonuclease B hydrolyses the cross-linkages between the purine nucleotide groups.

Isolation of adrenocortical compounds from normal male urine

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Large volumes of pooled, normal male urine were extracted with chloroform both before and after adjustment to pH 1. Further fractionation included recovery of the ketonic fractions, their partitioning between benzene and water and chro-

matography of the acetylated residues. Of the crystalline compounds recovered, one has been shown to be 17-hydroxy 11-dehydrocorticosterone which was isolated largely as the free compound in an over-all yield of 55.3 γ /l and another, the corresponding tetrahydro derivative, 3(α),21-di-acetoxy-17(α)-hydroxypregnanetriol-11,20-dione. Three other crystalline compounds and one non-crystalline substance have been partially characterized. Two of these are regarded as reduction products and the remaining two as possibly physiologically active compounds. These results will be discussed in relation to other relevant data.

Effect of ultraviolet irradiation on certain amino acids KLAUS SCHOCKEN (introduced by H. JENSEN) *Med. Dept. Field Research Lab. Fort Knox, Ky.*

The degree of decomposition of the following amino acids: phenylalanine, tyrosine, dihydroxyphenylalanine, histidine and tryptophane by ultraviolet irradiation has been studied quantitatively. The buffered amino acid solutions were irradiated with the unfiltered light of a Hanovia quartz mercury lamp at 30 cm. distance in test tubes of about 1 in. diameter, which were placed in a water bath. The degree of conversion of benzoic acid to hydroxybenzoic acid, of phenylalanine to tyrosine, and of tyrosine to dopa by ultraviolet irradiation has been determined. The significance of these findings will be discussed. Histidine has been found to be very sensitive to ultraviolet irradiation and was therefore used to study the effect of certain compounds which were expected to inhibit the decomposition effect of ultraviolet irradiation. Cysteine, cystine, homocystine and methionine were found to exert a pronounced inhibiting response. In addition, experiments on the influence of compounds which were expected to accelerate the irradiation effect have been carried out.

Precursors of purines MARTIN P. SCHULMAN,* JOHN M. BUCHANAN AND CHARLES S. MILLER* *Dept. Physiological Chemistry, School of Medicine, Univ. of Pennsylvania, Philadelphia*

There is appreciable disappearance of C^{14} -labeled 4-amino-5-imidazole carboxamide (synthesized by method of Miller and Gurin) when incubated with pigeon liver homogenates. When carrier hypoxanthine was added at the experiment's conclusion, radioactivity was found in the isolated hypoxanthine in quantities indicating that most of the disappearing carboxamide was converted to hypoxanthine. The hypoxanthine was precipitated by ammoniacal silver nitrate and purified by 3 passages through a starch column (butanol-water). Since rigorous purine characterization is necessary, further attempts are being made to separate the radioactivity from the purified hypoxanthine. Nevertheless hypoxanthine

contaminated with C^{14} -carboxamide in control experiments may be freed of radioactivity by the above procedure. Furthermore, radioactive uric acid was excreted by a pigeon administered C^{14} -carboxamide. Inappreciable isotope concentration was present in the respiratory CO_2 . Further experiments have shown that $NH_2CH_2C^{14}OOH$ and $HC^{14}OOH$ participate in hypoxanthine synthesis *in vitro* at a rate comparable to C^{14} -carboxamide utilization. A previous report (Sonne, Buchanan, Delluva) that acetate is involved in uric acid synthesis has not been substantiated. $CH_3C^{14}OOH$, synthesized from $C^{14}O_2$, failed to be incorporated into the purine ureide groups when administered to a pigeon or added to pigeon liver homogenates. When acetate was synthesized from isotopic $NaCN$ and $(CH_3)_2SO_4$, formic acid was a significant by-product and was undoubtedly responsible for the appearance of isotope in the ureide carbons of uric acid in the earlier experiments with C^{12} -acetate.

Nature of the hepatic protein lost during a 2-day fast JULIUS SCHULTZ* AND HARRY M. VARS *Harrison Dept. of Surgical Research, School of Medicine, Univ. of Pennsylvania, Philadelphia*

The total heat-coagulable protein of the livers of fed, 24-hour and 48-hour fasted rats were analysed for nitrogen, ash, glycogen, total-S, cystine-S, phosphorus and purine-N. In terms of the liver of a 'standard' 200-gm. rat, it was found that during the first 24 hours of fasting there was a loss of 268 mg. protein, 30 mg. nucleic acid, 3.6 mg. total-S and 0.4 mg. cystine-S. During the second 24 hours of fasting there was lost 183 mg., 5 mg., 2.2 mg., and 1.2 mg. respectively of the above constituents. This indicates that the proteins lost from the liver during the first day of fast contains little cystine-S while that lost during the second day contains much more. The nucleic acids lost during the first day were greater than that lost later. These data suggest that the composition of the 'labile' proteins of the liver may differ from that of the more metabolically 'stable' proteins.

Preparation of a soluble, purified succinic dehydrogenase CHARLES R. SCOTT (introduced by ELMER STOTZ) *Dept. Biochemistry, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

Succinic dehydrogenase, defined by its ability to catalyze the reduction of an electromotively active dye by succinate, has been prepared from pig heart muscle extract in a form which is not sedimented by centrifugation at 20,000 g . The rate of reduction of sodium 2,6-dichlorobenzenonendo-3-chlorophenol, measured aerobically and within a two-minute reaction period, was used for enzyme assay. The typical buffer extract of heart muscle is fractionated with ammonium sulfate in the presence of cholate, the appropriate fraction

subjected to partial heat denaturation, followed by further ammonium sulfate fractionation. The final clear, yellow solution contains the enzyme with some 20-fold purification over the heart muscle extract. It contains less than 0.04% cholate and some ammonium sulfate. The enzyme is precipitated by dialysis against water or buffer, but the activity is retained in a clear solution when dialyzed against strong ammonium sulfate solutions. The final preparation is free of cytochrome oxidase and cytochrome *c*, but shows a weak band at 560 m μ after dithionite reduction. Parallel studies of hemin content and enzyme activity at various stages of purification do not indicate any necessity of a hemin for succinic dehydrogenase activity. The apparently low flavin content has not yet justified a similar study of flavin and enzyme activity. The preparation no longer catalyzes the reduction of cytochrome *c* by succinate unless another heart muscle fraction is added.

Amino acids in two tuberculin protein fractions

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Henry Phipps Inst. Univ. of Pennsylvania, Philadelphia, and Dept. of Chemistry, Univ. of Birmingham, England

Protein fractions with different physico-chemical and biological properties have been isolated previously from unheated culture filtrates of a number of different strains of tubercle bacilli grown upon a non-protein synthetic medium. Two of these fractions with the most different properties, designated as A and C fractions, were chosen for a preliminary study of their amino acid composition. By the paper chromatographic method at least 10 amino acids and also one or two unidentified spots, possibly di- or tri-peptides, were identified in both A and C fractions. Quantitative chemical analyses of each fraction showed more tryptophan by the Bates' method, and more phenolic groups, by the Folin reagent, in unhydrolyzed samples of the A than of the C fraction. On hydrolysis, however, still more phenolic groups appeared in the A fractions, but not in the C fractions, indicating that some of the phenolic groups in the A fractions are inaccessible until released by hydrolysis. In analogy with work by many investigators on other physiologically active proteins, such as pepsin, papain, insulin, viruses, etc., it is interesting that this tuberculo-protein A fraction, which contains some of these apparently combined phenolic groups, is also the most potent fraction isolated, in regard to tuberculin activity and antigenicity. It is also the least chromogenic one, as well as the least stable in regard to solubility properties. It is, furthermore, from the A fraction that it is most difficult to separate carbohydrate, and while some carbohydrate can be easily removed, it is not yet certain how much may actually be strongly combined with the protein.

Effect of carbon dioxide on rate of denitrogenation in human subjects JULIUS SENDROY, JR. AND RODOLFO MARGARIA* *Naval Med. Research Inst., Bethesda, Md.*

Following exposure to pressure at 2 atmospheres (absolute) for 4 hours, the elimination of N₂ from the body has been measured in human subjects over periods of from 10^{a-b} minutes to 50-120 minutes of inhalation of an N₂-free gas mixture. The N₂ elimination over such periods following decompression, may be described as the resultant of simultaneous processes, the rate of which may be expressed as a function of time, by the sum of exponential terms of the type $Y = 10^{a-bt}$, of which 2 are given special consideration. On breathing a 3% CO₂ - O₂ mixture, the rate is not appreciably altered. A 5% CO₂ - O₂ mixture causes an increase of 70% in the velocity constant of the fast rate component, while it may slightly decrease the speed of the slow process. An increase of ventilation per se has no effect on the N₂ exchange over the time interval studied as is shown when the subject inhales a low CO₂ mixture (3%), or when the ventilation is increased by the introduction of a respiratory dead space. The effect of the 5% CO₂ mixture is attributed to an increased blood flow through some areas (watery tissues, muscles etc.) and to a decrease in others (fatty tissues). On inhalation of such a mixture, during the first 30 minutes of denitrogenation, 20% more N₂ is given off than when pure O₂ is breathed. It is suggested that CO₂ may possibly be effective in diminishing the incidence of decompression sickness in divers.

Blood volume of the rat measured by the tagged-erythrocyte dilution method L. M. SHARPE,* G. G. CULBRETH* AND J. R. KLEIN *Biology Dept. Brookhaven Natl. Lab., Upton, N. Y.*

Male rats of the Sprague-Dawley strain, weighing from 260 to 340 gm., were injected, via the superficial epigastric vein, with 0.5 ml. of fresh rat blood the cells of which had been tagged with Fe⁵⁹. After 1-2 hours the animals were again anesthetized and bled maximally from the aorta. From the radioactivities of donor and recipients blood, the average blood cell volume and its standard deviation were calculated to be 2.35 ± 0.18 ml./100 gm. body weight. The corresponding average blood volume and its deviation, estimated from the cell volumes and hematocrit, were 4.83 ± 0.31 ml./100 gm. The blood volume found is markedly less than most derived from plasma-labeling, but agrees well with that found in another strain of rat (BERLIN ET AL. *Proc. Soc. Exper. Biol. & Med.* 71, 1949) by means of cell-labeling.

C¹⁴-lactic acid as acetylating agent in intact rat and in rat liver and kidney slices WALTON W. SHREEVE (introduced by JOHN A. MUNTZ)

Dept of Biochemistry, School of Medicine, Western Reserve Univ, Cleveland, Ohio

Conversion of C^{14} α -labeled lactate to acetyl groups was studied in normal rats and in rat liver and kidney slices. Phenylaminobutyric acid was fed, or incubated *in vitro*, together with the labeled compound. The resulting acetylated foreign amine was isolated from the urine or incubation medium, the acetyl group split off, and the isotope content of its individual carbons determined. Entrance of α -labeled lactate (via pyruvate) into reactions of the Krebs cycle should generate pyruvate labeled to a considerable extent in the β as well as the α carbon. This expectation is based on the finding that after feeding α -labeled lactate, carbons 1 and 6 of the glucose derived from rat liver glycogen contain $\frac{1}{2}$ — $\frac{1}{3}$ as much isotope as found in carbons 2 and 5 (LORBER ET AL *Am J Physiol* 155 452, 1948). Of the isotope present in the acetyl groups isolated from the whole animal and the kidney slices, on the other hand, only 10% occurred in the methyl carbon. Acetylation by liver slices was negligible compared with kidney. From these findings it may be inferred that kidney is a main site of occurrence of the acetylation reaction under study, and that in this organ the major fraction of the isotope entering the acetyl groups from lactate does so without prior passage through the Krebs cycle, in contrast to the pathway from lactate to liver glycogen.

Reversible inhibition of the clotting of fibrinogen

SIDNEY SHULMAN* AND JOHN D. FERRY *Dept of Chemistry, Univ of Wisconsin, Madison*

The conversion of bovine fibrinogen to fibrin by thrombin can be markedly retarded by a number of reagents at moderate concentrations (2–5%). The normal clotting time (about 12 min at pH 6.3, ionic strength 0.45, 1 unit/ml thrombin) is prolonged to well over 24 hours by a characteristic minimum concentration of each reagent. These inhibitors can be classified in 3 groups: 1) neutral molecules, 2) anions and 3) cations. Their characteristic functional groups are hydroxyl, amino, sulfonic acid and sulfhydryl, but the whole molecular arrangement seems to be significant, since many of the inhibitors are closely related structurally, while certain other compounds, quite similar in structure, fail to produce inhibition. The inhibition can be shown to be reversible and not due to any permanent protein damage. Normal clotting results from dialysis of inhibited mixtures, furthermore, dialysis of a mixture of one of the proteins with reagent yields a solution that gives the normal clotting behavior with the other protein. Addition of the reagent after the mixing of fibrinogen and thrombin, at any time up to the moment of clotting, also results in reversible inhibition.

Metabolism of N-valeric acid in the intact rat, studied with γ - C^{14} , carboxyl- C^{13} labeled valerate IRWIN SIEGEL (introduced by JOHN A. MUNTZ) *Dept of Biochemistry, School of Medicine, Western Reserve Univ, Cleveland, Ohio*

Solutions of $CH_3C^{14}H_2CH_2CH_2C^{13}OONa$ and glucose were administered to fasted rats by stomach tube. The respiratory CO_2 was collected hourly for 3 hours, at the end of which time the liver glycogen was isolated, hydrolyzed to glucose, and the position of C^{14} and C^{13} in the glucose molecule determined. C^{13} appeared only in carbons 3 and 4, at too high a concentration to be explained by CO_2 fixation alone, while C^{14} was found predominantly and equally in carbons 1, 2, 5 and 6. The respiratory CO_2 contained about twice as much C^{13} as C^{14} , expressed as percentage of isotope administered. These results indicate clearly that the carboxyl and γ carbons of valerate follow different metabolic pathways. Of the various possible mechanisms, the only plausible one which is consistent with the data involves β oxidation to yield $CH_3C^{13}OOH$ and $CH_3C^{14}H_2COOH$. The distribution of isotope in liver glycogen following the feeding of carboxyl-labeled acetate and α -labeled propionate (LIFSON ET AL *J Biol Chem* 176 1263, 1948, LORBER ET AL *Federation Proc* 8 99, 1949) coincides with the patterns observed in the present study. The report by Atchley (*J Biol Chem* 176 123, 1948), who identified propionate and acetate in a kidney enzyme preparation incubated with valerate, is in harmony with the present results obtained in the intact animal.

Biological formation of formate and formaldehyde from methyl compounds in liver slices PHILIP SIEKEVITZ* AND DAVID M. GREENBERG *Division of Biochemistry, Univ of California Med School, San Francisco*

The formation of formate and formaldehyde was studied with C^{14} -labeled compounds. The radioactive compounds were isolated by the carrier method. The formate was determined by steam distilling the compound and oxidizing it to CO_2 with mercuric oxide, the formaldehyde was isolated by steam distillation into dimedon solution and recrystallization of the resulting methylenebis methone to constant specific activity. The methyl group of methionine and choline have been found to give rise to formate and to formaldehyde and can serve as a source of the β -carbon of serine. These reactions are inhibited by ethionine. Intraperitoneal injection of ethionine inhibits the *in vitro* production of formate from glycine but not the synthesis of serine from glycine. The α -carbon of glycine and the β -carbon of serine have been found to form both formaldehyde and formate. Formate production from the above 4 compounds,

except for choline, was greater in air than in nitrogen. With the exception of serine, formaldehyde production was higher in air than in nitrogen. The amount of labeled formaldehyde obtained was small compared to the formate formed.

Synthesis of some phosphate fractions in the threonine-deficient rat S A SINGAL, V P SYDENSTRICKER,* JULIA M LITTLEJOHN,* H T HAYES* AND S J HAZAN* *Univ of Georgia School of Medicine, Augusta*

In the attempt to determine the specific biochemical defect responsible for the fatty livers in rats resulting from threonine deficiency (SINGAL, SYDENSTRICKER AND LITTLEJOHN *Federation Proc* 8 251, 1949) radiophosphorus has been employed in the study of the turnover of inorganic, acid soluble, phospholipid and nucleoprotein phosphorus fractions in the liver, kidney and small intestine of the deficient and normal rat. The relative specific activity of the phospholipid and nucleoprotein fractions of the kidney and small intestine is not significantly different in the deficient and normal animal. The administration of a single dose of 0.2 mM DL-threonine does not alter the activities. In the liver the phospholipid activity is similarly unaffected by either threonine deficiency or by supplementary threonine. In the nucleoprotein fraction, however, the relative specific activity is significantly depressed 30% in the deficient rat. The administration of a single dose of threonine increases the uptake 26%. In the rat receiving an adequate quantity of the amino acid in the diet, supplementary threonine is without effect on the uptake of radiophosphorus.

Isolation of L-amino acid oxidase from moccasin venom THOMAS P SINGER, EDNA B KEARNEY* AND EVELYN A HEISLER* *Dept of Biochemistry, Western Reserve Univ School of Medicine, Cleveland, Ohio*

The preparation of the L-amino acid oxidase of moccasin venom involves the use of $\text{Ca}_3(\text{PO}_4)_2$ gels under rigorously controlled temperature, pH, and ionic strength. Impurities are removed by heating to 73° C for 5 minutes in the presence of L-leucine, followed by adsorption of inert proteins on $\text{Ca}_3(\text{PO}_4)_2$ gel at pH 6. The enzyme is next adsorbed at its isoelectric point on $\text{Ca}_3(\text{PO}_4)_2$ gel and the latter is washed with acetate buffer. The enzyme is eluted with 0.65 saturated $(\text{NH}_4)_2\text{SO}_4$ at its isoelectric point and precipitated by further addition of $(\text{NH}_4)_2\text{SO}_4$. In this manner, the oxidase is obtained in nearly pure form in good yield in a few hours working time. The purity of the enzyme has been studied by electrophoresis, diffusion, solubility measurements and in the ultracentrifuge. The absorption spectrum is characteristic of flavoenzymes, with maxima at 273, 389, and 465 m μ . These peaks are bleached in the presence

of L-leucine, even under aerobic conditions. The prosthetic group has been separated and identified as FAD by its absorption spectrum, by its fluorescence before and after hydrolysis with nucleotide pyrophosphatase or CCl_3COOH , by the quantitative measurement of the adenosine-5-phosphate content following enzymatic hydrolysis, and by the demonstration of the presence of adenine by paper chromatography. The prosthetic group quantitatively replaces FAD in the D-amino acid oxidase test, and it activates cytochrome reductase, a flavin mononucleotide enzyme, only after hydrolysis by nucleotide pyrophosphatase.

An inter-relationship between arginine and proline in metabolism of *Leuconostoc mesenteroides* P-60 R J SIRNY,* L T CHENG* AND C A ELVEHJEM *Dept of Biochemistry, Univ of Wisconsin College of Agriculture, Madison*

Investigations into possible causes of the marked lag observed in the standard curve response to proline under the routine conditions of microbiological analysis with the Henderson-Snell uniform medium (*J Biol Chem* 172 15, 1948) have revealed an unusually high requirement for arginine when proline is limiting and vice versa, in *Leuconostoc mesenteroides* P-60. The unmodified medium normally contains 400 μg L-arginine HCl and 200 μg L-proline in 2 ml, though 20 μg L-arginine and 10 μg L-proline are sufficient to give near maximal growth when one is made limiting with respect to the normal excessive amount of the other. However, when the level of L-arginine is lowered to 20 μg , no growth occurs with 10 μg L-proline. Maximal growth is not obtained until levels of 400 μg L-arginine are approached. Similarly, when L-arginine is held at 20 μg , growth increases progressively until levels somewhat above 200 μg proline are reached. This inter-relationship is not found with any of the other 16 amino acids when the low level of proline is used. Studies on the possibility of inter conversion of these amino acids have thus far shown that ornithine is ineffective in replacing arginine for the organism's needs with limiting proline. Further studies on the mechanism of this interrelationship are in progress.

Purine ribose nucleotide inhibition of desoxyribonucleic acid utilization by *Lactobacillus bifidus* HELEN R SKEGGS,* LEMUEL D WRIGHT, KATHERINE VALENTIK,* HELGA NEPPLE* AND JOHN SPIZIZEN* *Med Research Division, Sharp & Dohme, Glenolden, Pa*

The ability of ribonucleic acid (RNA) specifically to inhibit the utilization of desoxyribonucleic acid (DNA) by *Lactobacillus bifidus* (*Lactobacillus acidophilus* ATCC #4963) was described by Skeggs *et al* (*J Am Chem Soc* In press). RNA, degraded by alkaline hydrolysis, retained

its inhibitory ability. Further investigations have been made to determine whether any of the known products of RNA degradation could substitute for RNA in inhibiting the utilization of DNA by *L. bifidus*. The purines and pyrimidines, ribose nucleosides, uridylic acid and cytidylic acid have no effect on the ability of *L. bifidus* to utilize DNA. The purine ribose nucleotides, adenylic acid (adenosine-3-phosphoric acid or adenosine-5-phosphoric acid) and guanylic acid, competitively inhibit the utilization of DNA by the organism. The effects of the purine nucleotides can be demonstrated either by the test tube assay procedure previously described or by filter disk plate assay techniques. When vitamin B₁₂ is substituted for DNA in the nutrition of *L. bifidus* the effects of the purine nucleotides cannot readily be demonstrated.

Cytochrome c reductase (DPN) of heart muscle particles E. C. SLATER (introduced by S. OCHOA) *Molteno Inst., Univ. of Cambridge, England*

The aerobic oxidation of reduced DPN (DPNH₂) by Keilin and Hartree's heart muscle preparation was completely inactivated by treatment with 2,3-dimercaptopropanol (BAL) in air. The oxidation was restored by the addition of methylene blue. The reduction of cytochrome *C* by DPNH₂ in the presence of heart muscle preparation and cyanide was also inactivated by BAL-treatment. Spectroscopic studies indicated that cytochrome *b* was not on the main pathway for the aerobic oxidation of DPNH₂ but is concerned in a slow anaerobic oxidation of DPNH₂ by fumarate. This reaction is also inhibited by BAL. It is concluded that the cytochrome *c* reductase (DPN) on the heart muscle particles contains at least two components, one of which (diaphorase) is unaffected by BAL and the other is the BAL-labile factor previously found in the succinic oxidase system, acting between cytochromes *b* and *c*. The endogenous cytochrome *c* on the heart muscle particles must be reduced by DPNH₂ or succinate at 1200 times the rate at which the same concentration of added cytochrome *c* is reduced (in the presence of cyanide) in order to account for the observed rate of aerobic oxidation in the absence of added cytochrome *c* or cyanide.

Mechanism of anticonvulsant action of 2-diethyl, 1,3-propanediol I. H. SLATER, D. E. LEARY AND J. F. O'LEARY (introduced by S. W. CLAUSEN) *Dept. of Pharmacology and Toxicology and Dept. of Surgery, Division of Orthopedics, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

It has been suggested that monoethers and substituted ketals of glycerol which cause flaccid paralysis without loss of consciousness inhibit

multisynaptic pathways, particularly in the spinal cord. These agents are effective in the protection of animals from death from metrazol and strychnine convulsions. More recently, Berger has reported that 2-diethyl, 1,3-propanediol (DEP) appears to be an unusually effective agent for the protection of mice from metrazol and strychnine convulsions. We have extended these studies to larger animals. Cats anesthetized with dial given intravenous doses of 50 mg/kg of DEP did not convulse after large doses of either strychnine or metrazol. Conversely, when convulsions were induced in such cats with these drugs, it was possible to control the seizures with DEP. Similar observations were made after intraperitoneal injection in unanesthetized cats and rabbits. In cats anesthetized with dial, the i.v. administration of DEP in doses as high as 153 mg/kg caused no change in the knee jerk or in the response of a muscle to nerve stimulation. Doses of 50 mg/kg intravenously caused a distinct transient decrease in the amplitude of the flexor reflex response, a finding indicative of inhibition of multineurone reflex arcs in the spinal cord. Rapid i.e. injection of DEP was followed by slowing of the heart rate, a drop in mean arterial blood pressure and a slowing or cessation of respiration, findings more marked in ether than in dial anesthesia. After fatal doses, circulation continued several minutes after cessation of respiration.

Competitive effects of substrates for hexokinase MILTON W. SLEIN (introduced by C. F. CORI) *Dept. of Biological Chemistry, Washington Univ. School of Medicine, St. Louis, Mo.*

The question has been investigated whether yeast and brain hexokinase have one or more active centers for the utilization of the 3 fermentable hexoses. The order of affinity for the yeast enzyme (from determinations of K_s , the substrate-enzyme dissociation constant) was mannose > glucose > fructose and this was also the order of mutual inhibition of utilization of these sugars from mixtures. For the pair glucose-mannose it was shown that K_i mannose was the same as K_s mannose and K_i glucose the same as K_s glucose, indicating that they act as substrates and inhibitors at the same center of the enzyme. With brain or yeast hexokinase there was nearly 100% inhibition of fructose utilization by an equimolar concentration of either glucose or mannose, while fructose had little inhibitory effect on the utilization of the aldo sugars. This strong inhibition is largely explained by the fact that the concentration of fructofuranose (which is the form with which the enzyme reacts) is only a small fraction of the total fructose concentration (roughly 20% in the above experiments). The predominant hexokinase in brain appears to be of the yeast type and the competitive effects of substrates

described above would explain why mannose but not fructose causes an immediate relief of hypoglycemia. Sugars which are not utilized by yeast or brain hexokinase (e.g. galactose) do not act as inhibitors. The fructokinase of muscle (Federation Proc 6 245, 1947) which does not act on glucose is not inhibited by this sugar. Enzymes which act on fructose-1-phosphate (probably the primary phosphorylation product of fructose by liver fructokinase) have been found in liver and muscle but not in brain.

Effect of diet upon urinary amylase excretion

BENJAMIN W SMITH* AND JOSEPH H ROE
*Dept of Biochemistry, George Washington Univ
School of Medicine, Washington, D C*

The effect of fasting and of diets high in carbohydrate, protein, fat, purine, and alkaline residue, upon the urinary amylase excretion was determined, using 156 medical students as subjects. A control 24-hour sample of urine with the subjects on their usual diet was collected. The subjects were then placed upon the experimental diet for 4 days and urine was collected during the final 24-hour period. The amylase content of the urine samples was determined by the method of Smith and Roe (*J Biol Chem* 170 53, 1949). Urinary nitrogen values were determined as a control upon the dietary intake. Statistical analysis of the data showed a highly significant decrease in the urinary amylase excretion on the high carbohydrate and alkaline residue diets and an increase of borderline significance on the high protein diets ($P = 0.13$) and on fasting ($P = 0.06$). These data suggest that the metabolism of carbohydrates decreases, and of proteins increases, the excretion of amylase in the urine.

Partition chromatography of the vitamin B₁₂ group of factors

E LESTER SMITH, W F J CUTHBERTSON, A WALKER AND K A LEES
(introduced by F S DAFT) *Research Division,
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Fermentation Division, Glaxo Labs, Barnard
Castle, Co Durham, England*

We first obtained evidence of a second red clinically active anti-pernicious anaemia factor by partition chromatography of purified liver extracts with *n* butanol on columns of moist silica or starch. Subsequently we used strips or sheets of filter paper in place of the columns, following the separation of the red factors and coloured impurities visually, then (independently of WINSTEN AND EIGEN *J Biol Chem* 177 989, 1949) we developed an ultra-micro method whereby the factors were located by the 'zones of exhibition' induced on sheets of nutrient agar seeded with *L lactis* Dorner after contact with the developed strips and incubation. With this technique liver extracts and fermentation liquors gave 2 or 3 zones

of strong growth, and, further down the strip, one or more zones of diffuse growth. The substances responsible were tentatively identified by comparisons of R_f values with those of reference substances, using as solvents a range of higher alcohols and ketones. The order of separation with *n* butanol was (from slow to fast) vitamin B_{12b}, vitamin B₁₂ (giving dense zones), the desoxyribosides of cytosine, guanine, hypoxanthine, adenine, thymine (giving diffuse growth). By the visual technique, early samples of our own and of American vitamin B₁₂ gave a small slow-moving spot besides the main spot, but more highly purified English and American samples gave only single spots. By the ultra-micro technique, however, even these pure vitamin B₁₂ samples gave 2 (sometimes 3) zones on the agar, due to artefacts produced (presumably by oxidation) on the paper.

Solubilization and purification of cytochrome oxidase from heart muscle extract

E LUCILE SMITH* AND ELMER STOTZ *Dept of Biochemistry, Univ of Rochester School of Medicine and Dentistry, Rochester, N Y*

Cytochrome oxidase in heart muscle extract, commonly considered insoluble or associated with insoluble particulate material, has been prepared in a form which is not sedimented at 20,000 *g*. Pig heart muscle extract containing 0.8% sodium cholate was digested with crude trypsin or a fraction of fresh pancreas under carefully controlled conditions. This process increased the solubility of the oxidase, and the digest was subject to a fairly sharp fractionation of the enzyme with ammonium sulfate. The appropriate fraction was a clear brownish solution which had an oxidase purity about 7 times greater than the extract. The preparation contained approximately 3% ammonium sulfate and 0.5% sodium cholate. A further purification was effected by diluting the preparation to decrease the concentration of cholate and ammonium sulfate, then dissolving the precipitate formed in a mixture of ammonium sulfate and cholate. The cholate can be removed by dialysis against strong ammonium sulfate solution, and the enzyme remains in solution. The presence of ammonium sulfate was found to increase the solubility of cytochrome oxidase in the digested preparations, but not in the undigested heart extract.

Decomposition of chloramphenicol (chloromycetin) by bacteria

GRANT N SMITH AND CECILIA S WORREL (introduced by O D BIRD) *Research Labs, Parke, Davis & Company, Detroit, Mich*

An attempt has been made to determine the steps involved in the decomposition of chloramphenicol by bacteria sensitive to the drug using paper chromatography and chemical tests. The data from these studies indicates that there are

several possible pathways by means of which chloramphenicol can be degraded to compounds which no longer exhibit the strong antibiotic properties of the parent compound. At least 2 points of attack on the molecule are known with certainty. The aromatic nitro group may be reduced to an amine group or the amide linkage may be hydrolyzed with the formation of dichloroacetic acid and D(-)-threo 1-p-nitrophenyl-2-amino-1,3-propanediol. The order in which these reactions occur is determined by the individual organism. In the case of *E. coli*, the predominate reaction is the reduction of the nitro group of chloramphenicol to an aryl amine group. The amide linkage is then hydrolyzed with the formation of dichloroacetic acid and 1-p-aminophenyl-2-amino-1,3-propanediol. With other organisms such as *B. subtilis*, the chloramphenicol is hydrolyzed first to dichloroacetic acid and 1-p-nitrophenyl-2-amino-1,3-propanediol. The latter compound can then be reduced to 1-p-aminophenyl-2-amino-1,3-propanediol. Once the nitro group has been reduced to an aryl amine group no further change in this group appears to take place. No evidence could be obtained that the aryl amine group could be acetylated in the presence of ATP and acetate.

Proteolytic activity of rabbit muscle JOHN E. SNOKE* AND HANS NEURATH *Dept. of Biochemistry, Duke Univ. School of Medicine, Durham, N. C.*

Dilute KCl extracts of ground striated rabbit muscle exhibit proteolytic activity when tested against denatured hemoglobin as substrate. Activities were determined by incubating the extracts with the substrate for 1 hour at 35° and measuring the optical density of the supernatant solution at 280 mμ, after precipitation of the proteins by trichloroacetic acid. The proteolytic activity exhibits maximum stability at pH 6.0 and a sharp pH optimum at pH 4.0. Ferrous ions (0.04 M) cause a 5-fold increase in activity, manganese and magnesium being less effective in this respect. Zinc and barium ions, or cysteine, produce no activation. The amount of hemoglobin hydrolyzed is linear with time and the rate is proportional to the amount of extract used. Partial purification, with a concomitant increase in proteolytic activity, has been achieved by a process involving partial denaturation of the inactive proteins at 35° and fractional precipitation with trichloroacetate at pH 4. Active fractions thus prepared were, per mg. nitrogen, approximately 1/200 times as active at pH 4 as crystalline trypsin at pH 7.5.

Effect of advancing age on the cholesterol concentration of blood serum WARREN M. SPERRY AND MERRILL WEBB* *Dept. of Biochemistry, New York State Psychiatric Inst., New York City*
Recent reports of a marked increase in average

serum cholesterol concentration with advancing age (KELES, A. *Federation Proc.* 8: 523, 1949; GRAM, M. R. AND R. M. LEVERTON *Federation Proc.* 8: 384, 1949) are inconsistent with the hypothesis (SPERRY, W. M. *J. Biol. Chem.* 117: 391, 1937) that each individual maintains his serum cholesterol at a constitutional level from which large deviations do not ordinarily occur. Therefore, the cholesterol concentration of the serum was redetermined in most of the subjects of the investigation which led to this hypothesis, and which was carried out 13-15 years ago. In over half of the men no appreciable change in either direction from the earlier level was found. But in the remaining men and all but one of the women there were fairly substantial increases, ranging from about 10-30%. The average of the concentrations found in men in 1949 was 5.4% higher than the average obtained in the same subjects in 1934 to 1936, in women the corresponding value was 22.3%. The findings show that in some persons the serum cholesterol concentration is not maintained at as constant a level as had been thought, but they also indicate that an increase with advancing age is not obligatory. Changes in the method of determining cholesterol will be discussed in relation to the findings.

Studies of cellular calcifying mechanism ALBERT E. SOBEL, ALBERT HANOK* AND ALEXANDER WOLFFE* *Jewish Hospital of Brooklyn, Brooklyn, N. Y.*

Calcification *in vitro* of rachitic bones was used to further investigate the cellular mechanism of mineralization. Shaking rachitic rat sections with a solution containing 150 mEq/l of calcium chloride plus one of the following gave complete or almost complete inhibition of calcification, at a concentration of 2.5 mM/l of calcium and 0.161 mM/l of phosphate: 1) 0.1 mEq/l of beryllium chloride, 2) 0.5 mEq/l of cupric chloride, 3) 10.0 mEq/l of magnesium chloride, 4) 50.0 mEq/l of sodium chloride, 5) 100.0 mEq/l of strontium chloride, 6) 400.0 mEq/l of potassium chloride. Subsequent shaking with a solution containing 150 mEq/l of calcium chloride alone not only restored the calcifying power of the cells but increased the degree of calcification obtained under similar conditions before treatment of the sections. The inhibition due to beryllium was prevented by nickel chloride (5.0 mEq/l) and that of sodium by potassium chloride (5.0 mEq/l). Lower or higher concentrations of nickel and potassium were not as effective. The degree of inhibition of calcification appears to be a function of the ratio of calcium to the inhibitor indicating that this phenomenon obeys the Law of Mass Action.

Quantitative analysis of N¹⁵-labeled amino acids HERBERT A. SOBER,* MAX BRENNER* AND

JULIUS WHITE *Natl Cancer Inst, Natl Insts of Health, Bethesda, Md*

The investigation of nitrogen metabolism using N^{15} -labeled amino acids often depends on the isolation of pure amino acid fractions. A chromatographic procedure is described for the separation of some amino acids so that quantitative determinations of both the amino acid and isotope content may be performed. The procedure was designed for the determination of the amino acids in tissues. Conditions are described for the analysis of a mixture (ca 30 mg of nitrogen) consisting of aspartic acid, glutamic acid, glycine, alanine, urea and ammonia. The mixture is developed on a cation exchanger in the acid cycle (Dowex 50, 200-500 mesh), with increasing concentration of HCl. The effluent is divided into a regular series of small fractions (2 ml) with the aid of a fraction cutter. The constituent or constituents of each small fraction is determined by paper chromatography and the fractions combined accordingly. The combined fractions are analyzed for ammonia, nitrogen, etc and then analyzed for isotope content in the Consolidated Nier Mass Spectrometer. It is essential that at least a part of an amino acid appears uncontaminated with the component following it. The relative amounts of each constituent in a mixed fraction is determined by calculation using simultaneous equations containing the isotope content of each pure fraction. Recoveries of the components of synthetic mixtures have been obtained within 10% of theory. The application of this technique to the determination of free amino acids in tissue dialysates will be presented.

Effect of niacin on tryptophan requirement of the rat HARRY SPECTOR (introduced by HARRY L FEVOLD) *Nutrition Branch, Quartermaster Food and Container Inst for Armed Forces, Chicago, Ill*

Several investigators have stated that niacin increases the utilization of dietary tryptophan. The possible sparing of tryptophan by niacin has special nutritional significance in heat processed foods since it has been shown (PATTON, HILL AND FOREMAN *Science* 107: 623, 1948) that heat processing of foods in the presence of reducing sugars results in partial destruction of tryptophan. Effects of 3 levels of niacin (0, 2, and 10 mg %) were determined at 4 levels of tryptophan as provided by 6, 9, 12 and 18% of 'vitamin-free' casein. The nitrogen content of all the rations was made equivalent to that of 18% whole casein by the use of acid-hydrolyzed casein. The food intake of trios of rats, representing each level of niacin, was equalized during the first 4 experimental weeks. Five trios of Sprague-Dawley rats whose weights had been previously adjusted to about 66 gm were used at each level of tryptophan. At

all 4 levels of tryptophan the addition of niacin produced no greater gain in weight. The presence of niacin had no significant effect on the 24-hour urinary excretion of apparent-free tryptophan and nitrogen, at each tryptophan level. During the 5th and 6th weeks when all animals were allowed to eat *ad libitum* the addition of niacin, except at the level of 18% casein, resulted in a greater gain in weight associated with an increased food consumption. The significance of these data will be discussed.

Nutritional status of mice and their susceptibility to infection with *Shigella dysenteriae*

EUGENE M SPORN, EDWARD J SCHANTZ AND FRANK B ENGLE, JR (introduced by ALDEN K BOOR) *Camp Detrick, Frederick, Md*

Previous work in this laboratory (SCHANTZ SPORN AND ENGLE, Unpublished data) demonstrated that mice fed a semi-synthetic ration deficient in the vitamin B-complex were more susceptible to infection with *S. dysenteriae* than those fed a nutritionally complete ration. The present phase of the study was undertaken to determine whether individual vitamins were involved in this decreased resistance. Male weanling mice were fed diets lacking various members of the B-complex for extended periods, and then challenged with graded levels of *S. dysenteriae*. Mice fed diets deficient in riboflavin, pyridoxine, niacin, biotin or folic acid did not show any change in susceptibility to this organism. A thiamine deficiency was the only one of the 6 vitamin deficiencies examined which increased the susceptibility of mice. The lethal dose for mice on this deficiency was 1.2×10^5 organisms while the mice on the other rations required a dose of 1.2×10^8 . Experiments were conducted to examine the possibility that this increased susceptibility might be due to the emaciated condition of the mice. Pair fed controls for the thiamine deficient group, as well as mice restricted in food intake to drastically lower their weights, were maintained on a complete ration. There was no indication of an increased susceptibility in these animals. Mice that were fed a diet deficient in all members of the B-complex except thiamine showed no significant change in resistance. From these results, it is believed that thiamine is involved in some mechanism in the resistance of mice to *S. dysenteriae*.

Hormonal control of combination of insulin with isolated muscle W C STADIE, NIELS HAUGAARD* AND JULIAN B MARSH* *John Herr Musser Dept of Research Med, Univ of Pennsylvania, Philadelphia*

We have previously reported (*Am J Med Sc* 218: 265, 275, 1949) that the isolated rat hemidiaphragm, after a short preliminary equilibration in a medium containing insulin, utilizes glucose

and synthesizes glycogen at a greater rate than controls when equilibrated in a subsequent period in a medium containing glucose but no insulin. This was interpreted to mean that a chemical combination of insulin with structural units of the diaphragm takes place rapidly, that this combination is not easily reversed, and that the combined insulin exerts its customary effects on the metabolic pattern of the muscle. Furthermore, hormonal control of this chemical combination was indicated by the diminution of the reaction in the alloxan diabetic rat, and following injection of crude APE or crystalline growth hormone. Further studies on the hormonal aspects of this phenomenon are reported. Experiments on normal and adrenalectomized rats injected with purified growth hormone, ACTH, or adrenal cortical steroids, indicate that there is an interrelation between pituitary and adrenal hormonal factors in the regulation of the combination of insulin with muscle.

Coenzyme A-dependent transacetylation and transphosphorylation EARL R. STADTMAN (introduced by FRITZ LIPMANN) *Biochemical Research Lab, Massachusetts General Hospital, Dept of Biol Chemistry, Harvard Med School, Boston, Mass*

Extracts of *Cl. kluveri* contain a transacetylation enzyme which is measured by the catalysis of interchange of acetyl-bound with inorganic phosphate, using P^{32} (STADTMAN AND BARKER). This enzyme was considerably purified by ammonium sulfate fractionation. After several refractionations the enzyme becomes completely inactive but regains activity with the addition of coenzyme A (Co A). This purified fraction makes synthetic acetyl phosphate available as an acetyl donor to CO A-dependent liver acetyl-acceptor systems (acetoacetate, citrate and acetyl sulfanilamide synthesis). The fraction is free of ATP-acetate reaction. The apparent identity of the acetyl phosphate activating enzyme with the Co A-dependent transacetylation enzyme appears significant for the theory of acetyl transfer and of Co A function. A further surprising Co A-dependence was found with crude, resin-treated *Cl. kluveri* extract with reference to phosphate transfer from acetyl phosphate to propionate. The non-dependence of this reaction on adenylic acid was previously observed by Stadtmann and Barker, who studied this reaction. The Co A-dependent acetyl and phosphoryl-transfer functions appear in different protein fractions.

Preparation of 11-ketosteroids from methyl 3 α -acetoxy- Δ^9 11-cholenate HOMER E. STAEVELY (introduced by O. WINTERSTEINER) *Division of Organic Chemistry, Squibb Inst for Med Research, New Brunswick, N. J.*

Most of the methods for introducing the C_{11} -ketone group start with Δ^{11} 12-steroids. Hicks and Wallis (*J Biol Chem* 162 641, 1946) have reported the preparation of methyl 3 α -acetoxy-11-ketocholanoate from methyl 3 α -acetoxy- Δ^9 11-cholenate. In this paper the results of a similar investigation conducted in 1943 are reported. The semicarbazone of methyl 3 α -acetoxy- Δ^9 11-12-ketocholanoate was reduced by the Wolff-Kishner method to 3 α -hydroxy- Δ^9 11-cholenic acid, purified by chromatographing the crude 3 α -acetoxy methyl ester. After treatment with N-bromacetamide in aqueous acetone the total reaction product was debrominated with zinc-acetic acid. A product was isolated which appeared to be methyl 3 α -acetoxy-11-hydroxy- $\Delta^{8,9}$ -cholenate (Anal Calcd for $C_{27}H_{42}O_5$ C, 72.6, H, 9.5 Found C, 72.5, H, 9.8). This structure was proved by chromic acid oxidation to methyl 3 α -acetoxy- $\Delta^{8,9}$ -11-ketocholanoate (m.p. 145–148°, Anal Calcd for $C_{27}H_{40}O_5$ C, 72.9, H, 9.1 Found C, 72.4, H, 8.9) which had an absorption peak at 252 m μ , ϵ 5600. Only an $\alpha\beta$ -substituted $\alpha\beta$ -unsaturated ketone would absorb in this region. Thus debromination, rather than debromination, took place. When methyl 3 α -acetoxy- Δ^9 11-cholenate was reacted with N-bromacetamide and oxidized prior to treatment with zinc-acetic acid, a normal debromination occurred and methyl 3 α -acetoxy-11-ketocholanoate was isolated (m.p. 127–129°, $[\alpha]_D^{25} + 85^\circ$, Anal Calcd for $C_{27}H_{42}O_5$ C, 72.6, H, 9.5 Found C, 72.3, H, 9.3). Proof for this structure was obtained by hydrolysis of the acetoxy ester, re-esterification with diazomethane and chromic acid oxidation to methyl 3,11-diketocholanoate (m.p. 78–82°, Anal Calcd for $C_{25}H_{38}O_4$ C, 74.6, H, 9.5 Found C, 74.6, H, 9.6). The crude product would not crystallize until seeded with an authentic specimen.

Relation of the labile factor to the 'accelerator effect' of serum MARIO STEFANINI AND WILLIAM H. CROSBY (introduced by W. H. FISHMAN) *Ziskind Labs (Hematology Section) of Joseph H. Pratt and New England Center Hospitals and Dept of Medicine, Tufts College Med School, Boston, Mass*

One or more factors accelerating the conversion of prothrombin have been demonstrated in fresh serum. As some discrepancy exists on their nature and identity, it seems advisable to define them in terms of activity as 'serum accelerator effect'. While studying the relationship of these factors with other known coagulation agents, the concentration of the unconverted labile factor and the prothrombin activity were found inversely proportional to the accelerator effect in thrombin-free oxalated human serum. This was consistently observed in normal subjects and in patients with hemophilia, severe thrombocytopenic purpura or

under Dicumarol treatment Prothrombin activity was determined according to Quick *et al* (*J Lab & Clin Med* 34, 761, 1949), labile factor concentration according to Stefanini (*Am J Clin Path* In press) and accelerator effect of serum according to de Vries *et al* (*Blood* 4 247, 1949) Various amounts of bovine thrombin (from 0.5–10 Iowa units/ml) were then added to human fresh oxalated plasma, deprothrombinized according to Quick and Stefanini (*J Gen Physiol* 32 191, 1948) The serum thus obtained was incubated at 37° C for one hour, to assure complete neutralization of thrombin A marked accelerator effect developed in the sera Again the concentration of the labile factor was found to be inversely proportional to the accelerator activity Similar results were obtained with hemophilic and thrombocytopenic plasmas Our findings indicate that plasma contains the precursors of the agents responsible for the accelerator effect of serum One precursor is probably represented by the labile factor (factor V, plasma Ac globulin) and thrombin is required for its activation

Masking of carboxylate groups in native horse carbonylhemoglobin JACINTO STEINHARDT AND ETHEL M. ZAISER * *Dept of Chemistry, Massachusetts Inst of Technology, Cambridge, Mass*

'Titration curves' of carbonylhemoglobin are characterized by an anomalously high, almost abrupt increase in acid bound between pH 4 and 3, as compared with other proteins Since hemoglobin rapidly denatures in this region and back titration yields an entirely different curve, an effort has been made to determine the true titration curve by measuring pH and acid bound within 3 seconds after instantaneous mixing Data so obtained show only about one-third as much acid bound by carboxylate within this range as when otherwise determined The amount bound rises first very rapidly, and then much more slowly for some hours before attaining a stationary equilibrium value Changes in the increase in acid bound are paralleled, at each pH, by changes in the rate of simultaneous spectrophotometric effects The difference between the 3-second and long-time equilibrium curves reaches a maximum of 0.5 mm/gm by pH 3 It diminishes at lower pH where the liberation of titratable groups, and parallel spectrophotometric changes, proceed so rapidly that both techniques approach the same results Thus, at least 33 carboxylate groups, and probably many more, out of about 80 in this protein, are unavailable for combination with acid at pH below 4, until after exposure to acid (not necessarily below pH 4) has induced other changes The assumption that the liberation of all the unavailable groups is brought about by the binding of 2 protons by 2 of the initially available

carboxylates is shown to account for the observed phenomena

Sulfur-containing amino acids in growth with a 'labile methyl'-free diet containing vitamin B₁₂ JAKOB A. STEKOL, MARY A. BENNETT, KATHRYN WEISS,* PHILIP HALPERN* AND SIDNEY WEISS * *Lankenau Hospital Research Inst and Inst for Cancer Research, Philadelphia, Pa*

We reported previously that 30 days or older rats are able to grow on a diet which was free of all the known 'labile methyl group' donors but which contained vitamin B₁₂ and homocystine (*Abstracts, Am Chem Soc*, 116th meeting, Sept 18, 1949, 55C) The amino acid diet which was used in the above studies was complete with respect to all the essential amino acids, except for methionine, and it contained all the known vitamins, including folic acid and biotin We now find that on this diet, in the presence of vitamin B₁₂, homocystine produces even better growth response than equivalent amounts of homocystine Substitution of homocystine by L- and L-*allo*-cystathionine resulted in cessation of growth, which was resumed as soon as the cystathionines were replaced by homocystine On a diet containing ample methionine as the sole sulfur-amino acid, but which was free of vitamin B₁₂, poor growth was obtained, supplementing the methionine containing diet with vitamin B₁₂ resulted in good growth None of the experimental animals receiving vitamin B₁₂ died of kidney lesions in spite of the complete absence of 'labile methyl group' donors in the diet

Enzymatic synthesis of citric acid JOSEPH R. STERN* AND SEVERO OCHOA *Dept of Pharmacology, New York Univ College of Medicine, New York City*

The synthesis of citrate from oxalacetate, acetate (or acetoacetate), and ATP by enzyme solutions from pigeon liver has been previously reported (*J Biol Chem* 179 491, 1949) Both Lipmann's coenzyme A and Mg⁺⁺ or Mn⁺⁺ are required for activity Citrate, rather than *cis*-aconitate or *d*-isocitrate, is the product of the C₂ + C₄ condensation β -ketovalerate, β -ketocaproate, and β -ketooctanoate, like acetoacetate, can contribute a C₂ fragment for citrate synthesis in pigeon liver Citrate synthesis proceeds through at least 2 steps 1) ATP + acetate \rightarrow 'active' acetate, 2) 'active' acetate + oxalacetate \rightarrow citrate The 'active' acetate is closely related to acetyl phosphate The 'condensing enzyme' catalyzing step 2) is present in large amounts in yeast and in a variety of animal tissues (skeletal muscle, heart, liver, kidney, brain) It can be readily extracted with water and is relatively stable Both enzymes (1) and (2) are present in *E. coli* and *Azotobacter agilis* besides pigeon liver extracts

Preparations have been obtained from *E. coli* which contain a very active enzyme for step (1) but are almost devoid of condensing enzyme. Large amounts of citrate are synthesized when this preparation is combined with condensing enzyme from various sources.

Further studies on fractionation and photometric estimation of urinary estrogens BENJAMIN F. STIMMEL, *Rees-Stealy Med Research Fund, San Diego, Calif*

The hemipthalates of α -estradiol and estriol are quantitatively extractable from their ethyl ether solutions by aqueous potassium carbonate and sodium bicarbonate respectively. The esters produce typical Kober color products in proportion to their estrogen content. Advantage of these observations has been utilized to achieve additional purification of our chromatographic estradiol and estriol fractions (*J. Biol. Chem.* 162:99, 1946) from nonalcoholic contaminants. With the utilization of Girard's reagent T on our estrone chromatographic fraction, this modified procedure constitutes a more rigorous fractionation of the estrogens than can be achieved with our liquid chromatogram alone. The modified procedure is particularly effective in freeing nonpregnancy urinary estrogen residues (especially the estradiol fraction) from occasional purple pigments which cause overestimation of Kober titers. By this procedure comparative Kober and fluorometric (modified Jailer method) estrone, estradiol and estriol titers in human pregnancy urine generally are in good agreement. In nonpregnancy urine the fluorometric titers generally approximate one-third to one-half the corresponding Kober titers. There is evidence that nonestrogenic substances from the hydrolyzed urine are present in sufficient amounts to quench the full fluorescence exhibited by pure estrogens. By this procedure we have observed that the substance accountable for the Kober color product in pooled male urine to which had been added 16-ketoestrone immediately preceding zinc-hydrochloric acid hydrolysis cannot be 16-ketoestradiol nor estriol and is probably estradiol.

Relation of vitamin B_{12b} to vitamin B₁₂ and the biological activities of these compounds E. L. R. STOKSTAD, T. H. JUKES, JOHN A. BROCKMAN,* J. V. PIERCE,* H. P. BROQUIST* *Lederle Labs. Division, American Cyanamid Company, Pearl River, N. Y.*

Vitamin B_{12b} has been isolated from a fermentation product of *Streptomyces aureofaciens* as a red crystalline compound which exhibits different absorption spectra than vitamin B₁₂. The biological activities of vitamin B₁₂ and B_{12b} are the same for *Lactobacillus leichmannii* and for chicks. Catalytic hydrogenation of vitamin B₁₂ yielded

a compound which possessed the same ultraviolet, visible and infra red absorption spectra and the same biological activities for chicks and *L. leichmannii* as vitamin B_{12b}. These biological activities of the vitamin B₁₂ reduction product, described here, are in marked contrast to those of vitamin B₁₂, also produced by the hydrogenation of vitamin B₁₂ (KACZKA, WOLF AND FOLKERS *J. Am. Chem. Soc.* 71:1514, 1949). Vitamin B_{12a} was reported to be approximately 10-30% as active as B₁₂ for *L. leichmannii* and 30% as active for chicks. Evidence has been obtained which indicates that vitamin B₁₂ is involved in the methylation of homocystine to methionine in the chick. Chicks were placed on a ration composed of alcohol extracted soybean protein 25%, glucose 62.5%, cystine 0.2%, dimethylaminoethanol HCl, salts, corn oil plus vitamins A, D, and E, inositol, calcium pantothenate, niacinamide, riboflavin, pyridoxine, thiamine, pteroylglutamic acid, and biotin. On this diet, and in the presence of vitamin B₁₂, both homocystine and methionine were found to give a growth response. In the absence of vitamin B₁₂, only methionine gave a response while homocystine was without effect.

Nitrogen and ash content of soft tissues adjacent to bones and joints L. J. STROBINO (introduced by PAUL B. HAMILTON) *Dept. of Biochemistry, Alfred I. du Pont Inst., Nemours Fdn., Wilmington, Del. and Dept. of Orthopedic Surgery, Univ. of Pennsylvania, Philadelphia*

The present study represents a continuation of the investigations reported by Strobino and Farr (*J. Biol. Chem.* 178:599, 1949), on the nitrogen and ash content of beef and human bones. Analyses of calf bones showed an average of about 4.5% nitrogen and 67% ash on the dry weight. Samples of articular cartilage, tendon, ligament, periosteum and semilunar cartilage, carefully cleaned of extraneous material, were dried to constant weight and analysed for nitrogen and ash. Nitrogen was determined by the gasometric micro-Kjeldahl method of Van Slyke and ash was measured gravimetrically after heating in a muffle furnace at 600°C. Samples of the above tissues were taken from each of 10 calves less than 2 weeks old. All samples were taken from rear extremities between the knee and hoof. Averages of 10 determinations for nitrogen and ash as percentage of dry weight are as follows: articular cartilage, nitrogen 13.7, ash 7.0; tendon, nitrogen 17.1, ash 1.7; ligament, nitrogen 16.7, ash 1.9; periosteum, nitrogen 16.5, ash 1.4; semilunar cartilage, nitrogen 17.0, ash 1.6. As expected, these tissues show a much higher nitrogen and lower ash content than bone. Articular cartilage is intermediary in composition and the other tissues analysed show a remarkable similarity, with a high nitrogen and very low ash content.

Observations on plant amide enzyme system

P K STUMPF (introduced by H A BARKER)
Division of Plant Nutrition, Univ of California, Berkeley

An enzyme has been isolated from sugar pumpkin seedlings which specifically catalyzes the exchange reaction $\text{COOHCHNH}_2\text{CH}_2\text{CH}_2\text{CON}^{14}\text{H}_2 + \text{N}^{14}\text{H}_3 \leftrightarrow \text{COOHCHNH}_2\text{CH}_2\text{CH}_2\text{CON}^{15}\text{H}_2 + \text{N}^{14}\text{H}_3$. Since glutamylhydroxamic acid is formed when NH_2OH is substituted for NH_3 , the rate of its formation was employed for the majority of experiments to be reported. With either N^{14}H_3 or NH_2OH , the components of the system are 1) enzyme protein(s), 2) manganese, and 3) inorganic phosphate or arsenate. Manganese is the specific metal component. Mg, Zn, Cu, Co, Fe, Ba, and Al are ineffective. Furthermore, Zn and Cu are strong inhibitors of the system. The K_m value for Mn is about $2.5 \times 10^{-5} \text{ M}$. While phosphate or arsenate was found to be essential, ATP, ADP, and Co A could not replace them. Arsenate increased the rate of hydroxamic acid formation some 2-3 times above that of the phosphate system. Attempts to detect the accumulation of a glutamylphosphate intermediate in the enzyme reaction mixture in the absence of NH_2OH have failed. Further, glutamic acid, which should accumulate from a hydrolytic breakdown of the hypothetical phosphoryl derivative, could not be detected by paper chromatography in the reaction mixture. Finally, the enzyme is apparently highly specific for glutamine. The following compounds proved inactive: asparagine, glutathione, nicotinamide, pyrrolidone carboxylic acid, and glutamic acid.

Liberation of amino acids and active peptides from raw, heated and vitamin-free casein by tryptic digestion ROYAL A SULLIVAN, WILLIAM E DOWNEY, ELIZABETH K STANTON, ELIZABETH VAN WAGONER AND MARY JANE HANSELL (introduced by L K RIGGS) *Natl Dairy Research Labs, Oakdale, N Y*

The digestion of vitamin-free casein by enzymes has been studied by Pader, Melnick and Oser (*J Biol Chem* 172:763, 1948) after further dry heat treatment of the casein and by Hankes, Riesen, Henderson and Elvehjem (*J Biol Chem* 176:467, 1948) after further moist heat treatment, but in both cases the starting material may have been considerably altered from the protein found in milk. Casein pastes were prepared by acid precipitation of raw skim milk and washed in a supercentrifuge. After 6 days hydrolysis with Wilson's trypsin, it was found that 46% of the amino acids had been liberated from raw casein as compared with 35% from vitamin-free casein and 26% from commercial dried casein. Similarly, hydrolysates prepared from raw casein or from

vitamin-free casein contained only 11% of non-dialyzable nitrogen, while those prepared from commercial dried casein contained 18% of non-dialyzable nitrogen. The non-dialyzed residues were partially hydrolyzed by the addition of fresh trypsin. Microbiological assays of these hydrolysates for 18 amino acids showed distinct differences in the availability of individual amino acids from one type of casein to another. The total weight of available amino acids amounted to 78% for commercial dried casein, 77% for vitamin-free casein and 84% for raw casein.

Nucleotidases of liver MARJORIE A SWANSON (introduced by C ARTOM) *Dept of Biochemistry, Bowman Gray School of Medicine, Winston-Salem, N C*

The specificity of enzymes of the liver which split the nucleotides, especially adenosine triphosphate (ATP) has been investigated. The activity of two fractions of rat liver homogenate, the large granules or mitochondria, and the supernatant solution freed of granules, was tested against 4 substrates, at 5 pH values, both with and without added magnesium or calcium ions. Glycerophosphate and adenosine 3 monophosphate were more actively split by the supernatant than by the granules. The optimum pH was in the acid range. Adenosine-5-monophosphate (A-5-MP) was more actively split by the granules than by the supernatant. The activity was highest at neutral pH. Activity against these 5 substrates was little affected by the added ions. On the other hand, the rate of splitting ATP was about the same for the granules and the supernatant. The greatest activity was around pH 5.5 in the absence of added ions, and between 7.4 and 9 in the presence of Mg^{++} . At pH values above 5 the activity was also increased by Ca^{++} but not nearly to the same extent as by Mg^{++} . By fractional precipitation with $(\text{NH}_4)_2\text{SO}_4$ a precipitate was obtained which split ATP but not A-5-MP. Lysed human red cells also split ATP but not A-5-MP. More detailed studies on the partially purified liver preparation are in progress. From the results reported above, it appears that there is at least one specific enzyme splitting ATP. The effects of Mg^{++} , Ca^{++} and pH are specific for this enzyme and are not due to the action of these factors on other enzymes, such as A-5-MPase or non-specific phosphatases.

A study of the transamination reaction using isotopic nitrogen STUART W TANENBAUM* AND DAVID SHEMIN *Dept of Biochemistry, Columbia Univ, New York City*

An *in vitro* system employing pig heart breis has been used to study both the scope and mechanism of the transamination reaction. The addition of labeled amino acids and α -ketoglutarate to the

above with subsequent isolation and isotopic analysis of the products formed has enabled us to ascertain whether or not transamination has taken place. To serve as a model experiment, L-alanine (1.97% N^{15}) α -ketoglutaric acid, and non-isotopic ammonia in equivalent amounts were incubated with the pig heart breis at 37° at pH 7.4 for one hour. This experiment yielded L-glutamic acid (1.43% N^{15}) with practically the same isotopic content as that of the reisolated alanine (1.70% N^{15}). Samples of ammonia recovered had extremely low isotopic values, being of the order of 0.033% N^{15} . Thus it can be concluded that oxidative deamination coupled with reductive amination involving the transient formation of ammonia had not taken place, and that transamination via a Schiff's base type of intermediate has occurred. Similar experiments were performed with L-leucine as the isotopic amino acid. In this case, while the glutamic acid isolated had only roughly one-third the isotopic content of the recovered leucine, the N^{15} values of ammonia samples were negligible. This then demonstrates that leucine is capable of transamination with α -ketoglutarate. Further experiments with other labeled amino acids in this system are in progress. Using similar techniques, the role of the co-enzyme, pyridoxamine, in this process is also under investigation.

Synthesis of high molecular-weight protein-like substances by chymotrypsin HENRY TAUBER
Natl Insts of Health, Inst of Microbiology,
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U S Marine Hospital, Staten Island, N Y

It was recently found that an insoluble protein-like substance is synthesized when a small amount of chymotrypsin is added to a concentrated solution of Witte peptone. Such synthetic protein-like substances have now been produced from neutralized, protein-free concentrates of peptic digests containing about 45% solids on an ash-free basis of egg albumin, bovine albumin, fibrin and zein. No such action could be observed with crystalline trypsin. When 30 mg of crystalline chymotrypsin (in 4 cc of water) is added to 100 cc of the peptic digest of pH 7.3 and kept at 37°C , the solution turns viscous within a few hours and becomes a solid mass within 6 to 24 hours. With more chymotrypsin the reaction is more rapid. During peptic digestion, toluene is used as the antiseptic. During synthesis, sodium fluoride is employed. The synthetic protein-like substances contain a large number of different amino acids. They contain about 15.5% nitrogen. They are soluble in dilute acids and alkalis and form gelatinous precipitates on removal of these ions by dialysis. They are precipitated by protein precipitants and are insoluble at pH 4.0 to 8.5, with the exception of the zein product which is soluble between 6.2 and 8.5, and in more alkaline solutions.

The zein product is isoelectric close to pH 6.0. The average molecular weights of the synthetic products as determined in the analytical ultracentrifuge are estimated to be in the range 250,000–400,000. The protein-like substances are readily hydrolyzed by crystalline pepsin at pH 1.5, fairly well by crystalline chymotrypsin at pH 7.6, and less readily by crystalline trypsin at pH 7.6.

Specific volumes and molecular weights of D-glyceraldehyde phosphate dehydrogenase and aldolase JOHN FULLER TAYLOR *Dept of Biological Chemistry, Washington Univ School of Medicine, St Louis, Mo*

The partial specific volume, \bar{V} , of a protein is needed in order to calculate molecular weight from sedimentation and diffusion. We have investigated the use of the gradient tube of Linderström-Lang to measure the densities of protein solutions, concentrations of which were determined from dry weights. The requisite quantities of protein are small, the determination of density can be made as sensitive as desired, and labile enzyme proteins can be studied at low temperature to retard denaturation. As a test of the method, \bar{V}_{20} for human oxyhemoglobin was found to be 0.749, for crystalline bovine serum albumin (Armour) 0.734, corresponding to 0.749 and 0.736 found in the literature. For crystalline D-glyceraldehyde phosphate dehydrogenase (1) and aldolase (2) from rabbit muscle, \bar{V}_{20} by this method = 0.737 and 0.740 respectively. \bar{V}_{20} has also been calculated from the amino acid composition of these enzymes (VELICK AND RONZONI *J Biol Chem* 173:627, 1948) using the volume increments of the amino acid residues (COHN ET AL *J Biol Chem* 100:Proc LVIII, 1933). For both, the calculated \bar{V}_{20} = 0.743. The difference in the case of (1) may be related to the presence in the crystalline enzyme of diphosphopyridine nucleotide. Molecular weights calculated with these data are close to 118,000 (1) and 147,000 (2).

Factors affecting glycogen formation in vitro

CHING-TSENG TENG,* F MAROTT SINEX,* AND A BAIRD HASTINGS *Dept of Biological Chemistry, Harvard Med School, Boston, Mass*

The following medium has been used to study glycogen formation by rat liver slices *in vitro* from glucose and/or pyruvate. $K = 110$, $Mg = 20$, $Ca = 10$, $HCO_2 = 40$, $Cl = 130$ mV per liter. When pyruvate was present, it was substituted for an equivalent amount of Cl . A comparison of the amount of glycogen formed when the added substrates consisted of only glucose, only pyruvate, or mixtures of the two (all with equal concentrations of substrate carbon) showed that mixtures of the two substrates resulted in more glycogen than either one alone. The sources of the glycogen carbons were studied using glucose

uniformly labeled with C^{14} , prepared by Dr Martin Gibbs, and pyruvate labeled with C^{14} in the carbonyl carbon, prepared by Dr Manfred L Karnovsky. It was found that with labeled glucose or labeled pyruvate only as substrate, 80% of the resulting glycogen carbons were derived from the added substrate. By comparing experiments with a) labeled glucose plus unlabeled pyruvate and b) unlabeled glucose plus labeled pyruvate, it was found that about 40% of the glycogen carbon was derived from glucose carbon and about 36% from pyruvate carbon. Livers from adrenalectomized animals had an increased capacity to form glycogen from both glucose and pyruvate, whereas livers from diabetic-adrenalectomized animals had a diminished capacity to form glycogen from glucose but not from pyruvate.

Isolation of crystallized acetal phospholipid from brain S J THANNHAUSER, N F BONCODDO* AND G SCHMIDT *Tufts College Med School, Boston, Mass*

Ten pounds of calf brains are dehydrated with acetone and extracted with alcohol at 37°. The vacuum dried extract is extracted with petroleum ether, the dried supernatant is saponified with N NaOH (37°, 5 days). Acetic acid is added to pH 5, the solids are exhaustively washed with acetone and extracted with ether. A dilute methanol solution of the dried extract is precipitated with lead subacetate and ammonia. The supernatant is treated with anion exchanger after removal of the lead. The solution of the dried supernatant in ethanol is precipitated with Reinecke salt and shaken with Ag_2O . The alkaline filtrate is decolorized with charcoal and evaporated after treatment with H_2S . The acetone extracted material is dissolved in methanol, the solution is treated with cation exchanger and dried. The solution of this material in 5 volumes of propanol crystallizes in the refrigerator. Recrystallization from methanol after another treatment with cation exchanger yields 0.4-0.7 gm of acetal phospholipid. M.P. (decomp) 205°, softening 80°, sintering 150° C. 57.4%, H 9.88%, N 3.9%, P 6.75%. 2,4-dinitrophenylhydrazones of the liberated aldehyde M.P. 106-107° (Palmityl aldehyde 109-110°).

Influence of dietary protein on glutamine content of rat tissues HENRY TIGERMAN* AND ROBERT W MACVICAR *Dept of Agricultural Chemistry Research, Oklahoma Agricultural Exper Station, Stillwater*

Replacement of protein (20% casein or soybean sodium proteinate preparation) in the diet of the rat by zein caused a pronounced increase in the glutamine levels observed in liver, lung, kidney, heart, spleen, muscle and brain tissues. Supplementation of the zein containing ration with

tryptophan did not produce any material decrease. Replacement by casein of 50% of the zein, however, reduced the glutamine values to the basal level. In a series of analyses carried out on the tissues the results shown below were obtained (mg glutamine/100 gm fresh tissue).

	CASEIN	ZEIN-CASEIN	ZEIN
Liver	88	94	156
Lung	82	86	165
Kidney	107	111	192
Heart	203	210	297
Spleen	168	165	268
Muscle	170	178	253
Brain	77	82	116

In addition to these results, elevated glutamine levels have been found in tissues of rats which have been starved, fed a ration devoid of protein or an adequate ration containing 0.25% iodinated casein (Protamone). These data suggest a positive correlation between tissue glutamine content and the rate of body protein catabolism.

Effect of pteroylglutamic acid on the incorporation of radioactive glycine in chick liver homogenates JOHN R TOTTER, BARBARA KELLEY,* PAUL L DAY AND RAYMOND R EDWARDS* *Dept of Biochemistry, University of Arkansas School of Medicine, Little Rock, and the Inst of Science and Technology, Fayetteville*

In vitro experiments were conducted with glycine containing C^{14} (approximately 10 microcuries/mg) in the carboxyl group. Livers from chicks which had been maintained for 3½ weeks on a purified diet containing either 0 or 200 γ /100 gm of PGA were used. The procedure of Winnick and associates (*Arch Biochem* 21:235, 1949) for determining the uptake of radioactive glycine was followed. Liver homogenates were incubated with the labeled glycine for 60-90 minutes and the radioactivity of the phospholipid extracts and protein residues were then determined. Phospholipid extracts from liver homogenates of deficient chicks averaged 10.3 counts/minute/mg of phospholipid, while those from positive control birds averaged 21.2 counts/minute/mg. Determinations of the phospholipid phosphorus indicated that the positive control birds had only slightly higher phospholipids than did the deficient chicks. The protein residues showed surprisingly low counts, those from deficient chicks averaging 0.55 counts/minute/mg protein while the positive controls averaged 1.46 counts/minute. The *in vitro* addition of PGA had little effect on the rate of incorporation of radioactive glycine in either phospholipid or protein of the homogenate.

Estimation of blood ergothioneine concentration by the determination of bromine-labile sulfur OSCAR TOUSTER (introduced by WILLIAM J

DARBY) *Division of Nutrition, Depts of Biochemistry and Medicine, Vanderbilt Univ School of Medicine, Nashville, Tenn*

Ergothioneine has long been known to be a constituent of mammalian red blood cells, but its physiological significance has not been established. An approach to this problem would be a study of factors affecting its concentration in blood. Since the modified uric acid method of Behre and Benedict (*J Biol Chem* 82:11, 1929) and the diazo reaction of Hunter (*Biochem J* 22:4, 1928) are considered to have limitations in specificity and reliability (Behre *Biochem J* 26:458, 1932; Latner *Biochem J* 42:xxxv, 1948), we have developed a procedure differing in principle from previous methods used to determine blood ergothioneine concentrations. It is based upon the bromine oxidation of the sulfur of ergothioneine into inorganic sulfate and the subsequent colorimetric determination of the sulfate. The analysis involves the following steps: 1) saline-washed erythrocytes are heated with uranium nitrate solution to yield a colorless, protein-free extract, 2) the extract is treated with bromine for 30 minutes and the excess bromine removed by aspiration, 3) the sulfate is precipitated as benzidine sulfate, 4) the precipitated benzidine is determined colorimetrically with sodium β -naphthoquinone-4-sulfonate. Inorganic sulfate and thiocyanate, which respond to this test, are present in negligible amounts in the extracts. Recoveries average 70% and duplicate analyses on different samples of the same blood are in fair agreement. The determination can be run on 1.5 cc of blood and is being employed to study ergothioneine blood levels in man.

Cholesterol esterase of rat intestinal mucosa

C. R. TREADWELL, LEON SWELL* AND J. E. BYRON* *Dept of Biochemistry, George Washington Univ School of Medicine, Washington, D. C.*

Highly active esterifying and hydrolyzing cholesterol esterase systems have been demonstrated in glycerol-water (1-1) extracts of rat intestinal mucosa by methods previously developed in this laboratory (*J Biol Chem* 180:615, 1949; 182:479, 1950). Depending on the substrate present, the enzyme catalyzed the synthesis or hydrolysis of cholesterol esters. The experiments indicated that the enzyme was a constant component of the mucosa of normal rats and was not removed by prolonged washing of the lumen of the intestine with saline under pressure. The hydrolyzing and esterifying systems had the same properties and characteristics as those of the pancreas. Bile salts were an essential component of both systems. The activities were not influenced by the substitution of citrate for phosphate buffer in the substrate mixtures. The cholesterol content of the

previous diet did not influence the occurrence or amount of the enzyme. A comparison of the enzyme activity in the mucosa of normal rats with rats with 95% of the pancreas removed suggested that the pancreas was the primary source of at least a major part of the cholesterol esterase of the mucosa.

Protein permeability through thin organic layers

H. J. TRURNIT (introduced by DAVID B. DILL)
Med Division, Army Chemical Center, Md

A. Rothen (*J Biol Chem* 168:75, 1947) found specific reactions in immunological systems where antibody and antigen are separated by inert, supposedly impermeable, materials. He suggested the existence of hitherto unknown molecular forces between large molecules. In order to uncover the mechanism of Rothen's effect, we repeated his experiments and obtained identical results. We then changed the physical pattern of the screening material, using multiple Formvar screens instead of single cast screens. A similar approach was made by Singer (*Federation Proc* 8:251, 1949). We found that multiple screens inhibit the specific reaction more than single cast screens of the same total thickness. Also, colloidal screens are more effective than Formvar screens. We then realized that so far no control had been made on the action of phosphate buffer itself. This has been the diluting agent for sera and antisera in Rothen's experiments as well as in ours. The control experiment proved that this buffer removes part of the antigen through the screen whereas distilled water, saline solution or veronal buffer have no such effect. The antigen penetrates the screen and can be transferred to a conditioned control plate. This plate shows a specific adsorption after treatment with antiserum, whereas the original plate gives correspondingly less adsorption. These results offer a simple explanation for Rothen's effect. A new technique developed during this study will be outlined.

Blood sugar and serum inorganic phosphate after the ingestion of D-glucose, D-fructose or D-xylose

D. A. TURNER (introduced by A. B. MACALLUM) *Dept of Biochemistry, Univ of Western Ontario, London, Canada*

Changes in the concentration of serum inorganic phosphate and of the concentration of the appropriate carbohydrate in the blood of normal human subjects have been determined after an oral dose of D-glucose, D-fructose or D-xylose. For each sugar there was a characteristic change in the concentration of serum inorganic phosphate. Changes in the concentration of blood xylose after xylose administration were much more reproducible than either the changes in the concentration of glucose after a test dose of glucose, or changes

in the concentration of fructose after a test dose of fructose Tween 80 (Sorbitol) had no effect on the serum inorganic phosphate or blood carbohydrate when given by mouth at the same time as the test dose of glucose. Similar results were obtained for fructose and xylose. These findings are discussed from the point of view of the possible metabolism of xylose and its possible phosphorylation during absorption. The xylose tolerance test would appear to be a much better test of absorption from the gastro-intestinal tract in man than either the glucose or the fructose tolerance test.

A micro technique for identification of organic compounds using isotopic indicators and paper chromatography SIDNEY UDENFRIEND (introduced by MILDRED COHN) *Dept of Biological Chemistry, Washington Univ Med School, St Louis, Mo*

Isotopic indicators and paper chromatography, as used in the isotopic derivative method of amino acid analysis, can be employed for rigorous identification of microgram quantities of compounds. A reagent is synthesized in two isotopic forms, with isotopes that can be determined accurately, one in the presence of the other. The unknown substance and the compound being used for comparison are converted to derivatives of the reagents, each with a different isotope. The two derivatives are then mixed and subjected to chromatography on paper. If the two derivatives are identical then one band results in which the proportions of the two isotopes remain constant throughout. This is ascertained by cutting the band into consecutive transverse strips and measuring the isotope proportions in each. If the substances are not identical then the isotope ratio will vary from one end of the band to the other. The technique has been applied to the identification of a substance isolated from paper chromatograms of tissue extracts, having an R_f similar to γ -aminobutyric acid. An authentic sample of S^{35} labelled pipsyl γ -aminobutyric acid was mixed with the I^{131} labelled pipsyl derivative of the unknown. One band resulted with ratios of I^{131} to S^{35} of 0.433, 0.449, 0.439, 0.433, in consecutive strips. A mixture of S^{35} -pipsyl leucine with I^{131} -pipsyl isoleucine yielded one band with ratios of 3.35, 2.23, 1.90, 1.83, 1.38, in consecutive strips. Similarly, S^{35} -pipsyl valine and I^{131} -pipsyl norvaline yielded one band with consecutive ratios of 0.078, 0.136, 0.253, 0.498, 1.07, 1.26, 3.17.

Influence of cortisone on proline oxidation W W UMBREIT AND N E TONHAZY * *Merck Inst for Therapeutic Research, Rahway, N J*

Proline oxidation by unwashed isotonic rat kidney homogenates is markedly decreased by the second day after adrenalectomy and remains at a low level thereafter. Treatment of the adrenalectomized animals (maintained on saline) with 0.5 to 1 mg cortisone daily maintains the activity of the proline oxidizing system or restores it to normal if treatment is instituted even weeks after adrenalectomy. The proline is oxidized to glutamate which is oxidized to completion via the tricarboxy acid cycle. Adrenalectomy and cortisone treatment do not markedly affect the ability of kidney homogenates to oxidize glutamate or other members of the cycle. The effect of cortisone appears to be primarily upon the level of the proline oxidizing enzyme. In adrenalectomized animals, the rate of proline oxidation is related to the amount of cortisone supplied.

Carbon dioxide fixation in dicarboxylic acids M F UTTER *Dept of Biochemistry, Western Reserve Univ School of Medicine, Cleveland, Ohio*

Previously it has been shown that pigeon liver preparations incorporate CO_2 in oxalacetate in the presence of ATP. Ochoa and collaborators have shown fixation of CO_2 in L-malate by a malate-TPN enzyme also obtained from pigeon liver. The relationship of these two processes is not understood. By examining the relative specific activities of the two acids after CO_2 fixation under various conditions an insight into the problem has been obtained. When dialyzed pigeon liver extracts are incubated with $C^{14}O_2$, oxalacetate, malate, and ATP, the specific activity of the oxalacetate is 3-4 times that of malate after short incubations. The ratio approaches 1 after longer incubation, presumably through the action of DPN-malic dehydrogenase. Prior treatment of the extract with a brain preparation capable of destroying DPN increases the specific activity ratio of oxalacetate to malate to 9. These results make it very unlikely that malate is a precursor in the fixation of CO_2 in oxalacetate. However, when TPN is substituted for ATP, the converse situation occurs with the specific activity of malate 3-4 times that of oxalacetate after short incubations and an approach to 1 after longer incubation. The foregoing results may be explained by either of the two following hypotheses: a) two separate mechanisms for CO_2 fixation exist, an ATP-dependent system for oxalacetate and a TPN-dependent system for malate, b) CO_2 is fixed in some intermediate compound which may be directed toward oxalacetate by ATP or toward malate by TPN.

A semi-micro synthesis of C^{14} -carboxyl labeled acetate J T VAN BRUGGEN,* CECIL K CLAY COMB,* TYRA T HUTCHENS,* AND EDWARD S WEST *Dept of Biochemistry, Univ of Oregon Med School, Portland*

Previously described syntheses of acetate by carbonation of a methyl grignard reagent have

generally involved macro quantities of reagents. In addition, specialized equipment not generally available to the small isotope laboratory has been required. An apparatus (slide 1) is described, which is easily assembled by a semi-skilled glass blower from inexpensive, readily available parts. The other essential equipment required is a water aspirator and a small liquid nitrogen cooling bath or its equivalent. Using the technique presented, $\frac{1}{2}$ to 1 millimolar quantities of carboxyl labeled acetate can be synthesized in 85 to 90% yields in a minimum of time. By controlled timing and addition of reagents in the completely closed system, a good yield of product is assured, and complete control of the isotopic agent is maintained. The apparatus may be adapted readily to the synthesis of many other similar products.

A modified method of estimation of xanthine oxidase activity in tissue homogenates J. C. VAN METER AND J. J. OLESON (introduced by I. S. DANIELSON) *Lederle Labs. Division, American Cyanamid Company, Pearl River, N. Y.*

In the course of testing the effect of diet on rat liver xanthine oxidase using the method of Axelrod and Elvehjem (*J. Biol. Chem.* 140: 725, 1941), some anomalous results were obtained. A number of 'zero' livers similar to those described by Richert, Edwards and Westerfeld (*J. Biol. Chem.* 181: 255, 1949) were found. This phenomenon was attributed to the presence of endogenous substrate in the liver and a low enzyme concentration. It was possible by the use of 2-amino-4-hydroxy-6-formylpteridine (AHFP) as a specific xanthine oxidase inhibitor (Lowry, Bessey and Crawford *J. Biol. Chem.* 180: 399, 1949) to negate the effect of the endogenous substrate present in the liver. Factorial experiments to determine the effect of AHFP, hypoxanthine, xanthine and the time interval after addition of substrate were performed. It was found that AHFP as an inhibitor and hypoxanthine as a substrate, alone and in conjunction, produced statistically significantly higher enzyme titers than those obtained by the original method. The mean rate of oxygen uptake approaches a maximum forty minutes after addition of substrate and remains approximately constant for the next sixty minutes. This latter corresponds to the linear part of the curve of oxygen uptake versus time after addition of substrate, used by Axelrod and Elvehjem for the calculation of enzyme activity. On the basis of the above findings, a modified method for the estimation of xanthine oxidase activity in tissue homogenates is proposed.

A comparison of D-3, glyceraldehyde phosphate dehydrogenase from yeast and from skeletal muscle SIDNEY F. VELICK AND SIDNEY UDEN-

FRIEND * *Dept. of Biological Chemistry, Washington Univ. Med. School, St. Louis, Mo.*

That configurational differences exist between glyceraldehyde phosphate dehydrogenases crystallized from yeast and from rabbit muscle is shown by the demonstration of Krebs and Najar that they are immunologically distinguishable. We have analyzed the yeast enzyme for several amino acids by microbiological procedures for comparison with previous analyses of the muscle enzyme by Velick and Ronzoni. In addition we have carried out parallel analyses of several amino acids by the isotope derivative indicator method of Kesten, Udenfriend, and Levy on enzymes from the two sources. Some of the results are summarized as gm/100 gm protein. The first figure is for muscle, the second for yeast: GLU 5.7, 4.2, ASP 10.1, 9.4, ALA 7.1, 7.3, GLY 5.9, 4.9, PRO 3.4, 3.9, METH 2.7, 2.8, VAL 12.0, 11.2, ARG 5.2, 6.0, HIS 5.0, 3.5, LYS 9.4, 10.7, THR 5.2, 6.0, SER 4.4, 4.7, AMIDE N 0.95, 0.72. In both proteins there is a considerable excess of free basic over free acidic groups and yet both proteins are isoelectric in phosphate buffers in the acid region. This apparent anomaly is due to the strong affinity of the enzymes for phosphate ions. The characteristic features in composition of the muscle enzyme are shared by the enzyme from yeast. However, many of the differences observed are significant and it may be concluded, in agreement with other evidence, that the two proteins are similar but not identical. The terminal amino groups are being investigated.

Peptides released in the enzymatic conversion of ovalbumin to plakalbumin C. A. VILLEE, K. LINDERSTRØM-LANG* AND M. OTTESEN * *Chemical Dept., Carlsberg Lab., Copenhagen, Denmark.*

The conversion of ovalbumin to plakalbumin by an enzyme from *Bacillus subtilis* was discovered by Linderstrøm-Lang and Ottesen. In this reaction, approximately 1% of the protein is split off and appears as a mixture of peptides. By means of starch column chromatography these peptides and their hydrolysates were separated and analyzed. The peptides were obtained by precipitating the protein, after a given reaction period, with 10% trichloroacetic acid and extracting the TCA with ether. The aqueous solutions of peptides were then concentrated by lyophilization and aliquots were placed on columns. A variety of solvents were tried but for routine purposes a mixture of 1 butanol 2 n-propanol 1 0.1 N aqueous HCl followed by 2 n-propanol 1 0.5 N aqueous HCl was used. For the first 5 hours of reaction, the peptides upon hydrolysis yielded only alanine, valine, glycine and aspartic acid. No glutamic acid was found. When the unhydrolyzed pep-

tides were placed on columns they were separated into 3 distinct fractions, A, B, and C. The appearance of fractions A and C in the reaction is correlated with the ammonium sulfate titration, shown by Linderström-Lang and Ottesen to be a measure of the conversion of ovalbumin to plakalbumin. In contrast, fraction B is produced by a second step, not correlated with this conversion. The fractions isolated from one column were dried, hydrolyzed with 6 N HCl, and analyzed on other columns. Fraction C proved to contain alanine alone, fraction A contained all 4 amino acids in the ratio 3 alanine 1 valine 1 glycine 1 aspartic acid and fraction B contained the 4 amino acids in equimolar quantities.

Glutamo- and asparto-transferases HEINRICH WAELSCH, ERNEST BOREK, NATHAN GROSSOWICZ* AND MOGENS SCHOU* *Depts of Biochemistry, N Y State Psychiatric Inst and Columbia Univ, New York City*

Studies on the recently discovered enzyme systems (116th meeting, Am Chem Soc, Sept, 1949) which exchange the amide group of glutamine and asparagine with hydroxylamine have been continued. The enzymatic synthesis and splitting of γ -glutamo- and β -aspartohydroxamic acid occurs with washed cells and cell free extracts of several microorganisms, as well as with pigeon brain and liver homogenates. The enzymes were studied in detail in cell-free extracts of *Proteus vulgaris*. The addition of phosphate to extracts, dialysed for 48 hours, did not change the rate of enzymatic synthesis. The expected liberation of ammonia during the enzymatic synthesis is difficult to establish quantitatively since the extracts possess weak glutaminase and strong asparaginase activity. The pH dependence curve of glutamohydroxamic acid synthesis (optimum ca. 8) reflects only the pH dependence of the synthetic process. In the case of aspartohydroxamic acid the curve is a composite of the pH dependence of synthesis and of splitting, since the extracts show considerable activity in splitting aspartohydroxamic acid. Over the whole pH range studied there is no formation of chromogenic material, detectable with the ferric chloride reaction, if glutamine is replaced by glutamic acid. However, aspartic acid at low pH levels produces considerable chromogenic material. The enzymatic synthesis of glutamohydroxamic acid is inhibited by a variety of amino acids. The mechanism of this inhibition will be discussed.

Distribution of N^{15} in the amino acids of *Rhodospirillum rubrum* supplied N_2^{15} A C WAGENKNECHT* AND R H BURRIS *Dept of Biochemistry, Univ of Wisconsin College of Agriculture, Madison*

Seventeen amino acids and ammonia were separated quantitatively from an acid hydrolysate of *Rhodospirillum rubrum* cells by chromatography on starch columns. The most abundant amino acids were glutamic acid, alanine, valine, lysine, glycine, arginine and leucine-isoleucine. An illuminated culture of *R. rubrum* was grown for 3 days on a medium containing 20 mg/liter of ammonium nitrogen, malic acid was the carbon source. The culture then was aerated with N^{15} -enriched N_2 for 30 minutes. The cells were inactivated with acid, recovered and hydrolyzed with acid. The amino acids separated on a large starch column were analyzed for their N^{15} content. The compound showing greatest N^{15} enrichment was ammonia, followed by the alanine-glutamic acid mixture, aspartic acid and the leucine-isoleucine mixture. The monoaminomonocarboxylic acids and arginine were intermediate in N^{15} content and the lysine, histidine and cystine were lowest in N^{15} enrichment. This distribution of N^{15} among the amino acids of *R. rubrum* was very similar to that observed earlier when *Azotobacter vinelandii* was allowed to fix N^{15} -enriched N_2 .

Oxidation by cytochrome oxidase of reduced cytochrome c W W WAINIO, P PERSON, S J COOPERSTEIN AND B EICHEL (introduced by R H BARNES) *Bureau of Biological Research, Rutgers Univ, New Brunswick, N J, and Dept of Anatomy, Western Reserve Univ School of Medicine, Cleveland, Ohio*

A 'solubilized' preparation of cytochrome oxidase has been previously reported. The addition of 2% of sodium desoxycholate, and then 3% of sodium desoxycholate to the precipitate, followed in each instance by high-speed centrifugation, yields a preparation of high activity. The Qo-protein of the preparation used here was 2650 at 25°. The oxidation of reduced cytochrome c was followed spectrophotometrically and the effect of various factors studied. Phosphate buffers (Na_2HPO_4 - KH_2PO_4 , pH 7.4) at the highest concentrations studied (0.1 to 0.2 M final concentration) inhibited the oxidation. The rates at the lower concentrations (0.005 to 0.05 M final concentration) were constant. The effect of pH was studied in phosphate buffers of 0.033 M final concentration over the range 8.6 to 4.6 at intervals of approximately 0.2 of a pH unit. The pH optimum was at 6.67 ± 0.15 . Sodium desoxycholate inhibited the oxidation at concentrations approximately 75 times that added with the oxidase. Concentrations 37 times that added with the oxidase had no effect.

Effect of epinephrine on glucose uptake, glycogen formation and lactic acid production in isolated rat diaphragm OTTO WALAAS AND EYA WALAAS (introduced by G T CORI) *Dept of Biological*

Chemistry, Washington Univ School of Medicine, St Louis, Mo

Methods for the determination of microgram quantities of glycogen were reexamined. Owing to its solubility in 70% alcohol (about 6 $\mu\text{g/ml}$), serious losses of glycogen occur, unless alcohol volumes are kept below 1 ml and reprecipitation of glycogen is omitted. Diaphragms from rats of about 100 gm of body weight were soaked for 20 minutes in ice-cold Ringer's solution in order to remove preformed lactic acid. Of 2.84 mg of glucose taken up per gram of diaphragm per hour (average of 16 experiments), 48% were recovered as glycogen and 25% as lactic acid. In the presence of epinephrine (1 $\mu\text{g/ml}$ of medium), glucose uptake was 1.9 mg (average of 10 experiments), glycogen decreased 13% from its initial value and lactic acid formation increased 43% over that found in normal diaphragm, all statistically significant changes. O_2 consumption and R.Q. were not significantly different from normal. The low recovery (40% of glucose uptake) is probably the result of accumulation of hexosemonophosphate. Diaphragms of rats injected subcutaneously one hour previously with 0.02 mg of epinephrine showed a very low initial glycogen content, during incubation (in the absence of epinephrine) glucose uptake was slightly increased (3.22 mg, average of 10 experiments), glycogen deposition was not changed significantly from normal, while lactic acid formation was 20% below normal, a difference of marginal significance. These rather minor changes probably reflect alterations in the hormonal balance produced by the injection of epinephrine.

Synthesis of visual pigments from the retinenes

GEORGE WALD AND PAUL K. BROWN (introduced by J. T. EDSALL) *Biological Laboratories, Harvard Univ., Cambridge, Mass*

The light-sensitive pigment of rod vision, rhodopsin, is synthesized *in vivo* in two ways from the yellow products of its bleaching, retinene and protein, and from the colorless products, vitamin A_1 and protein. The regeneration from retinene₁ occurs slightly even in aqueous solution, though with a yield of at most 15% (Kühne, Hecht *et al.*). We have found that when cattle or frog rhodopsin in aqueous solution is flooded with synthetic retinene₁, it regenerates about 70% in the dark after bleaching by light. Rhodopsin is also synthesized by mixing its colorless protein moiety in aqueous solution with synthetic retinene₁. There is no evidence that other molecules participate in this reaction. It is a spontaneous, i.e. an energy-yielding process, the bleaching of rhodopsin requires energy, usually in the form of light. The synthesis is inhibited competitively by formaldehyde, and also by such retinene-

trapping reagents as hydroxylamine. When rhodopsin-protein is mixed in aqueous solution with synthetic retinene₂, a light-sensitive pigment is formed with its spectrum displaced in the direction of porphyropsin. Porphyropsin-protein, mixed with both retinenes, yields about the same results as rhodopsin-protein. These experiments in fact involve the synthesis *in vitro* of rhodopsin from crystalline vitamin A_1 , and of a comparable light-sensitive pigment from highly purified vitamin A_2 . The vitamins A were oxidized to the corresponding retinenes, not by a retinal process, but on manganese dioxide. The pathways of these syntheses however bear a significant relation to those which occur in the retina.

Isolation of crystalline conalbumin

ROBERT C. WARNER AND IONE WEBER * *Dept. of Chemistry, New York Univ. College of Medicine, New York City*

The conalbumin of the hen's egg white was first isolated in a purified form by Longworth, Cannan and MacInnes (*J. A. Chem. Soc.* 62, 2580, 1940). Their procedure entailed a precipitation below pH 4 in the presence of salt and was demonstrated to result in a product differing from the original protein in electrophoretic behavior. A method for isolating this protein without exposure to low pH has been reported by Bain and Deutsch (*J. Biol. Chem.* 172, 547, 1948) which yields an electrophoretically homogeneous, amorphous product. Conalbumin has now been obtained in crystalline form both as the ferric iron complex and as the iron free protein. The conalbumin was purified by salt and alcohol fractionation after the addition of excess iron. In most of this work, the filtrate from the usual crystallization of ovalbumin by ammonium sulfate was used as the starting material. Alcohol fractionation of egg white without prior removal of ovalbumin or globulins has also been employed. The purified protein was crystallized as needles or elongated prisms from 10% ethyl alcohol at the isoelectric point in the presence of 0.02 M sodium chloride. After removal of the iron, the protein can be crystallized in a different crystalline form by a similar procedure. The reddish brown crystalline iron complex contains two moles of ferric iron per mole of conalbumin. It is electrophoretically homogeneous above pH 4 in 0.1 ionic strength buffers.

Effects of narcotics and convulsants on rat tissue metabolism

JAMES L. WEBB* AND K. A. C. ELLIOTT *Montreal Neurological Inst., McGill Univ., Montreal, Canada*

The effects of a number of narcotic and convulsant drugs have been studied on the metabolism of isotonic suspensions of brain, of teased testis, and of kidney cortex slices, in bicarbonate

buffered medium. Accompanying the well-known inhibition of oxygen uptake of brain tissue by narcotics, a marked increase occurs in lactic acid formation. The rate of glycolysis increases with increasing narcotic concentration reaching a maximum when the respiration is inhibited 50 to 60%, and then decreases. The maximum may exceed the rate of anaerobic glycolysis. Total glucose utilization (calculated) is greatly increased. Under anaerobic conditions the glycolysis is not appreciably affected until the narcotic concentration is about the optimal for aerobic glycolysis. The concentration required for maximum aerobic glycolysis, 15 mM for sodium pentobarbital (Nembutal), varies from drug to drug but bears some relation to the narcotic potency of the drug. In the presence of Nembutal, succinate stimulates oxygen uptake but does not affect the glycolysis while ascorbate stimulates oxygen uptake and abolishes the extra aerobic glycolysis. L-glutamate oxidation was inhibited by Nembutal. Results with testis were similar to those with brain. With kidney cortex slices similar inhibition of respiration was obtained with or without glucose, and oxidation of L-glutamate, and the concomitant acid disappearance, were strongly inhibited. Metrazole, coramine and picrotoxin have little effect on brain tissue metabolism until a relatively high concentration is reached, when respiration is somewhat inhibited (60% with 50 mM metrazol) with very little increase in glycolysis.

Photochemical oxidation of amino acids, β -lactoglobulin and lysozyme in the presence of methylene blue. LEOPOLD WEIL AND JEANNE MAHER * *Eastern Regional Research Lab, Philadelphia, Pa*

Amino acids irradiated with visible light in the presence of traces of methylene blue undergo photo-oxidation to a varying degree. It was found by the manometric technique that tryptophane, tyrosine, histidine, methionine and cysteine were rapidly oxidized but the rest of the amino acids acted sluggishly or not at all. Irradiation of crystalline β -lactoglobulin resulted in complete disappearance of tryptophane and a parallel decrease in solubility when 16 mols of oxygen per mol of protein had been taken up. Tyrosine decreased only after the complete elimination of tryptophane. Results obtained with irradiated crystalline lysozyme strongly indicate that tryptophane is an essential part of this enzyme. Changes observed in the ultraviolet spectrum during irradiation were in line with the decrease of tryptophane and tyrosine. Under anaerobic condition, no reaction took place, indicating that these reactions were entirely due to oxidation.

An investigation of an electrophoretically homogeneous mucoprotein isolated from normal human plasma. HENRY E. WEIMER,* JOHN W. MEHL AND RICHARD J. WINZLER *Dept of Biochemistry and Nutrition, Univ of Southern California School of Medicine, Los Angeles*

A mucoprotein isolated from pooled, normal, human plasma by ammonium sulfate fractionation has been found to be electrophoretically homogeneous at pH values from 1.5-8.5. The material was found to be isoelectric at pH 1.8. The low isoelectric point is believed to be due to the presence of sulfuric acid ester groups in the molecule since there is an excess of sulfur over the cystine-cysteine and methionine content, and after mild acid hydrolysis of the material a positive test for sulfate can be obtained with barium chloride and benzidine reagents. This mucoprotein migrates with the α -1 globulins at pH 8.5 and can be demonstrated as a separate component in untreated serum at pH 4.5. At this pH it migrates toward the anode with a mobility of 4.2×10^{-5} CM²/sec/volt. Increased serum levels have been demonstrated electrophoretically in the serum of patients with cancer, with pneumonia, and with rheumatoid arthritis. This mucoprotein is a component of serum polysaccharides and of seromucoid investigated by other workers. Chemical analysis of the material yielded the following values: hexose-16.4%, hexosamine-11.9%, nitrogen-10.3%, lipid-3.6%, sulfur-1.02%, ash-1.8%. The amino acid composition as determined by microbiological assay is similar to that of the other plasma proteins. The molecular weight determined from analytical data is approximately 51,000.

Oxidation of isotopic palmitic acid in animal tissues. SIDNEY WEINHOUSE, RUTH H. MILLINGTON* AND MURRAY E. VOLK * *Research Inst and Dept of Chemistry, Temple Univ, Philadelphia, Pa*

Supplementing previous studies with short chain fatty acids, the metabolism of isotopically (carboxyl) labeled palmitic acid was studied in surviving animal tissues. In slices and whole homogenates of rat liver, and in washed homogenates supplemented with adenosine triphosphate, palmitate undergoes two metabolic transformations, complete oxidation to CO₂ and conversion to acetoacetate. Both processes are of approximately equal magnitude in contrast with the behavior of short chain acids which, under the same circumstances, are converted predominantly to acetoacetate. The labeled palmitate carboxyl carbon was found distributed approximately equally between the carbonyl and carboxyl carbons of acetoacetate. In washed homogenates fumarate in low concentrations enhanced the oxidation of palmitate as measured by incorporation of isotopic carbon in the respiratory CO₂.

and at the same time diminished production of acetoacetate. Oxidation of palmitate was observed in pigeon-breast and heart homogenates and in slices and homogenates of rat kidney and heart, but little or no oxidation occurred in rat brain or skeletal muscle homogenates. The data provide unequivocal evidence for extrahepatic oxidation of long-chain fatty acids and indicate that the enzymes which act on the higher fatty acids are the same ones responsible for the catabolism of the short-chain acids.

An enzymic approach to determination of crude fiber A WEINSTOCK AND G HARVEY BENHAM (introduced by MARTIN E HANKE) *Dept of Biology, Illinois Inst of Technology, Chicago, Ill*

The crude fiber content of foodstuffs as determined by the Weende method does not represent a true value because much of the indigestible matter is dissolved during the acid-alkali hydrolysis. Enzymic digestive methods have been found to give a close correlation to the digestibility or feeding value of the material under question. Methods suggested by Horwitt, Remy, Woodson, and others take from five to eight days for completion. A method is described using potent commercial enzyme preparations such as Rhozyme S and Polidase whereby values for the true indigestible matter are obtained in a reduced time interval. Starch is solubilized at pH 4.9 and 50°C in one hour, and protein in a further 20 hours. The digestion residues are filtered through alundum extraction thimbles, dried, weighed, ashed, and reweighed, the loss in weight being the crude fiber. Values of 4.0% indigestible matter were obtained in yellow corn meal compared with 0.9% by the Weende method. These results were duplicated using known mixtures. It is repeatedly found that the Weende method gives values about 3-4 times too low. Consequently the enzymic approach described gives values which are consistent with the actual content of indigestible matter by a method which is simple and readily adaptable to routine work.

Synthesis of labile methyl groups by animal tissues in vivo and in vitro ARNOLD D WELCH* AND WARWICK SAKAMI *Depts of Biochemistry and Pharmacology, Western Reserve Univ, School of Medicine, Cleveland, Ohio*

In previous work (Sakami and Wood, unpublished) the methyl carbon of acetone has been found to appear in labile methyl groups and to contribute to the formation of the formate or formate derivative from which the β -carbon of serine is formed. The formation of 'formate' from acetone suggested that synthesis of labile methyl groups might occur over this pathway. Conversion of formic acid to labile methyl has been ob-

tained in rats injected repeatedly with C^{14} -formate. The crude protein fraction was separated from the tissues and hydrolyzed with HI to release the methyl group of methionine as CH_3I , radioactivity of $(CH_3)_4NI$ formed from this was not altered by recrystallization. Rat liver slices were incubated in the presence of C^{14} -formate, homocysteine, dimethylaminoethanol, folic acid and vitamin B_{12} . After removal of unreacted $HCOOH$ and hydrolysis, methionine was isolated and degraded with HI, $(CH_3)_4NI$, with radioactivity unaltered by recrystallization, was obtained. Choline chloride, isolated from the slices via the reneckate and chloroplatinate, was converted to $(CH_3)_4N$ and recovered as $(CH_3)_4NI$ the radioactivity of which was unaltered by recrystallization. In progress are studies of homogenates and of the possible role of folic acid and vitamin B_{12} in the synthesis by various tissues of compounds containing methyl groups.

Ratio of sickle cell anemia to normal hemoglobin in sicklemics IBERT C WELLS AND HARVEY A ITANO (introduced by L ZECHNEISTER) *Gates and Crellin Labs of Chemistry, California Inst of Technology, Pasadena*

The discovery was made in these laboratories that the hemoglobin of persons having sickle cell anemia differs from normal hemoglobin and that both hemoglobins occur in persons with sickle cell anemia (Science 110 543, 1949). It was inferred that sickle cell anemia was inherited by an individual from sickle cell parents. The results of the genetic study of J V Neel (Science 110 64, 1949) are in accord with this conclusion. Since sickle cell anemia (SCA) hemoglobin and normal hemoglobin occur together in the erythrocytes of sickle cell persons, such individuals offer the possibility of studying the genetic control of hemoglobin synthesis. It was reported by us (Science 110 543, 1949) that in a pooled sample of blood from 5 sicklemics the SCA hemoglobin constituted 39% of the total. To ascertain whether this percentage is characteristic of sicklemics or not, the percentage of SCA hemoglobin in the blood of 20 sicklemics has been measured using the procedure previously employed. These were adult persons selected by chance. Among these individuals, the SCA hemoglobin composed from 24% to 43% of the total hemoglobin. Approximately one-third of the cases fell in the range 39% to 42%. From these results it appears that there is no ratio of the two hemoglobins which is characteristic of sicklemics. Factors which might affect the ratio are being studied.

Metabolism of intravenous testosterone in the human C D WEST* AND L T SAMUELS *Dept of Biological Chemistry, University of Utah College of Medicine, Salt Lake City*

Crystalline testosterone in serum albumin solu-

tion has been administered intravenously to several human subjects in doses up to 200 mg. Following this administration it is possible to measure and follow the blood levels of testosterone and its metabolites. The testosterone concentration in the blood decreases rapidly usually to zero during the first hour after injection. Approximately 60% of the administered testosterone can be accounted for as increased amounts of conjugated 17-ketosteroids in the urine during the first 24 hours. The peak excretion of urinary 17-ketosteroids occurs during the first 2 hours when an average of 30% is excreted. Small amounts of non-conjugated 17-ketosteroids can be demonstrated in these post-injection urines. Conjugated 17-ketosteroids can be demonstrated in the blood during this period of increased urinary excretion. Smaller amounts of non-conjugated 17-ketosteroids were also found in the blood. Conjugated α - β unsaturated steroids can be demonstrated in the urine for several hours after injection. In the immediate post-injection urines small concentrations of non-conjugated α - β unsaturated steroids are found. In patients with liver disease the rate of metabolism of intravenous testosterone is depressed.

Composition of rat liver xanthine oxidase and its inhibition by tetraethylthiuramdisulfide (antabuse) W. W. WESTERFELD, DAN A. RICHERT* AND RAYMOND VANDERLINDE* *Dept. of Biochemistry, Syracuse Univ. College of Medicine, Syracuse, N. Y.*

In vitro addition of tetraethylthiuramdisulfide (antabuse) to a rat liver homogenate inhibited its xanthine oxidase activity from 50% to 100%. Milk xanthine oxidase alone or when added to a liver homogenate containing antabuse was not inhibited. Methylene blue added to the aerobic system overcame the antabuse inhibition of the liver enzyme; dehydrogenation of xanthine was unaffected but autooxidation of xanthine oxidase was inhibited by antabuse. Heating the liver homogenate at 56° for 5 minutes destroyed the antabuse sensitivity of the xanthine oxidase without otherwise affecting the enzyme. Longer heating also reduced its capacity to react with atmospheric oxygen without affecting its dehydrogenase activity. Rat liver xanthine oxidase behaved as a single entity during its purification; the purified enzyme responded to antabuse, methylene blue, and 56° heating in the manner described for the enzyme in the homogenate. The results indicate that rat liver xanthine oxidase is similar to the milk enzyme, but is attached to some other constituent that confers antabuse-sensitivity upon the complex. By analogy with succinoxidase the possibility that this identified constituent is phospholipid is being investigated.

Succinoxidase activity was completely inhibited by antabuse and was destroyed by heating at 56° for 5 minutes. By contrast, the d-amino acid oxidase activity of a rat liver homogenate was not affected by antabuse, methylene blue, or heating at 56°C.

Xanthine metabolism in the rat J. N. WILLIAMS, JR.,* PHILIP FEIGELSON,* AND C. A. ELVEHJEM *Dept. of Biochemistry, College of Agriculture, Univ. of Wisconsin, Madison*

Liver xanthine oxidase activity in the rat has been observed to be highly dependent upon the level of dietary protein. This variation with diet offered a means of observing the relationship between *in vitro* and *in vivo* activity of this enzyme. Two groups of rats were given 6% casein and 18% casein + 0.25% methionine rations, respectively, for 3 weeks. The animals were then injected intraperitoneally with 25 mg xanthine per 100 gm body weight and the urines collected at intervals for one week. The animals were then killed for *in vitro* liver xanthine oxidase determinations. Allantoin excretion was determined in the urine collections to obtain an *in vivo* estimation of xanthine oxidase activity. Contrary to expectation, the rats with very low *in vitro* liver xanthine oxidase activity, (those fed the 6% casein ration) showed an output of allantoin about double that of the animals with normal liver activity. Moreover, the allantoin excretion accounted for less than 10% of the injected xanthine in both groups. Attempts to account for the injected xanthine as any of the purines both in urine and feces failed. Our results indicate that *in vitro* measurements of xanthine oxidase may not be an indication of the actual rate of xanthine oxidation in the intact animal. It appears that exogenous xanthine may be metabolized by a pathway other than that of simple oxidation to allantoin and subsequent urinary excretion.

Chromogenic values of ketosteroids in the Zimmermann reaction HILDEGARD WILSON (introduced by KONRAD DOBRINER) *Sloan-Kettering Inst. for Cancer Research, New York City*

The Zimmermann reaction is employed for the quantitative determination of individual ketosteroids isolated from human urine to establish steroid excretion patterns. Since various ketosteroids give different chromogenic values, the use of a single reference standard such as androstosterone does not give absolute values for the amounts of each steroid excreted. Therefore the relative intensities of ketosteroids commonly found in human urine were determined by the Zimmermann reaction as modified by Callow at 25°C and also by an adaption for micro amounts at 0°C. When the colors were developed at 25°C and read after 50 minutes the chromogenic values

relative to androsterone (=100) were as follows dehydroisoandrosterone, 100, Δ^2 or Δ^3 -androsten-17-one, 106, androstane-3,17-dione, 116, Δ^4 -androsterone-3,17-dione, 129, 11-hydroxyandrosterone, 62, etiocholanolone, 112, etiocholanone-3,17-dione, 114, Δ^5 -etiocholanolone, 117, 11-ketioetiocholanolone, 117, pregnan-3 α -ol-20-one, 15, and allopregnan-3 α -ol-20-one, 15. The results also indicate wide variations in the rates of reaction. Androstane-3,17-dione, etiocholanone-3,17-dione, Δ^5 -etiocholanolone and 11-ketioetiocholanolone have especially rapid color development. With both techniques the greatest similarity was found at the time of maximum color. Other results and experimental details will be discussed.

Incorporation of labeled amino acids into the protein of embryonic and tumor tissue homogenates THEODORE WINNICK *Radiation Research Lab, State Univ of Iowa Med School, Iowa City*
When homogenates of fetal rat liver or transplanted mouse mammary tumor were incubated with carboxyl- C^{14} -labeled glycine or alanine at 37° under oxygen atmosphere, the uptake of C^{14} by protein was several times as great as when homogenates of normal adult rat or mouse liver were employed. The turnover of glycine and alanine residues in the fetal liver and tumor homogenates was about 0.1 to 0.2% per hour. The incorporation process in the embryonic system was stimulated by adding certain members of the Krebs tricarboxylic acid cycle. Malonate was inhibitory. The isolated proteins lost no $C^{14}O_2$ when heated with ninhydrin. After complete acid hydrolysis all the C^{14} was released by ninhydrin. Protein from adult liver homogenate, incubated with glycine- C^{14} , lost most of its radioactivity upon subsequent treatment with dilute NaOH. The C^{14} was apparently concentrated in a labile peptide fraction, associated with this protein. By contrast, proteins from homogenates of fetal liver and tumor tissue retained their C^{14} when treated with alkali. The available evidence indicated that, in these systems, the C^{14} represented true incorporation of amino acids into protein structure.

Relation between propagation of mouse encephalomyelitis virus and uptake of radiophosphate by minced brain from mice RICHARD J. WINZLER, H. E. PEARSON* AND MAX E. RAFELSON* *Depts of Biochemistry and Nutrition, and of Microbiology, Univ of Southern California School of Medicine, and Lab Division of Los Angeles County Hospital, Los Angeles*

It has previously been shown that minced one-day-old mouse brain was capable of supporting the propagation of Theiler's GD VII mouse encephalomyelitis virus *in vitro*. Virus-infected cultures incorporate inorganic phosphate into

their phospholipids and nucleoproteins at rates significantly greater than uninfected controls. Minced brain from mice older than 10 days is unable to support virus growth. A study of the rates of oxygen consumption and of P^{32} uptake by minced brain tissue from mice of different ages in the presence and absence of virus shows that older mouse brain has a higher initial QO_2 than one-day-old brain, but the QO_2 declines at a very much more rapid rate than the one-day-old tissue. The incorporation of inorganic phosphate into the lipids and proteins in a 24-hour incubation period is much greater in the one-day-old brain than in older brain. The stimulating effect of the virus on these processes is no longer apparent in brain from mice older than 7 days, and corresponds to the ability of the tissue to support virus growth.

Location in protoporphyrin of carbon atoms derived from α -carbon of glycine and carbon atoms of acetic acid JONATHAN WITTENBERG* AND DAVID SHEMIN *Dept of Biochemistry, Columbia Univ, New York City*

It has been shown that 8 carbon atoms in protoporphyrin are derived from the α -carbon atom of glycine and that most or all of the remaining carbon atoms are derived from both carbon atoms of acetic acid. This paper presents experiments in which the positions of the carbon atoms in protoporphyrins derived from the α -carbon atom of glycine and from acetic acid are located. C^{14} -labeled hemin, prepared by incubating duck blood with C^{14} -methylene labeled glycine, was degraded by a procedure which allowed the isolation of individual carbon atoms. It was found that one-half of the total radioactivity of the porphyrin was present in the 4 pyrrole rings. Only the α -carbon atoms of the pyrroles on the same side as the vinyl and propionic acid side chains were labeled. The other one-half of the total radioactivity was accounted for by the methene bridge carbon atoms. From the data it is concluded that all 4 methine bridge carbon atoms are derived also from the α -carbon atom of glycine, that these carbon atoms have equal radioactivity and that their individual radioactivities are equal to the radioactivity of the individual labeled carbon atoms of the pyrrole rings. Also two samples of C^{14} -labeled hemin were prepared by incubating duck blood with carboxyl labeled acetate and with methyl labeled acetate separately. The locations of the carbon atoms which are derived from both carbon atoms of acetate will be presented. Hypotheses of porphyrin formation are discussed.

Requirements for organic phosphate in *in vitro* oxidation of 6-carbon fatty acids ROBERT F. WITTER (introduced by ELMER STOTZ) *Dept of Biochemistry, Univ of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

By measurement of the rates of acetoacetate formation with properly fortified rat washed liver particles, the requirements for inorganic phosphate and adenine nucleotides for several 6-carbon acids, namely, hexanoate, 2- Δ -hexenoate, sorbate, β -hydroxyhexanoate, and β -ketohehexanoate, were investigated. In order to study the effect of high ATP concentration on the rate of sorbate oxidation, factors influencing the maintenance of the initial (added) ATP level were studied. An elevated 10-minute hydrolyzable phosphate level could be maintained using an enzymatic source of ATP, namely, a rabbit muscle protein fraction and 3-phosphoglycerate, whereas without the enzymatic source the initial level of ATP decreased to a low value. With the enzymatic source of ATP it was possible to use considerably higher concentrations of sorbate than under the usual conditions. Control experiments with added pyruvate showed that part of the above effects of the enzymatic source of ATP might be due to the enzymatic production of pyruvate from phosphoglycerate. Dinitrophenol blocked fatty acid oxidation and caused a decrease in the 10-minute phosphate to a low value, even with the enzymatic source of ATP. Under anaerobic conditions, where added pyruvate was ineffective, the enzymatic source maintained the level of 10-minute phosphate, and here again dinitrophenol caused the same effect as under aerobic conditions. This anaerobic effect of dinitrophenol is under further investigation.

Studies on tobacco chewing WM A WOLFF AND W E GILES * *Tobacco Research Lab, Bowman Gray School of Medicine, Winston-Salem, N C*

The purpose of this study was to determine the dosage and metabolism of nicotine resulting from the chewing of tobacco, and to compare the findings with determinations made after cigarette smoking. Experiments were made on 21 normal adult males, habitual tobacco users, who chewed during a test period of 6½ to 8 hours. Tobacco of known nicotine content was prepared from selected batches which had gone through all steps of the manufacturing process except being pressed into plug form. The nicotine content of the chewed tobacco and of the spittle was determined by the A O A C silicotungstate method, that of the blood and urine, by a method previously reported (*J Biol Chem* 175: 825, 1948). The difference between the nicotine content of the original tobacco sample and that of the spittle, including tobacco chewed, was considered as the amount of nicotine absorbed. The amount of tobacco chewed ranged from 15.6 to 58.4 gm, that of the nicotine absorbed, from 8.0 to 87.7 mg. Nicotine levels in the blood were determined before and after the period of chewing, and ranged from 0 to 0.56 mg/l. The increase during the chewing period averaged 0.05 mg/l, a value slightly lower than

those obtained with comparable doses of nicotine from smoking. The amount of nicotine excreted in the urine was 0.55-3.43 mg. Calculated as percentage of the total nicotine dose the excretion is about the same as found with cigarette smoking.

Effect of temperature on P^{32} transport in fertile and infertile eggs JEROME J WOLKEN (introduced by M A LAUFFER) *Dept of Biophysics, Univ of Pittsburgh, Pittsburgh, Pa*

Radioactive phosphorus as sodium phosphate was injected into the white of fertile and infertile hen's eggs and incubated at temperatures from 12° to 41°C for time intervals from 0 to 120 hours. The rates of transport were determined by a measure of activity and specific activity of P^{32} in the shell, white, yolk and embryo. It was found that P^{32} does not come rapidly to equilibrium between the white and yolk except in stored infertile eggs. The rates of change of activity in the fertile eggs are greater than those for the infertile eggs, and equilibrium is not established even after 120 hours at 41°C. Except for the first 9 hours of incubation, transport of P^{32} varies linearly with temperature, but is accelerated in the fertile eggs between 30° and 41°C, the optimum temperature range for enzymatic reactions and embryonic growth. The form of the equation at all temperatures in both the fertile and infertile systems is identical to that which describes the rate of a first order reaction. The velocity rate constant for the infertile system was found to be temperature dependent, and to behave as any ordinary physical transport phenomenon. But in the fertile system, the velocity rate constant is independent of temperature, and no mechanism has yet been postulated to explain this behavior. A theoretical interpretation of the processes of fertilization offers the possible explanation that the velocity rate constant varies with the effective volume of the yolk and the permeability of the yolk membrane.

Photosynthesis studies with isotopic CO_2 HARLAND G WOOD, GEORGE O BURR AND CONSTANCE E HARTT * *Dept of Biochemistry, Western Reserve Univ School of Medicine, Cleveland, Ohio, Experiment Station, H.S.P.A., Honolulu*

The photosynthetic fixation of isotopic CO_2 by beans, sugar cane and rye has been studied. The sugars from 80% alcohol extracts have been degraded by bacterial fermentation of the hexose unit. With sugar cane the 3,4 positions contained the highest, the 2,5 the next, and the 1,6 positions the lowest isotopic concentration and in general the relative difference was greater with shorter time of photosynthesis. With beans and rye the isotope in the 2,5 and 1,6 positions was nearly equal and occasionally was as high as the 3,4 positions. The 80% alcohol soluble material from

rye of 1 minute exposure in light and 100 minute exposure in the dark has been fractionated by extraction with benzene at pH 7.0, then continuous extraction with ether at pH 2.5 and finally fermentation of the sugars. On a percentage basis the isotope distribution was as follows (Italics are for dark fixation) benzene extract, 8.2 and 19.1, ether soluble acids, 6.9 and 49.2, sugars, 5.2 and 12.6, and fermentation residue, 79.2 and 17.4. Of the isotope administered 0.10 and 0.14% was fixed in the 80% alcohol extracts. The large amount of isotope in the residue fraction with light might be due to a unique photosynthetic product, however other interpretations will be considered.

D-amino acid formation occurrence and properties of an alanine racemase W. A. Wood* AND I. C. GUNSALUS *Lab. of Bacteriology, Indiana Univ., Bloomington*

The finding that *Streptococcus faecalis*, strain R, grown with vitamin B₆ contains D-alanine, whereas cells grown with D-alanine as replacement for vitamin B₆ do not form this vitamin, indicates a role of vitamin B₆ in D-alanine formation. Investigation of the mechanism of D-alanine formation has shown that a racemase is involved, for which pyridoxal phosphate serves as the co-enzyme. Vacuum dried cells of *S. faecalis*, strain R, harvested from a complex medium containing 0.3% cerelese, racemize 2 to 6 micromoles of alanine/mg cells/hr. A cell-free racemase was prepared from the dried cells by sonic oscillation and fractionated by ammonium sulfate precipitation and adsorption on and elution from calcium phosphate gel. Racemase activity was followed manometrically with L-alanine as substrate in the presence of excess D-amino acid oxidase. Both the vacuum dried cells and the cell-free enzyme were partially deficient in pyridoxal phosphate and the purified racemase was almost completely resolved. With the purified enzyme in the presence of pyridoxal phosphate, D- or L-alanine was converted to the racemic mixture. Other amino acids were not racemized by these preparations. Pyridoxamine phosphate did not act as a coracemase. Formation of alanine by transamination with amino acids or pyridoxamine phosphate and pyruvate did not occur, nor did pyruvate, α -ketoglutarate, or oxalacetate stimulate the rate of racemization. No pyruvate could be detected during racemization. Assay of representative bacterial species grown under various conditions indicates a wide distribution of alanine racemase. Attempts to demonstrate this enzyme in rabbit tissue homogenates have not revealed activity within the range of assay.

A study of non-competitive antagonism with chloromycetin and related analogs of phenylalanine

D. W. WOOLLEY *The Rockefeller Inst. for Med. Research, New York City*

The antibacterial effects of chloromycetin were found to be antagonized by phenylalanine. This amino acid was recognized to be a structural analog of the antibiotic agent, and to differ from it in 4 ways. Most of these types of change had previously been used individually to convert metabolites into substances which antagonized their action competitively. The antagonism of chloromycetin and phenylalanine was non-competitive and could be shown only within a restricted range of concentration of the antibiotic agent. In order to study the structural features which might confer on a competitive structural analog, a non-competitive type of action, phenylalanine was changed progressively one step at a time, by the introduction into it of the alterations which make it differ from chloromycetin. Thus, just the nitro group, or the dichloroacetyl radical, or the β -hydroxyl group, or the primary alcohol was built into phenylalanine, and then two, and then three, of these changes were made simultaneously. Tests with *Escherichia coli* and with *Lactobacillus casei* showed that the compounds which differed in only one respect from the metabolite were either inert as growth inhibitors, or were competitive antagonists of low potency. With two or three simultaneous alterations, more powerful antagonists were obtained, but with these the competitive feature was evident only at rather low concentrations. Large amounts were not reversed at all by phenylalanine. Chloromycetin, which has four structural dissimilarities to the metabolite, was strictly a non-competitive antagonist. These facts were viewed in relation to the understanding of non-competitive antagonism between structural analogs.

Evaluation of rates of metabolic processes from excretion data in isotopic feeding experiments HSIEN WU (introduced by EMMETT B. CARMICHAEL) *Biochemistry Dept., Med. College of Alabama, Birmingham*

When a labeled compound A is taken orally and the label is excreted in another compound B, the rate of appearance of B in urine depends on a) the rate of absorption of A from the alimentary canal, b) the rate of conversion of A to B in the metabolic pool and c) the rate of excretion of B by the kidneys. It is possible, therefore, to evaluate all these rates from the excretion data of labeled B after feeding the labeled compound A. On the assumptions that labeled and unlabeled molecules behave metabolically alike and that a steady state obtains, equations are formulated for the rates of absorption, conversion and excretion. The solution of these equations leads to an expression which relates the isotopic ratio of B excreted to the rate of absorption of labeled A.

(K), the rate of turnover of A (Q_A), the fraction of this rate due to conversion of A to B, the rate of turnover of B (Q_B) and the rate of excretion of B. The expression is complicated, but it can be easily solved by taking advantage of the fact that when Q_A is much smaller than K and Q_B , as it is in the case in isotopic feeding experiments, certain terms in the expression become negligible a few hours after feeding. Thus, Q_A can be very simply evaluated first after which the other rates can be evaluated. The expression arrived at in the present study puts the theory underlying the evaluation of rates on a sounder basis and at the same time makes the calculation simpler than hitherto.

Role of dietary proteins in development of sex organs of young rats under stimulus of a gonadotrophin RICHARD S. YAMAMOTO* AND BACON F. CHOW, *Dept. of Biochemistry, School of Hygiene and Public Health, Johns Hopkins Univ., Baltimore, Md.*

The development of sex organs under the added stimulus of a gonadotrophin involves the synthesis of tissue proteins which might be affected by dietary proteins. In this study, we fed *ad lib* 23 days old rats of both sexes, 5 diets containing 20% proteins of different nutritive values (casein, lactalbumin, soy bean, wheat gluten and stock diet). A 6th diet containing no protein was also included. On the 27th day, one half of the rats on each diet was given subcutaneously a daily dose of 10 units of Follutein (to males) or 0.10 unit of Gonadogen (to females). The remaining animals served as dietary controls. All animals were killed on the 33rd day. Their sex organs were removed, weighed and analyzed for total nitrogen. The results demonstrate 1) injection of gonadotrophin produced several-fold increase in the sex organs including those of animals on a nitrogen-free diet. Such growth obviously took place at the expense of other body tissues. 2) Injection of gonadotrophin to animals on diets containing proteins produced hypertrophy of the sex organs but a loss of body weight. 3) The degree of hypertrophy of sex organs was related to the nutritive values of the protein. 4) The ratios of weights of these organs to body weights at necropsy were significantly different depending on the dietary proteins. These data suggest that the response of normal rats to the stimulus of sex hormones is dependent on the protein intake.

Accumulation of a substance possessing niacin activity by a mutant strain of Neurospora CHARLES YANOFSKY* AND DAVID M. BONNER, *Osborn Botanical Lab., Yale Univ., New Haven, Conn.*

Although kynurenine and 3-hydroxykynurenine have been proposed as intermediates in the con-

version of tryptophan to niacin in *Neurospora* (BEADLE, G. W. ET AL. *Proc. Nat. Acad. Sci.* 33: 155, 1947; MITCHELL, H. K., AND J. F. NYC, *Proc. Nat. Acad. Sci.* 34: 1, 1948) evidence has not been presented as yet which would critically evaluate this hypothesis. The accumulation of either of these substances by mutant strains or their presence in culture filtrates or mycelial extracts has not been reported. To establish, with some degree of certainty, the participation of these compounds in the synthesis of niacin, their presence must be demonstrated. Niacin-less mutant strains capable of utilizing 3-hydroxyanthranilic acid, an established intermediate in niacin synthesis (BONNER, D. M. *Proc. Nat. Acad. Sci.* 34: 5, 1948), but incapable of using tryptophan, might be expected to accumulate kynurenine or 3-hydroxykynurenine. A mutant strain has been obtained which has these growth requirements and does accumulate large quantities of a substance possessing niacin activity. This substance has recently been isolated and tentatively identified as α -N-acetyl kynurenine. Its activity is too slight to justify considering it a normal intermediate in niacin synthesis. However, since this substance is structurally similar to kynurenine, it would seem reasonable to assume that the isolated compound results from kynurenine accumulation. This work clearly demonstrates the participation of kynurenine in the biosynthesis of niacin in *Neurospora*.

Analysis of commercial adrenal cortex extracts by paper partition chromatography ALEJANDRO ZAFFARONI, ROBERT B. BURTON AND E. HENRY KEUTMANN (introduced by ALEXANDER L. DOUNCE), *Depts. of Biochemistry and Medicine, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

In analyzing corticosteroid mixtures by the method of paper partition chromatography described previously by us (*Science* 111: 6, 1950), known compounds are required for use as standards. In order to use commercial adrenal cortex extracts as reference material, characterization of their major steroid components was undertaken. Analyzing relatively small quantities of extract, three commercial preparations were studied. The following α -ketolic steroids were found: 17-hydroxycorticosterone, 17-hydroxy-11-dehydrocorticosterone, corticosterone, 11-dehydrocorticosterone, and four other compounds whose probable identity will be discussed. Evidence for the presence of 11-desoxycorticosterone in very low concentration will be given. The various data to be presented in support of the identity of the compounds found will include chromatographic behavior of free and esterified forms, ultraviolet absorption spectra, absorption spectra of 2,4-dinitrophenylhydrazones, specific color reactions and formation of other derivatives.

Mechanism of oxidative deamination E A ZELLER, G A FLEISHER AND D C UTZ (introduced by M H POWER) *Division of Biochemistry, Mayo Foundation, Rochester, Minn*

The behavior of crude diamine oxidases (DO) from animal tissues, plants, (sprouting clover), molds (*Nocardia asteroides*) and bacteria were investigated. The substrates putrescine, cadaverine, histamine, agmatine, spermine, 1,3-diaminopropanol* (those with asterisks tried for the first time), 3-[aminobutyl]-pyridin,* and promin* were attacked with variable velocities by the DO from different sources. Similarly the degree of inhibition caused by pyridoxamine,* streptomycin, carbonyl reagents, pyocyanine, arcaine, 2-(4'-amino phenyl) piperidine* showed marked differences from one DO to another. Such enzymes like the DO from several sources will be designated 'homologous' in reference to the use of this term in comparative anatomy. The variations within a group of homologous enzymes may become of some importance for the understanding of the action of chemotherapeutic agents on the biochemical systems of microorganisms and their host respectively. The oxidation of diamines by *Nocardia* extracts was not followed by deamination, but the deamination took place by addition of DO-free extracts of rat kidney. These results are corroborated by those obtained with ophiol-L-amino acid oxidase. The reaction product from L-phenylalanine turned out to be different from phenylpyruvic acid, according to chemical, microbiologic and spectroscopic data. A model for the oxidative deamination in general and for DO specifically will be discussed.

Effects of anions, cations and amino acids on bovine alkaline phosphatases CHARLES A

ZITTLE AND EDWARD S DELLA MONICA * *Eastern Regional Research Lab, Philadelphia, Pa*

Alkaline phosphatase from intestinal mucosa of the calf and that from cow's milk were studied with phenylphosphate as the substrate. The phenol released by the enzymes was determined colorimetrically. About 0.1 mg of the milk preparation in a volume of 12 cc was required for an assay, $\frac{1}{25}$ th as much of the intestinal preparation was sufficient. Inhibition of the alkaline phosphatases by the following compounds was determined: the anions phosphate, arsenate, carbonate, tetraborate and pyrophosphate, the cations ethanolamine and ammonium, and the amino acids glutamic acid and lysine. The type of inhibition was determined with tetraborate, ethanolamine and the amino acids. From these studies and those of others, an inhibition pattern emerges that suggests that the milk and intestinal phosphatases differ. Milk phosphatase was more inhibited by the cations and by lysine than was the intestinal phosphatase, whereas the reverse was true with the anions and with glutamic acid. Our studies have shown that tetraborate was a competitive inhibitor, an effect shown by phosphate and carbonate also. Ethanolamine was a noncompetitive inhibitor, as was the ammonium ion. Inhibition by glutamic acid was largely noncompetitive, the small amount of competitive inhibition was no more than that shown by glycine. Lysine was also a noncompetitive inhibitor. Bodansky found that rat intestinal phosphatase was more inhibited by glutamic acid than were the bone and kidney phosphatases, the reverse was true of inhibition by lysine. Milk phosphatase is probably more closely related to bone and kidney phosphatases than it is to intestinal phosphatase.

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Histamine-like actions of curari on submaxillary glands of the cat JOHN ABAJIAN* AND N B DREYER *Depts of Anesthesiology and Pharmacology, Univ of Vermont, Burlington*

During the course of clinical anesthesia where D-tubocurarine is used to facilitate muscular relaxation one occasionally sees marked cyanosis and hypoxia in spite of adequate pulmonary ventilation. There is resistance to manual inflation even with an intratracheal catheter in place. This would seem to indicate obstruction in the lower respiratory tract, reputedly due to bronchospasm from histamine liberated on injection of curari. Histamine is also liberated from the tissues of the forearm on intra-arterial injection of curari. The use of anti-histaminic drugs to combat this curari shock—bronchoconstriction and hypotension—has been suggested. To test this hypothesis the submaxillary gland of the cat was used. This gland secretes saliva on intra-arterial injection of histamine. Actions of Histamine and D-tubocurarine were compared. In cats under chloralose, (100 mg/kg) the salivary ducts were cannulated and the chorda tympani and cervical sympathetic were sectioned. All drugs were given intra-arterially into the common carotid in amounts never exceeding 25 cc total dose. Artificial respiration was maintained in all experiments. Histamine, 10-20 μ g and D-tubocurarine, 25-60 μ g, caused a flow of saliva. D-tubocurarine on repeated injections gave lessened responses. Anti-histaminics like Thonzylamine and Neo-antergan abolished the salivary responses to both histamine and D-tubocurarine. In addition D-tubocurarine caused ganglionic block shown by stimulating the nerves of the gland. These results would seem to support the hypothesis that the injection of D-tubocurarine liberates histamine from the tissues.

Effect of morphine in vivo and in vitro on glycolysis of rat tissues L G ABOOD (introduced by J M COON) *Dept of Pharmacology, Univ of Chicago, Chicago, Ill*

Glycolysis was studied on tissues of rats which were chronically and acutely treated with morphine. The chronically morphinized rats were given morphine SQ daily for a period of 10 weeks with progressively increasing doses up to 250

mg/kg. Acutely treated animals were given 50 mg/kg morphine 3 hours before they were killed. The rate of glycolysis from glucose, as determined by CO_2 and lactic acid formation, increased 65% in the chronically treated animals and 50% in the acutely treated animals. A linear relationship between the dose of morphine and the increase in glycolysis was found in animals acutely treated. This increase in glycolysis was observed in brain, liver and kidney of acutely and chronically treated animals and the skeletal muscle of chronically treated animals only. With a final concentration of 10^{-3} M morphine sulfate a 50% activation of glycolysis was produced *in vitro* in all these tissues except skeletal muscle, in which no change was noted. The mechanism of action of this increased glycolysis has not been elucidated to date. Morphine was tested *in vitro* on hexokinase, aldolase, ATP-ase, and lactic dehydrogenase, and was found to be without effect. An increase of aldolase was found in the tissues of chronically morphinized animals, but it was not enough to account for the increased glycolysis. Morphine is oxidized anaerobically *in vitro*, possibly to pseudomorphine, and the effect of this reaction on glycolysis is now being considered.

Comparison of central and peripheral nervous system effects of certain anti-muscarinic local anesthetic agents BENEDICT E ABREU, FREDERICK H MEYERS* AND WILLIAM M ALEXANDER* *Research Dept, Pitman-Moore Co, Indianapolis, Ind, and Division of Pharmacology and Experimental Therapeutics, Univ of California Med School, San Francisco, Calif*

Previous observations (Federation Proc 5 161, 1946) indicated that certain anti-muscarinic compounds administered in non-lethal dosage produced effects which could be construed as being due to central nervous system stimulation. However, it was also shown in this investigation that the central nervous system effects of agents known to be stimulant in the dog and other mammals could not be differentiated from those produced by anti-muscarinic agents, which uniformly caused ataxia and muscular weakness in the dog. Amino alcohol esters of the following acids have been studied: tropic, mandelic, phenyl- α thienylgly

colic, fluorene - 9 - carboxylic, diphenylacetic, 1-phenyl cyclopentane-1-carboxylic and 4 n-hexyloxy benzilic. All were less effective than atropine in depressing intestinal motility or the pupillary response to light. However, all were more capable than atropine of producing skeletal muscular weakness and ataxia. Though all of these agents possess local anesthetic activity, no correlation appears to exist between this type of action and such central nervous system depressant effects as ataxia and skeletal muscular weakness.

Some effects of procaine and diethylaminoethanol in auricular flutter in the dog G. H. ACHESON AND BARBARA BROWN * *Pharmacology Dept., Univ. of Cincinnati College of Medicine, Cincinnati, Ohio*

In dogs under dial-urethane anesthesia, with chest open and artificial respiration, stable auricular flutter was established by the method of Rosenblueth and García Ramos. Auricular electrograms and ECG's were recorded. In vagotomized animals procaine (1-8 mg/kg) slows the rate of auricular flutter with little change of ventricular rate, whereas diethylaminoethanol (16-64 mg/kg) slows both auricular and ventricular rates. Both compounds slow conduction velocity in the non-fluttering auricle. Reversion from flutter to sinus rhythm occurs less regularly and at lower auricular rates with procaine than with diethylaminoethanol. At doses of the 2 compounds which produce equivalent slowing of rate of auricular flutter (1 and 32 mg/kg, respectively), the 'maximal rate' of the non-fluttering auricle *in situ* is decreased less by procaine than by diethylaminoethanol. In animals with intact vagi the slowing of the rate of auricular flutter produced by procaine is accompanied by an increase of ventricular rate, whereas diethylaminoethanol slows both auricle and ventricle as above. Stimulation of the cut vagus accelerates the rate of auricular flutter and markedly decreases the ratio of ventricular rate to auricular rate. These effects are largely suppressed by procaine, but only occasionally decreased by diethylaminoethanol.

Analeptic effect of succinate, malonate, citrate and acetate on pentobarbital sodium JOSEPH P. ADAMS, JR. * AND EDWARD LARSON *Dept. of Physiology and Pharmacology, Univ. of Miami Med. Research Unit at Veterans' Hospital, Coral Gables, Fla.*

Certain compounds suggested by Soskin and Taubenhaus and others (*J. Pharmacol. & Exper. Therap.* 78:49, 1943, *Ibid.* 83:45, 1945) for analeptic activity, and other carbohydrate like compounds have been studied. Male Wistar rats, properly nourished, weighing 150-350 gm each were fasted 15-17 hours. The control group (29 rats) which received sodium pentobarbital 50 mg/

kg injected subcutaneously, averaged 100 minutes for duration of anesthesia and 142 minutes for duration of the loss of the righting reflex. No rats were used more frequently than once a month to avoid tolerance. The test groups each received an additional intraperitoneal injection of sodium succinate, sodium malonate, sodium citrate or sodium acetate, 1 gm/kg, as a 10% solution. The latter injections were made simultaneously with the pentobarbital. To determine the onset of and recovery from anesthesia, the paw of one of the extended hind legs was stimulated by a pinch at 2-minute intervals. Statistical analysis (J. H. BURN *Biological Standardization* 1937, p. 29) showed that succinate had a slight analeptic effect if any ($S.D. = 3.02$ for 28 rats) on the duration of anesthesia, while acetate showed a marked effect ($S.D. = 3.02$ for 28 rats) on the duration of anesthesia. None of the salts used had any significant effect on the duration of the loss of the righting reflex induced by pentobarbital except citrate which prolonged it significantly.

Comparative effects of epinephrine and arterenol-isopropyl arterenol mixtures RAYMOND P. AHLQUIST *Dept. of Pharmacology, Univ. of Georgia School of Medicine, Augusta*

It has been repeatedly demonstrated that although epinephrine is less active than arterenol as a pressor agent it is more active as an excitatory agent (vasoconstriction, uterine stimulation, contraction of retractor penis and nictitating membrane, etc.). One explanation for the smaller pressor effect of epinephrine is that unlike arterenol it is a very active vasodilator, and that this vasodilation diminishes its pressor effect. To test this theory various mixtures of racemic arterenol and isopropyl-arterenol were compared with racemic epinephrine on the arterial pressure, femoral arterial blood flow, and uterine and intestinal activity in the anesthetized dog. The following mixtures were compared with 0.001 M epinephrine.

MIXTURE	ARTERENOL	ISOPROPYL ARTERENOL
1	0.002 M	0.0005 M
2	0.003 M	0.0005 M
3	0.002 M	0.0003 M

Mixture 3 most closely approximated epinephrine. The most obvious difference was noted in the duration of the pressor response, the mixtures showed a more rapid decline from the pressure peak. This can be explained in part by different rates of inactivation of the 3 amines. These results give support to the theory that the pressor response to epinephrine is diminished by its simultaneous vasodilating action.

Cardiovascular and respiratory effects of β -diethylaminoethyl 4 n-hexyloxybenzilate HCl
 WILLIAM M. ALEXANDER AND WILLIAM K. McDONALD (introduced by BENEDICT E. ABREU)
Research Dept., Pitman-Moore Co., Indianapolis, Ind.

The cardiovascular and respiratory effects produced by intravenous administration of β -diethylaminoethyl 4 n-hexyloxybenzilate, a new local anesthetic, were studied and compared with dibucaine in both unanesthetized and anesthetized dogs. The effects produced by both drugs paralleled each other very closely. In general, the hexyloxybenzilate was one tenth as active as dibucaine in producing cardiovascular and respiratory effects. In unanesthetized dogs, 1.0–3.0 mg/kg of the benzilate ester produced a moderate increase in cardiac rate, smaller doses had no apparent effect. When 5.0 mg/kg or more was given, cardiac arrhythmias and a progressive decrease in heart rate resulted. Respiration was unaffected by doses of 3.0 mg/kg or less. Doses of 5.0 mg/kg or more often caused the animal to pant. Both drugs exerted a powerful vasodepressor action in dogs anesthetized with ether. The duration and degree of vasodepression was directly related to dosage. These blood pressure changes were not produced by vagal or other cholinergic stimulation, since they were not altered by bilateral vagotomy or adequate atropinization. With a dose of 1.0 mg/kg of the benzilate ester, there was a moderate increase in heart rate. After doses of 3.0–10.0 mg/kg there was a progressive decrease in heart rate which was associated with arrhythmias and transitory heart blocks. Identical effects are produced by doses of dibucaine about one tenth as great. Accordingly, these drugs appear to be direct cardiac depressants. Both respiratory rate and depth were decreased by vasodepressor doses of these drugs. Fatal doses appear to cause respiratory failure.

Effect of rutin and related compounds on experimental frostbite in rabbits ANTHONY M. AMBROSE, DOROTHY J. ROBBINS* AND FLOYD DEEDS
Pharmacology Division, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Dept. of Agriculture, Albany, Calif.

Following the demonstration by Fuhrman and Crismon (*J. Clin. Investigation* 27: 364, 1948) that orally administered rutin was effective on experimental frostbite in rabbits, similar studies were undertaken by us with rutin and related flavonoids. Experimental frostbite was produced in rabbits by the technique described by Fuhrman and Crismon. The various flavonoids were administered by stomach tube, one dose (100 mg/kg) immediately after freezing and daily doses on 3

following days. In 12 rabbits receiving rutin we have confirmed the observations of Fuhrman and Crismon. Other flavonoids—quercitrin (7 rabbits), quercetin (7), methyl hesperidin chalcone (4), 2-3-dihydroquercetin (6), and an extract of muscat raisin seeds (7) (prepared by Dr. C. E. Sando of this Bureau) all appeared to offer some degree of protection as compared to no protection in 20 controls. In the untreated rabbits tissue loss was the greatest, most of the frozen foot was involved. In the flavonoid-treated rabbits loss to the frozen foot was confined chiefly to the toes, with partial to complete loss of phalanges, and in a few instances with slight scarring of the dorsum. The suggestion of Fuhrman and Crismon that the protection afforded by rutin may be due to changes in the pattern of local blood flow may also be applicable to the other flavonoids studied on frostbite, and on the trypan blue escape time previously reported by us (*J. Pharmacol. & Exper. Therap.* 97: 115, 1949).

Release of nucleic acids from cell components by heparin NORMAN G. ANDERSON AND KARL M. WILBUR (introduced by FREDERICK BERNHEIM)
Dept. of Zoology, Duke Univ., Durham, N. C.

Heparin in low concentration has been found to increase the viscosity of rat liver brei, while high concentrations caused gelation. Removal of the nuclei from the brei prevented the observed increase in viscosity. Suspensions of nuclei exhibited an increase in viscosity on the addition of heparin further indicating that the nuclei were responsible for the viscosity increase of the brei. After centrifugal removal of the nuclei, desoxyribonucleic acid (diphenylamine reaction) was found in the supernate. Similar experiments with purified isolated mitochondria indicated that heparin causes the release of ribonucleic acid (orcinol reaction) without increasing viscosity. It is suggested that heparin with its strongly acidic sulfonic acid groups may displace nucleic acids from the basic proteins of the nucleus and the mitochondria. Dilute rat liver homogenates in sucrose-phosphate solution (pH 7.15) reached a maximum viscosity 4–8 minutes after the addition of heparin (70 μ g/ml, 23° C). The viscosity then fell, indicating depolymerization by enzyme action. The findings are discussed in relation to the inhibitory effect of heparin on cell division and the possible significance of high concentrations of heparin in the blood.

Anti-curare activity of 3-acetoxy phenyltrimethylammonium methylsulfate (Nu-2017) in man
 JOSEPH F. ARTUSIO, JR.,* WALTER F. RIKER, JR. AND W. CLARKE WESCOE
Depts. of Surgery (Anesthesia) and Pharmacology, Cornell Univ. Med. College and New York Hospital, New York City

Previous reports have described the pharmacology of Nu-2017 (*J Pharmacol & Exper Therap* 97 190, 208, 1949). The potent anti-curare action of this compound, previously described in cats, has now been demonstrated in man. Anesthetized surgical patients were given intravenous doses of D-tubocurarine chloride sufficient to reduce their respiratory minute volumes (RMV) to 40-60% (A), 20-40% (B), or 0-20% (C), of the control values. At the points of deepest curarization, various doses of Nu-2017 were given intravenously. The anti-curare action was evident within one minute, and maximal within 2, in every patient regardless of the dose of Nu-2017 injected or the depth of curarization. In the patients of group A a dose of 5.0 mg of Nu-2017 was sufficient to restore the RMV to the control level where it remained. In the patients of group B a Nu-2017 dose of 5.0-10.0 mg increased the RMV to approximately 80% of control. A second smaller dose restored the RMV to the control level. In the patients of group C a dose of 5.0 or 7.5 mg of Nu-2017 did not produce adequate ventilation although repetition of the dose produced a prompt restoration of the RMV to approximately 80% of control. In this group a large dose (15.0 mg) raised the RMV to over 60% of control where it remained constant, a second smaller dose of Nu-2017 returned the RMV to control. The only significant side-effect was a moderate cardiac slowing with the higher doses. None of these doses produced any signs of synergism with curare. The anti-curare action of related agents will be discussed.

Chemo- and pressor-reflexes from perfused heart and lungs of dogs DOMINGO M. AVIADO, JR., T. H. LI, W. KALOW, M. HESS and G. TURNBULL (introduced by C. J. LAMBERTSEN) *Lab of Pharmacology, Univ of Pennsylvania, Philadelphia*

Previous reports of reflexes from the heart and pulmonary circulation have been the subject of controversy chiefly because of the methods used. This investigation was undertaken using methods that we believe are less objectionable. With the aid of perfusion pumps and one or more donor dogs, each part of the cardio pulmonary circulation was perfused individually so that the flow, pressure and gas contents of the blood could be kept under reasonably complete control. Each factor could then be independently changed without directly affecting respiration and circulation. Veratridine which stimulates reflexes (*Federation Proc* 8 5 1949) was used for testing intact innervation of the perfused organ. Although veratridine still reflexly inhibited respiration and circulation through apparent chemoreceptors in the perfused lung, no chemo reflex sensitive to changes

in blood oxygen and CO₂ was demonstrable. Pulmonary congestion caused a reflex drop in systemic blood pressure and reflex inhibition of respiration. These receptors appear to be in the pulmonary veins and are connected centrally by the ipsilateral vagus. Raising pressure in the right side of the heart caused a bradycardia, contrary to the report of Bainbridge and others. There was an accompanying drop in systemic blood pressure independent of bradycardia. After vagotomy, the bradycardia disappeared and tachycardia appeared instead. There was a powerful reflex bradycardia from raising pulmonary arterial trunk pressure, also dependent on an intact vagus.

Hypothermic agents. II Effects on pyruvate utilization by brain JAMES A. BAIN AND R. M. KOHLENBRENNER (introduced by C. C. PFEIFFER) *Depts of Psychiatry and Pharmacology, Univ of Illinois College of Medicine, Chicago*

The effects of 3 hypothermic drugs were tested on fortified rat brain homogenates which were capable of uptakes of 10 microatoms of oxygen, 10 micromoles of pyruvate or oxalacetate and 20-30 μ M of inorganic phosphorus/50 mg (wet weight) tissue/30 min with P/O ratios of approximately 2.5. B-45 (4-amyln N-benzohydril pyridinium bromide) gave 50% inhibition of the system at 10^{-5} M, Privityne (alpha naphthyl methyl imidazoline HCl) at 5×10^{-4} M, and pyruvamide at concentrations twice the concentration (18 mM) of the pyruvate used as substrate. Inhibition of carbohydrate metabolism at this level is suggested as a possible mechanism of action of these agents in lowering body temperature.

A rapid and simple method for carbon dioxide analysis in anesthetic atmospheres ANN BARDEEN* AND O. S. ORTH *Depts of Anesthesia and Pharmacology, Univ of Wisconsin Med School, Madison, Wis*

Methods in general use for the analysis of carbon dioxide in anesthetic atmospheres have required either the transfer of gas samples to the apparatus or the use of quite complicated electronic instruments. By adaptation of the 'pocket model' of the Dwyer CO₂ indicator, made commercially for testing flue gas, it has been possible to obtain analyses in 30-60 seconds. The method is essentially a manometric procedure for measuring absorption of CO₂ by alkali. The samples are taken directly into the apparatus from the mixture in the breathing bag, mask, pharynx, or trachea of anesthetized patients. The accuracy has been determined by comparison with the Van Slyke manometric method. In the operating room, the apparatus has been used to study the elimination of CO₂ during anesthetic administrations, with a variety of techniques and with different rates of

gas flow Preliminary results indicate the practical utility of this instrument where rapid, simple analyses of CO_2 are desired Other applications of use for this compact, convenient analyser will be the study of the effect of altered metabolic states and decreased respiratory exchange on carbon dioxide elimination, the determination of the exhaustion of carbon dioxide absorbents, and the measurement of CO_2 accumulation in incubators and oxygen tents

Electrical action of adrenergic blocking agents

T C BARNES *Dept of Pharmacology, Hahnemann Med College and Hospital of Philadelphia, Philadelphia, Pa*

Sympathomimetic drugs are soluble in tri-glyceride oils on which parasympathomimetic drugs are inactive (*Science* 104 569, 1946, *Federation Proc* 8 272, 1949) Tyramine is used to detect adrenergic oils (0.05% gives a phaseboundary potential of 60 mv negativity on tributyrin) Adrenolytic drugs tested dissolve in adrenergic oils and establish a negative phaseboundary potential which blocks sympathomimetic agents by competitive inhibition 5 mg % veriloid (Coe Chemical) gave 60 mv negativity on equal parts triacetintributyrin suggesting that the *Veratrum viride* alkaloids probably reduce blood pressure by action on sympathetic nerve endings 0.05% C-7337 or 2-(N-p' - tolyl-N (m-hydroxyphenyl)-aminoethyl)-imidazole HCl has outstanding phaseboundary potential producing 136 mv negativity on tributyrin (compared with 45 mv for priscoline) Benodane or 2(1-Piperidylmethyl)-1,4-Benzodioxan produced 60 mv negativity on tributyrin (which however returned to zero in 15 minutes) A sympathetic cellular receptor was represented by a narrow tube of triacetin 3 mm in diameter on which 0.125% dibenamine produced the same potential (46 mv) as on a wide surface of triacetin 16 mm in diameter Drug molecules dissolved in a spot on a living cell would produce the same potential as when spread over the entire surface When the model receptor contained F dibenamine, benzedrine and other sympathomimetic drugs were without effect

Effect of carbon dioxide on phaseboundary potential of acetyl-beta-methylcholine in the presence of brain esterase

T C BARNES *Dept of Pharmacology, Hahnemann Med College and Hospital of Philadelphia, Philadelphia, Pa*

Five mg % of methacholine in Locke's solution containing 12 mg % bicarbonate (pH 8.0) gave phaseboundary potential of 25 mv negativity at a guaiacol interface in the oil-cell (*Science* 104 569, 1946) Five gm of fresh ground dog cerebral cortex was placed in each of two 400 cc portions which were stirred for 6 hours at 27°C with CO_2

bubbled through one The phaseboundary potentials were then determined in the oil-cell The original 25 mv was retained with CO_2 (pH 5.3) but the solution not treated with CO_2 (pH 7.9) had a potential of 15 mv negativity In another experiment 1 mg % methacholine with 30 mg % bicarbonate (pH 8.3) gave 10 mv negativity which was retained 5 hours in the presence of dog brain and CO_2 (pH 7.1) but reduced to zero by brain not treated with CO_2 (pH 7.8) In another run 17 mg % methacholine with 25 mg % bicarbonate in equal parts Locke solution and saline (pH 8.1) gave 40 mv negativity which was retained 4 hours in the presence of cat brain and CO_2 (pH 5.3) but reduced to 30 mv negativity by brain not treated with CO_2 (pH 8.1) The data may explain the slowing of the EEG in the acapnia of hyperventilation and the effects of CO_2 therapy in other conditions

Method of sensitizing guinea pigs to horse serum that yields after challenging of animals a 100% lethal response

WALTER E BARRETT (introduced by B N CRAVER) *Research Dept, Division of Macrobiology, Ciba Pharmaceutical Products, Inc, Summit, N J*

The problem of assaying the value of drugs in protecting guinea pigs from anaphylactic shock is rendered somewhat simpler if a method of sensitization be available that invariably leads to such a high titer of antibodies that a later injection of a shocking dose of the antigen, given after a suitable interval, always proves fatal Such a method is admittedly open to the objection that without immunological tests one does not know how much more than a surely fatal dose of antibody is present in each animal A possible second objection is that animals so sensitized might not be suitable for revealing the protective effect of a drug of low potency This second objection seem inapplicable to the antihistaminics, and the first objection can be met by employing in each test of an unknown drug a compound of known activity The method that realized the goal sought was the intra-hepatic injection of 2 doses of 0.05 ml of horse serum They were injected at the same time but in different places The results with other methods, including the use of adjuvants and vehicles (cf FREUND, F AND K McDERMOTT *Proc Soc Exper Biol & Med* 49 548, 1942), will be presented Wide variations in dosage and in routes of administration failed to accomplish what the intrahepatic method achieved The comparative anti-anaphylactic activities of Pyribenzamine and Antistine as determined by this method will be presented

Studies with gitalin (amorphous) for treatment of patients with congestive heart failure

R C BATTERMAN, A C DEGRAFF, L B GUTNER,*

O A ROSE,* AND J HOWE* *Dept of Therapeutics, New York Univ College of Medicine, New York City*

Studies with amorphous gitalin (mixture of glycosides obtained from the aqueous extract of *digitalis purpurea*), indicate that the preparation possesses advantages over other cardiac glycosides. The therapeutic ratio (therapeutic dose/toxic dose) obtained by multiple dose digitalization reveals an index of 36.9 as compared with 66.5, 58.0 and 60.6 for digitalis leaf, digitoxin and digoxin, respectively. The therapeutic range as determined by the percentage of patients presenting toxicity by doubling the minimal maintenance dose was 43.4. This greater range for amorphous gitalin as compared to digitalis leaf was demonstrated in 18 patients receiving both preparations for either maintenance or toxicity. For digitalis leaf, the percentage of patients who became toxic upon doubling the maintenance dose was 66.6, while that for gitalin was 33.3. Calculation of anticipated toxic dose of digitalis leaf on the basis of equivalent maintenance doses for gitalin and digitalis leaf indicates a 40.4% increase in therapeutic range for gitalin. Patients manifesting toxicity to digitalis leaf may take an equivalent dose of gitalin without toxicity. Digitalization was attained with ease. Maintenance was satisfactory and predictable. Dissipation was not as slow as digitoxin but not as rapid as digoxin. Toxicity although similar in type and incidence to other glycosides was less severe than digitoxin. On the basis of therapeutic range, predictability of dosage, and dissipation, amorphous gitalin is the digitalis preparation of choice for the usual treatment of the patient with congestive heart failure.

Acute toxicity of veriloid for some laboratory animals ROBERT O BAUER,* GEORGE L MAISON AND J W STUTZMAN *Dept of Pharmacology, Boston Univ School of Medicine, Boston, Mass*

The acute toxicity of Veriloid (J W Dart Labs' purified, reproducible extract of *Veratrum viride*) was tested in albino mice, rabbits of mixed sex and male Wistar rats. The LD₅₀ data are summarized in the following table:

	LD ₅₀	CONFIDENCE LIMITS (P = 0.05)	ROUTE
63 Rabbits	18.7	15.4-22.6	Oral
291 Rats	12.2	10.9-13.8	Oral
40 Mice	3.2	2.5-3.9	I.P.

The I.P. LD₅₀ value of Veratrone (Parke, Davis and Company) in albino mice—2.45 (1.8-3.2) mg/kg—when compared with the LD₅₀ value of Veriloid by the same route in the same species gave a po-

tency ratio of 1.26 (Veriloid/Veratrone). The 19/20 confidence limits of 0.9 to 1.76 indicate no statistically significant difference between the two LD₅₀ values. Rats and mice poisoned with Veriloid die within 2 hours regardless of route of administration. In the rabbit death may occur up to 24 hours with an average of 2-3 hours. Death is preceded by a series of predictable events. Within 5-8 minutes after administration of a toxic dose of Veriloid there is severe retching and profuse salivation. The hyperirritability that follows the retching usually leads to clonic convulsions. An occasional animal died without convulsing. Gross pathologic examination reveals only hyperemic adrenals.

Studies with low vapor concentrations of carbon tetrachloride labeled with carbon fourteen II Absorption and elimination upon skin exposure of monkeys W H BEAMER, D D MCCOLLISTER, G J ATCHISON AND H C SPENCER (introduced by D D IRISH) *Biochemical Research Lab and Spectroscopy Lab, Dow Chemical Co, Midland, Mich*

Monkeys received skin exposures to 1150 ppm (7.24 mg/l) radioactive carbon tetrachloride for 270 minutes and to 485 ppm (3.06 mg/l) for 240 minutes. Precautions were taken to prevent inhalation of the radioactive vapors. At the end of the 1150 ppm exposure, the monkey blood contained carbon-14 activity equivalent to 0.30 mg CCl₄/l of blood and the expired air contained activity representing 0.003 mg CCl₄/l of air. Complete elimination of radioactive material from the monkey body was rapid. Carbon-14 activity was not measurable in the blood after 48 hours nor in expired air after 5 days. The results from the skin exposure to 485 ppm were of a similar, but proportionately lower, order. Radioactivity was not measurable in blood 24 hours after exposure nor after 2 days in expired air. After the 270 minute skin exposure to 1150 ppm, the carbon-14 concentration of the monkey blood was equivalent to that reached in 24 minutes during the inhalation of 46 ppm radioactive carbon tetrachloride. Similarly, the blood concentration reached in the 240 minute skin exposure to 485 ppm was equal to 10 minutes of the inhalation of 46 ppm. Thus, skin exposures to vapor concentrations up to 1150 ppm carbon tetrachloride do not appear to constitute an industrial health problem.

Measurement of pathological pain in distinction to experimental pain HENRY K BEECHER, ARTHUR S KEATS* AND FREDERICK C MOSTELLER* *Anesthesia Lab of Harvard Med School at Massachusetts General Hospital, Boston, and Dept of Social Relations, Harvard Univ, Cambridge, Mass*

This report is a further development of the

method employed by Denton and Beecher for the study of pain relief by analgesics. The 978 subjects employed in elaborating this method over the last 3 years were postoperative patients in pain. Six members of the methadone series, morphine, pentobarbital sodium and saline have been used in developing the method and all were administered as unknowns both to the observers and to the patients. The order of administration of drugs was randomized according to sound statistical practice. The minimum number of patients (about 25) necessary for the determination of analgesic potency as well as the upper and lower limits of analgesic activity have been established. Saline has provided the lower and morphine sulfate 10 mg/150 lbs body weight, the upper since exceeding this dose does not give increased frequency of relief in routine trials. The use of morphine itself as an unknown gives an extraordinarily good check on the method. The accumulative data show that a variation in sensitivity to analgesic effects of morphine exists among patients, and the distribution of this variation is approximately normal. This observation provided the basis for a mathematical model which would provide a single or point estimate of the proportion of patients helped by a drug, when more than one drug was administered to the same patient. This model enabled us to eliminate some difficulties in our experimental design. From selected portions of the data, curves have been constructed relating the probability of being helped to the difficulty of being helped.

Pharmacological properties of 2,2-diethyl-1,3-propanediol (DEP) F M BERGER, B J LUDWIG* AND C RUSSO * *Wallace Labs, Inc, New Brunswick, N J*

DEP was prepared by the reaction of 2 ethylbutyraldehyde and formaldehyde in alcoholic potassium hydroxide solution. This substance is a white crystalline compound melting at 62°C, readily soluble in most organic solvents and soluble in water to the extent of 10% at 25°C. The toxicity of DEP is very low by all routes of administration. After large doses, DEP causes loss of the righting reflex and induces paralysis. Paralysis is preceded by excitation suggesting that the compound affects higher levels of the central nervous system than mephenesin. The anticonvulsant activity of DEP was evaluated according to the timed intravenous infusion method of Orloff *et al* (*Proc Soc Exper Biol & Med* 70:254, 1949). After intraperitoneal administration of DEP in doses of 200 mg/kg ($\frac{1}{2}$ of the mean paralyzing dose), metrazol in doses of 250 mg/kg can be injected i.v. without producing persistent convulsions. DEP also elevates the threshold to convulsions produced by strychnine and by picro-

toxin. It possesses stronger anticonvulsant properties than trimethadione and mephenesin. DEP is well absorbed, even after oral administration. It is rapidly inactivated in the body and has only a short duration of action. The lack of effect of DEP on multineuronal reflexes is rather interesting in view of its strong antagonistic action to strychnine.

Electrical action of glyketal R BEUTNER *Keeley Inst, Dwight, Ill*

Like mephenesin (tolserol, myanesin), glyketal relaxes skeletal muscle by depression of multi-neuron reflexes and crossed extensor reflexes (F M BERGER, 1949). When injecting this new drug in the lymph sac of frogs, it was found to diminish the electrical activity of the brain, also that of muscles and the heart. Diminution of brain activity by tolserol has previously been reported by Spiegel and Wycin in humans, being most pronounced in the electrical activity of the hypothalamus. Glyketal, like tolserol, produces a general lowering of the potential of the EEG and of the voltage of currents, the rate declined, in some cases to 3/sec. This diminution of EEG activity may be another factor in the quieting action of these drugs in tension states, alcoholism, muscle spasm etc.

'Benemid' (p-(di-n-propylsulfamyl)-benzoic acid)
—An anticatabolite its pharmacological properties. KARL H BEYER, VIRGIL D WIEBELHAUS,* HORACE F RUSSO,* HAROLD M PECK* AND SAMUEL E MCKINNEY * *Dept of Pharmacology, Med Research Division, Sharp & Dohme, Inc, Glenolden, Pa*

A principal mode of inactivation of a number of essential or therapeutically useful organic acids, such as p-aminobenzoic acid (PAB), salicylic acid, acetylsalicylic acid or p-aminosalicylic acid (PAS), is by conjugation with glycine to form the corresponding amides. In general, these amides are inactive therapeutically, and are eliminated rapidly by the kidneys. It has been found that 'Benemid' (p-(di-n-propylsulfamyl)-benzoic acid) and related compounds decrease the destruction of such organic acids by inhibiting their conjugation. In this sense, the agent may be thought to function as an anticatabolite. Since it permits a greater physiological economy of the therapeutic agent by inhibiting its inactivation or elimination, various uses for the agent are being explored in antibiotic chemotherapy, PAS therapy of tuberculosis, salicylate therapy of rheumatic fever and in the field of steroid physiological chemistry. 'Benemid' is absorbed rapidly following its oral administration to laboratory animals and to man. Following a single oral dose of the drug, a determinable and functionally useful plasma concentration persists in the dog for over

44 hours The compound *per se* is excreted so slowly that its renal clearance cannot be estimated, there being little or no drug in the urine It is metabolized very slowly and its metabolic products may be estimated in the urine The drug appears to be well tolerated by man and animals and is essentially non-toxic at useful dosages

Observations on effects of procaine, and diethylaminoethanol upon hemoglobin D D BONNYCASTLE, JOSEPH M WHITE* AND CARL S HELLIJAS* *Dept of Pharmacology and Toxicology, Yale School of Medicine, New Haven, Conn, and Dept of Anesthesiology, Hartford Hospital, Hartford, Conn*

It has been observed by one of us in some clinical cases receiving intravenous procaine, that cyanosis occurred in the nail beds, and that the blood, arterial and venous, oozing from the operative site appeared darker than normal, this was in spite of adequate ventilation with 100% oxygen This change in blood has been reproduced experimentally in the dog *in vivo* and in dog blood tested *in vitro* The hydrolytic products of procaine have been examined for this action and diethylaminoethyl alcohol alone was found to produce the change Studies in the dog upon the production of this change and its physiological importance, with respect to gaseous exchange, have been carried out These studies indicate that the color change is due to a modification of the hemoglobin, which change, when marked, results in a reduced oxygen carrying capacity of the hemoglobin Some observations have also been made upon blood samples obtained from humans receiving large quantities of procaine intravenously Attempts have been made to determine the mechanism of this change as well as the nature of the altered hemoglobin

Bromsulfonphthalein as a tool for study of liver physiology R W BRAUER, J S KREBS* AND R L PESSOTTI* *Dept of Pharmacology and Radiobiology, Louisiana State University School of Medicine, New Orleans*

Bromsulfonphthalein (BSP) uptake from the blood stream in dogs has been studied by means of the continuous infusion technique A steady level of blood and bile BSP can be attained by this means Dye extraction at infusion rates of 0.2 mg/kg/min or less occurs at a constant rate near 12% of circulating dye per minute With further increase of infusion rate a rapid decrease of extraction efficiency is observed Bile collection during such infusion has shown 1) that colorimetrically determined BSP concentrations of 1000 mg % represent the limit attainable in the dog 2) That colorimetric BSP excretion occurs at rates from 30 to 70% of the infusion rates Analysis of the

livers in such experiments shows that while some BSP accumulation occurs in that organ, a considerable amount of BSP remains unaccounted for Using S^{35} tagged BSP under similar conditions nearly quantitative recovery of S^{35} infused can be made from liver, blood and bile Chromatographic analysis of bile BSP shows that the S^{35} activity can be recovered in 4 fractions, different from BSP, none of which are colorless Quantitative relations among these components will be discussed in relation to infusion rates, and to conditions modifying biliary BSP excretion such as liver injury or bile stasis The significance of these findings in connection with the basic mechanism involved in BSP excretion and their bearing on the question of extrahepatic BSP uptake are considered

Protective action of sodium catechol disulphonate in acute poisoning by compounds of vanadium and cobalt H A BRAUN AND L M LUSKY* *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D C*

Disodium catechol disulphonate (DCD) injected intramuscularly in aqueous solution proved effective in the treatment of acute poisoning by ammonium metavanadate in rabbits, rats and pigeons In animals treated with DCD the fatal dose of the vanadate was about twice that of the untreated animals Treatment must be prompt, i.e. within 3 minutes However, if either sodium bicarbonate or citrate was injected simultaneously with the DCD, treatment might be delayed up to one hour Sodium succinate had no such effect The intraperitoneal LD_{50} of DCD for the rat was 3.75 gm/kg DCD appeared in the urine in from 30-45 minutes and continued to be excreted for about 2 hours Since rats injected intramuscularly with the fatal dose of cobalt sulfate survived for about 15 minutes, prophylactic treatment with DCD was resorted to Animals treated with DCD 30 minutes previous to the injection of cobalt sulfate survived otherwise fatal doses of this salt DCD was ineffective in poisoning by compounds of selenium, thallium and mercury

Some effects of procaine and diethylaminoethanol on the auricle and ratio of ventricular rate to auricular rate in the dog BARBARA BROWN, (introduced by G H ACHESON) *Pharmacology Dept, University of Cincinnati College of Medicine, Cincinnati, Ohio*

In dogs under dial-urethane anesthesia with chest open and artificial respiration, the right auricle was driven by electrical stimuli at varying rates (from 170/min up) Auricular electrograms and ECG's were recorded Before and after vagotomy the auricle responds regularly to stimuli up to 480-620 per min, the threshold increasing as frequency rises Intravenous procaine (8 mg/kg)

and diethylaminoethanol (32 mg/kg) decrease the maximal rate which the auricle will follow to 330-540 per min (8-33%). Diethylaminoethanol increases the threshold at rates of stimulation down to the lowest rate studied whereas procaine does not change the threshold in the lower range of frequencies. Both compounds decrease auricular conduction velocity. In this preparation, before and after vagotomy, the ratio of ventricular rate to auricular rate (V/A ratio) remains 1.0 up to auricular rates of 280-360 per min and reaches 0.5 or less at 420-550 per min. Procaine increases this ratio when it is less than 1.0 and usually shortens the P-R interval, whereas diethylaminoethanol decreases the ratio and usually lengthens P-R interval. The marked decrease of V/A ratio produced by vagal stimulation or by intravenous acetylcholine (0.08-0.12 mg/kg) was more effectively prevented by procaine than by diethylaminoethanol.

Effect of BAL on distribution of intravenously administered zinc using Zn^{65} H. D. BRUNER
Dept. of Pharmacology, Univ. of North Carolina, Chapel Hill, and Med. Division, Oak Ridge Inst. of Nuclear Studies, Oak Ridge, Tenn.

In studies on the toxicity of intravenously administered $ZnCl_2$ (1% sol., pH 6.1, L.D. 60-90 = 0.22 mm/kg) it was observed that 2 mol equivalents of BAL in propylene glycol given before or with the zinc completely eliminated the acute toxicity, but did not prevent the delayed death 72 to 96 hours later. In fact, BAL seemed to accentuate the renal damage, the chief cause of death. Subsequently, the distribution of zinc has been studied 72 to 96 hours after a single intravenous injection using Zn^{65} for tracing. Twelve rats received approximately 0.012 mm/kg of Zn^{65} intravenously of which 4 received simultaneously 50 mol equivalents of BAL in peanut oil and two got 62 mol equivalents spaced at 0, 24, and 48 hours. Four additional rats received 0.02 mm/kg of Zn^{65} plus 0.165 mm/kg of stable Zn, enough to give severe renal damage, two of the four received 3.3 mol equivalents of BAL intramuscularly at the time of the zinc. Control and treated pairs of animals were viviperfused 75 to 92 hours later, and tissues and separated excreta counted after pseudo-wet ashing with HNO_3 . In the control rats 31-42% of the zinc was excreted via the feces with 4% or less appearing chiefly on the first day. After BAL, urinary excretion increased up to 20 times and fecal excretion decreased. The red cell content of Zn^{65} doubled in the BAL rats. The content in 17 other organs and tissues examined, particularly the kidneys, was not affected by BAL. The high dose of zinc roughly doubled the amount in the kidney, but otherwise the data do not support the pathological interpretation.

Considerable tolerance of excitability of excised muscle to drastic changes in electrolyte concentration of immersion fluid E. H. BRUNQUIST AND J. B. HENDERSON (introduced by RICHARD W. WHITEHEAD) *Dept. of Physiology and Pharmacology, Univ. of Colorado School of Medicine, Denver*

Paired sartorius muscles (frog) were maintained in an extended condition, under a slight amount of tension, in test tubes containing 20 to 25 cc of immersate. One series of such preparations was maintained at 13° C during the survival of excitability, another series was kept at 25° C. Periodic determinations of the threshold to faradic stimulation were the criteria of alterations in excitability. Survival in conventional Ringer's fluid was compared on the one hand with survival in the same fluid diluted to varying extents with distilled water, or, on the other hand, with survival in Ringer's fluid modified by added amounts of NaCl or of all of the salts of Ringer's fluid. Dilution of Ringer's fluid to half strength did not reduce the average survival time, but a doubling of the concentration of all of the salts very definitely shortened survival of excitability.

A further study of the uterine action of aqueous extracts of corpus luteum HAROLD H. BRYANT,* C. JELLEFF CARR AND JOHN C. KRANTZ, JR.
Dept. of Pharmacology, Univ. of Maryland School of Medicine, Baltimore

A method of testing the uterine activity *in situ* of aqueous extracts of corpus luteum has been devised. Experiments were conducted on guinea pigs and rabbits. Intravenous injections of aqueous corpus luteum extracts were shown to decrease the tone and amplitude of uterine contractions in estrogen-primed animals. The relaxant response was not elicited under these conditions by progesterone or chlorobutanol. The latter is used as a preservative in corpus luteum preparation and relaxes the uterus *in vitro*.

Metabolic fate of evipal MILTON T. BUSH, THOMAS C. BUTLER AND H. LEO DICKISON
Dept. of Pharmacology, Vanderbilt Univ. School of Medicine, Nashville, Tenn.

We have reported previously (J. Pharmacol. 69: 277 and 72: 5) the isolation from dog urine, after administration of evipal, of three products. We presented evidence that these substances were 5-methyl-5-(Δ' -cyclohexenonyl) barbituric acid, I, 5-methyl-5-(Δ' -cyclohexenonyl)-1-methyl barbituric acid, II, and a substance isomeric with the latter, III. I (m.p. 213-217°C decomp.) has now been obtained from the urine of 2 dogs in amounts equivalent to 20% of the administered *nor*-evipal (5-methyl-5- Δ' -cyclohexenyl barbituric acid). This same substance (I) has also been

obtained from the urine of 2 other dogs in amounts equivalent to 5% of the evipal administered. Substance *II* has been obtained in 15% yield from these latter dogs, but *III* only from one of them, in 0.5% of the amount of evipal given. We have now established that *I* and *II* actually are ketones. The ovime from *I* was obtained in 60% yield, of pure substance, m.p. 250–260° decomp., the ovime from *II* in 50% yield, of pure substance, m.p. 185–7°C. Elementary analyses for C, H, and N are in reasonably good accord with the theoretical values for both these ovimes. The available supply of *III* was mostly used for analyses before an ovime preparation was made.

Isolation of β -nitropropionic acid from cultures of an *Aspergillus flavus* MILTON T. BUSH AND OSCAR TOUSTER * *Depts. of Pharmacology and Biochemistry, Vanderbilt Univ. School of Medicine, Nashville, Tenn.*

Studies of the production of penicillin-like substances by an *A. flavus* led to the incidental isolation of an apparently pure organic acid from a crude 'toxic fraction A' (*J. Pharmacol.* 84: 275, 1945). This substance (*I*) was reported to have m.p. 67.5–68.5° corr., neutralization equivalent 124, and apparent ionization constants pK'_1 3.7 and pK'_2 9.1. Elementary analyses were subsequently obtained which indicated the simplest empirical formula $C_3H_5NO_4$. The recent report (CARTER AND MCCHESENEY *Nature* 164: 575, 1949) that hiptagenic acid, a compound long ago obtained from a plant glycoside, has been found to be β -nitropropionic acid (*II*) (a type of compound occurring rarely in nature) led us to consider this structure for *I*. We have synthesized *II* from silver nitrite and β -iodopropionic acid (LEWKOWITZ *J. prakt. Chem.* [2], 20: 169, 1879) and also from sodium nitrite and propiolactone. That the compounds *I* and *II* are identical we have shown by the following comparisons: the melting points and various mixed melting points were identical, the results of nitrosation tests (Victor Meyer) for primary nitro groups were the same, titrations with sodium hydroxide, using glass electrodes, gave identical curves, which showed pK'_1 3.7 and pK'_2 9.0–9.1. Both compounds showed, in these titrations, the slow reaction with NaOH in the high pH ranges characteristic of primary nitro compounds. It remains to be determined whether the β -nitropropionic acid was actually produced by the mold, which seems very likely because of the large amount present, or was already present in the medium.

A contribution to the study of mechanism of action of cardiac glycosides ANNE CAMERON (introduced by B. N. CRAVER) *Research Dept.,*

Division of Microbiology, Ciba Pharmaceutical Products, Inc., Summit, N. J.

Feline hearts were perfused after the method of Langendorff with modifications previously described (*J. Pharmacol. & Exper. Therap.* 93: 135, 1948), and to the perfusion fluid were added the following drugs in the concentrations indicated, expressed as $\mu\text{g/ml}$: atropine sulfate, 1; Decamethonium bromide 50; papaverine hydrochloride 0.5; procaine hydrochloride, 15; Pyribenzamine hydrochloride, 2; quinidine sulfate, 5; 2[N-p-tolyl-N-(m'-hydroxy phenyl)-aminomethyl]imidazoline phosphate (C-7337), 0.5; m-tolyl-oxoacetamide hydrochloride (Su-212), 0.1. The concentrations employed were well tolerated, and, save for Decamethonium bromide, were in general sufficient, as demonstrated by previous experiment, to exert the characteristic effect of each compound. After an interval of perfusion with the drugs indicated, the sensitivity of the heart to Digifoline was ascertained, and was found to be essentially that of a heart perfused with normal fluid. These results and the fact that cardiac glycosides exhibit no qualitative differences in their effects upon the hearts of all the common laboratory species of animal would suggest that these drugs act directly on the contractile mechanism of the muscle cell or very close thereto. This circumstantial evidence lends support to the studies of Horvath *et al.* on the action of cardiac glycosides on the polymerization of Actin (*Nature* 164: 792, 1949).

Studies on the new 4-hydroxycoumarin anticoagulant no. 63 R. T. CAPPS,* W. D. BATTLE,* O. O. MEYER* AND O. S. ORTH *Depts. of Pharmacology and Medicine, Univ. of Wisconsin Med. School, Madison, Wis.*

The toxic effects of Dicumarol which appeared after its clinical introduction led to the synthesis of derivatives of the 4-hydroxycoumarin type having anticoagulant action. The 4-hydroxycoumarin anticoagulant no. 63 (2-methyl-2-methoxy-4-phenyl-5-oxodihydropyrano-3,2-c(1)benzopyran) which was found to be most promising, i.e. least toxic with approximately the same hypoprothrombinemia-inducing capacity as Dicumarol (SCHEEL *et al.*, 1949), has been further tested in dogs and has been given clinical trial. Dogs were fed single doses of 5, 10, 20, 50, and 100 mg/kg and daily doses of 5 mg/kg. No deaths occurred from single oral doses. Maximum hypoprothrombinemia, as determined by the Link-Shapiro modification of Quick's method using 100% plasma, was obtained in 3 to 5 days and prothrombin times returned to normal in 5 to 7 days. Six of 7 dogs which were fed 5 mg/kg/day died after 6, 9, 9, 18, 20 and 30 administrations, respectively. One dog survived 30 doses.

Pathological findings included hemorrhagic pleural effusion, hemopericardium, subcutaneous hemorrhages and massive uterine hemorrhage. Microscopic findings are presented. Effects of vitamin K and K_1 oxide and animal age on prothrombin reaction also will be reported. Capsules of 4-hydroxycoumarin anticoagulant no. 63 were administered to patients in single and repeated dosages. A single oral dose of 2 mg/kg usually was found to produce a desired prothrombin time within 24 to 48 hours which then could be maintained with repeated smaller administrations. The prothrombin times returned to pretreatment level in an average of 9 days. No evidence of hemorrhage, changes in differential blood count, urinalysis or hepatic function tests was noted.

Cholinergic effects of F 2268 EDWARD A. CARR, JR.,* MAX KARSCH* AND DOUGLAS S. RIGGS
Dept. of Pharmacology, Harvard Med. School, Boston, Mass.

The ethylal of γ trimethylammoniumpropylpanediol (Fournau Compound #2268) was found by Bovet to be the most potent known parasympathomimetic agent with regard to action on the dog's heart. Bovet showed that this compound produced typical muscarinic effects on a variety of tissues, but he was unable to demonstrate any ganglionic stimulating action. In view of the structural relationship of F 2268 to Methacholine—the former representing a cyclic isomer of the latter—we compared the effects of these 2 drugs given intravenously to cats under dial-urethane anesthesia. F 2268 and Methacholine were found to be quantitatively very similar in their ability to lower blood pressure. F 2268 appeared only slightly more potent in producing sinus bradycardia, but the duration of the slowing caused by F 2268 was very much greater than the Methacholine-induced bradycardia. When the dose of each was 10 micrograms, the mean duration of the bradycardia was 17 minutes for F 2268 and 38 seconds for Methacholine. Other effects on the heart, such as second- and third-degree auriculo-ventricular block and auricular fibrillation, appeared with greater frequency at a given dose of F 2268 than of Methacholine. When 10 mg of F 2268 was given to atropinized cats, a rise in blood pressure occurred. Thus, in contrast to Methacholine, F 2268 has a definite ganglionic stimulating action. No appreciable tachyphylaxis to the effects of F 2268 on blood pressure and sinus rate could be demonstrated.

Potentiation of curarizing action of diacetylcholine (succinylcholine) by aliphatic dicarboxylic acid aminoethyl amides JULIO C. CASTILLO* AND EDWIN J. DE BEER
The Wellcome Research Labs., Tuckahoe, N. Y.

At the meetings of the Pharmacology Society held at Indianapolis in November 1949, the authors showed that diacetylcholine has a very evanescent curare-like action which can be greatly prolonged by the injection of an anticholinesterase such as eserine. Evidence was also presented which indicates that the prolongation of action is due, at least in part, to the inhibition of the enzyme or enzymes responsible for the destruction of diacetylcholine. Examination of the aliphatic double amides of the general formula $\text{CO-NH-CH}_2\text{-CH}_2\text{-N(CH}_2\text{)}_n\text{-I}$ (CH_2) $_n$ $\text{CO-NH-CH}_2\text{-CH}_2\text{-N(CH}_2\text{)}_n\text{-I}$ reveals that although these compounds are virtually devoid of curariform action, they nevertheless potentiate the curare-like action of diacetylcholine in the cat and prevent the inactivation of the same by cat's serum *in vitro*. In these respects the amides act like eserine, but they differ from the latter in that they do not enhance the muscarinic action of acetylcholine on the dog's blood pressure and fail to antagonize curare. In addition, the amides fail to prevent the inactivation of acetylcholine by cat's serum *in vitro*. These differences suggest that the enzyme or enzymes which destroy diacetylcholine are probably not those responsible for the inactivation of acetylcholine.

Parasympathetic blockade by alpha-alpha-diphenyl-gamma-dimethylamino valeramide HCl RALPH J. CAZORT (introduced by KLAUS R. UNNA)
Dept. of Pharmacology, Univ. of Illinois College of Medicine, Chicago

Alpha - alpha - diphenyl - gamma - dimethyl amino valeramide HCl (BL-139) is a new antispasmodic with effects on the parasympathetic nervous system resembling those of atropine. On intravenous administration in mice it is twice as toxic as atropine. The action of BL-139 on guinea pig and rabbit ileum was predominantly neurotropic, being effective against acetylcholine contractions but, like atropine, exhibiting little effect against barium chloride induced contractions. BL-139 in the same concentration as atropine produced maximal dilatation of the rabbit pupil. However, duration of mydriasis was shorter. In man the compound was equal to atropine in suppressing salivation after oral administration and caused blurring of near vision in smaller doses than atropine. Small doses of BL-139 slowed the pulse rate, but doses larger than those of atropine were needed for acceleration. A similar quantitative difference in the effect on the pulse rate between atropine and BL-139 was observed on intravenous injection in unanesthetized dogs. BL-139 increased the subcutaneous LD_{50} of physostigmine in mice 98%, atropine 113%. Decrease in frequency and increase in voltage of brain waves in curarized cats were obtained alike with BL-139.

and atropine. Thus, BL-139 shows a remarkable similarity to atropine in potency and action. Its structural relationship to other parasympathetic blocking agents will be discussed.

Cardiac glycosides of *Tanghinia venenifera* K

K CHEN AND FRANCIS G HENDERSON *Lilly Research Labs, Eli Lilly and Company, Indianapolis, Ind*

Tanghinia venenifera is a shrub resembling oleander indigenous to the coastal regions of Madagascar. The natives there have prepared a poison, known as 'Tanghin,' from the kernels of the seeds, and used it as an ordeal which has resulted in a considerable loss of population. Only recently Frèrejacque and Hasenfratz (*Compt rend Acad d c* 222 149 and 815, 1946, 223 642, 1946, 226 268, 1948) successfully isolated tanghinin, desacetyltanghinin, tanghiniferin and veneniferin. By acetylation both tanghinin and desacetyltanghinin yield acetyltanghinin. Our experiments were directed to prove the cardiac action of these 5 glycosides. By the usual methods of study each of the glycosides was found to have a digitalis-like action in either amphibians or warm-blooded animals. They were thus subjected to cat assay according to our previous procedure (*J Am Pharm A* 25 579, 1936). The following figures were obtained with 10 cats each.

GLYCOSIDE	MEAN (GEOMETRIC) LETHAL DOSE \pm S.E., $\mu\text{g/kg}$
Tanghinin	352.4 \pm 38.8
Desacetyltanghinin	231.1 \pm 14.1
Acetyltanghinin	909.7 \pm 57.6
Veneniferin	369.6 \pm 22.0
Tanghiniferin	944.3 \pm 77.0

Relation of methylated xanthine retardation of development to cholinesterase values RALPH HOLT CHENEY *Dept of Biology, Brooklyn College, Brooklyn N Y and the Marine Biological Laboratory, Woods Hole, Mass*

Fertilized eggs and 8 specific developmental stages of *Arbacia punctulata* were observed for 3 days in sea water containing di- and trimethylated xanthines (pure alkaloids) in a series of concentrations from maximum solubility down to $\text{m}/10,000$. Retardations in developmental time schedules were greatest at the 2 celled stage and during the blastula-pluteus sequence. Concentrations less than completely inhibitory molarities were more drastic on the blastula-pluteus stages. Earlier effects may be due to a competitive substrate-enzyme-drug relationship or possibly to the surface action of methylated xanthines. Later effects may be due primarily to the inhibition of an enzyme, cholinesterase, essential to larval development. Relationship does exist between ChE activity and function during embryonic

development. Augustinsson and Gustafson have shown in the sea urchin, *Paracentrotus lividus*, that ChE activity is absent in fertilized eggs during the first few hours but a sudden rise occurs at the 20-hour period and progresses to high values in the pluteus larva. Assuming ChE activity is similar in *Arbacia*, this is the time when caffeine is most inhibitory. That a methylated xanthine, caffeine, is inhibitory to ChE has been demonstrated in nerve tissue by Nachmansohn, and, in muscle by Nachmansohn and Schneemann. Inter-relationships between methylated xanthine effects and cell division and growth probably involve other factors (ATP-ase) but the correlation between the high activity value of ChE normally during the stages at which these compounds are most inhibitory is of interest to cell life and to the pharmacology of these drugs.

Positive inotropic and other actions of fluoroacetate MAYNARD B CHENOWETH AND KOMOL PENGSRITONG * *Dept of Pharmacology, Univ of Michigan, Ann Arbor*

Considerations arising from current experiments suggested the possibility that sodium fluoroacetate (FA) might exert a positive inotropic action on the hypodynamic papillary muscle. It has now been demonstrated that 10^{-5} M FA exerts a marked positive inotropic effect on hypodynamic rabbit papillary muscles while those of the cat respond similarly to 10^{-3} M FA. Lower concentrations are but slightly effective while 10-fold higher concentrations are distinctly effective but result in subsequent depression. The prior presence of acetate (0.01 M) in the bath totally prevents the actions of FA on the muscle. Addition of ouabain in concentrations known to produce a marked and prolonged inotropic action (rabbit, 1.5 million or 3×10^{-7} M, cat, 1.10 million or 1.5×10^{-7} M) after suitable amounts of FA is almost completely without effect in glucose Ringer but does stimulate the muscle when the actions of FA have been prevented by acetate. Quadrupling the concentration of calcium ions in the bath produces a less than usual inotropic response after FA. The inotropic response to epinephrine does not appear to be much affected by prior presence of FA. Possible explanations for these phenomena will be discussed.

Chemotherapy of allergic arteritis in the rabbit JAMES CLAMPIT (introduced by D M GREEN) *Dept of Microbiology, Division of Biological Research, G D Scarle & Co, Chicago, Ill*

Consistent extensive arteritis was produced in the rabbit by intravenous injection of normal horse serum in a dose of 10 cc/kg on the first and 5th days of the experiment. The animals were autopsied on the 15th day. The most extensive lesions were found in the mesentery, heart, liver

and lung and occasionally in every tissue examined Daily medication was begun usually on the 7th or 8th day and continued until the end of the experiment ACTH was effective in preventing the development of arteritis Several antihistamines and sodium salicylate had some activity while rutin, estrone, diethylstilbestrol, testosterone, histamine and ascorbic acid were inactive at moderate dose levels

Hormonal treatment of the adult cat that assures a uterus in vivo highly responsive to oxytocics
 MARY LEE CLARY (introduced by B N CRAVER) *Research Dept, Division of Microbiology, Ciba Pharmaceutical Products, Inc, Summit, N J*

The voluminous literature on the action of hormones on the uterus has been recently reviewed (REYNOLDS *Physiology of the Uterus* (2nd ed), 1949) Methods have been described for rendering the immature lapin uterus responsive (WICK AND POWELL, 1942) or for rendering that same species of uterus more sensitive to progestational action (TRIPOD AND MEIER, 1948), but they seemed inapplicable to the present problem The following conclusions were reached after scores of experiments correlating vaginal changes with uterine responses a) Cats exhibiting the cornification of estrus are suitable for immediate use in assaying oxytocics b) Cats exhibiting vaginal proestrus are suitable after 3 days if injected intramuscularly with 10 mg T D of Ovocycin Dipropionate in oil c) Cats in other stages of the cycle require two injections of the estrogen, one on the 1st day and one on the 3rd day, after which they may be used for assay on the 7th day The cytological changes of estrus developed before the uteri had become responsive, which suggests that the myometrial changes induced require longer for their development d) Ovariectomized cats treated 2 weeks after operation by the procedure indicated in no 3 yielded poorly responsive uteri, the results were better if progesterone were injected from the 9th to 13th days after the first dose of estrogen Efforts to improve the motility of the uterus during the day of the experiment have been fruitless These efforts included the injection of variable doses of aqueous solutions of estrogen, of progesterone, of androgens, and of concentrated solutions of the vitamin B complex

Absorption, plasma levels and excretion of mescaline in the dog J COCHIN,* L A WOODS AND M H SEEVERS *Dept of Pharmacology, Univ of Michigan, Ann Arbor*

In this study we utilized a specific and sensitive colorimetric method, the details of which will be published elsewhere, capable of determining mescaline in concentrations above 0.5 $\mu\text{g/cc}$ of plasma and 2.0 $\mu\text{g/cc}$ of urine Plasma levels

of mescaline are compared after oral, intramuscular and intravenous administration to the dog Following intravenous injection of 20 mg/kg (base equivalent) of mescaline sulfate, there is initially a rapid decrease in plasma level This decrease is due probably to a redistribution of the drug from the plasma to various tissues and fluids of the body Immediately after injection the animal exhibits the expected autonomic signs, cerebral depression and hyperreflexia of the limbs After 20-30 minutes, the plasma concentration levels off at about 6 to 10 $\mu\text{g/cc}$ Thereafter, disappearance of mescaline from the plasma is gradual, being complete in 6-8 hours Rate of recovery from the pharmacological effects of the drug parallels the decay curve In contrast to the report of Slotta and Müller (*Ztschr f physiol chem*, 238, 14, 1936) mescaline is found in the urine of the dog Twenty-four hour recovery of urinary mescaline amounts to 25 to 40% of the administered dose Positive identification of this product was made by preparation of the crystalline picrate derivative and comparison of the mp and mixed mp with authentic mescaline picrate 3,4,5-Trimethoxyphenylacetic acid was isolated in dog urine confirming the work of Slotta and Müller

Studies on acute beryllium poisoning in guinea pigs KENNETH W COCHRAN,* MARCELLA MAZUR* AND KENNETH P DuBOIS *Univ of Chicago Toxicity Lab, and Dept of Pharmacology, Univ of Chicago, Chicago, Ill*

Recent investigations in several laboratories have shown that beryllium inhibits alkaline phosphatases We have observed that a depression of serum alkaline phosphatase occurs in rats following acute poisoning by beryllium and that the extent of this inhibition is dependent upon the dose of beryllium In order to obtain additional information concerning the relationship between phosphatase inhibition and acute toxicity of beryllium we have employed guinea pigs which were found to be less susceptible than rats to acute beryllium poisoning The 30-day LD_{50} was 6.3 mg/kg of beryllium as beryllium chloride administered intraperitoneally to guinea pigs whereas the LD_{50} for rats was about 0.6 mg Be/kg The alkaline phosphatase activities of all normal guinea pig tissues assayed, using sodium B glycerophosphate as the substrate, were lower than those of the corresponding rat tissues Beryllium was a less potent inhibitor of alkaline phosphatases of guinea pig tissues than of rat tissues The alkaline phosphatase activity of guinea pig serum averaged 0.058 mg P/gm/hr and the concentration of beryllium producing 50% inhibition *in vitro* was 3.6×10^{-4} M, while the corresponding values for rats were 0.390 mg P/gm/hr and 1.8

$\times 10^{-6}$ M Be The following alkaline phosphatase activities were found for other guinea pig tissues: duodenum, 21.4, kidney, 16.9, and liver, 0.49 mg P/gm/hr. The molar concentrations of beryllium producing 50% inhibition of these guinea pig tissues were as follows: duodenum, 2.5×10^{-6} , kidney, 4.0×10^{-6} , and liver, 2.8×10^{-4} . No inhibition of the serum alkaline phosphatase was observed in guinea pigs 3 hours after doses of beryllium as high as 17 mg/kg. Thus there was a correlation between the species susceptibility to acute beryllium poisoning and phosphatase inhibition.

Effect of some sodium sulfonates on tuberculosis in mice VERSA V. COLE, H. R. HULPIEU AND S. H. HOPPER* *Depts. of Pharmacology and Public Health, Indiana Univ., Indianapolis*

Mice were injected intravenously with 0.2 mg/mouse of a wet culture of *M. tuberculosis* (H37). The mice were divided into groups of 10 each and fed various concentrations of surface active agents. Records of weight and food intake were kept. The mice were killed at from 10 to 19 days and the lungs and liver were graded histologically from 0 to 4 plus. The 7 agents studied were classified on the basis of their effects on tuberculous involvement at concentrations which had slight or no effects on weight and food intake. The following showed an increase in the amount of tuberculosis found in the lungs: decyl benzene sodium sulfonate, dodecyl benzene sodium sulfonate, dibutyl phenyl phenol sodium disulfonate, monobutyl phenyl-phenol sodium monosulfonate. Inconsistent results were obtained with monobutyl diphenyl sodium monosulfonate. A decrease in the amount of tuberculosis in the lungs was shown by isopropyl naphthalene sodium sulfonate and N-methyl oleamide of sodium ethyl sulfonate. The effects in the liver were less marked but in the same direction as those in the lungs. When dibutylphenyl-phenol sodium disulfonate and monobutyl phenyl-phenol sodium monosulfonate were fed with promizole their unfavorable effects on tuberculosis were eliminated. When isopropyl-naphthalene sodium sulfonate was fed with promizole its effect was changed from favorable to unfavorable. N-methyl oleamide of sodium ethyl sulfonate showed the same effect with promizole as without promizole.

Factors influencing bile flow in the dog DONALD L. COOK,* DORIS A. BEACH,* ROBERT G. BIANCHI,* W. F. HAMBOURGER AND D. M. GREFEN* *Pharmacology Dept., Division of Biological Research, G. D. Scarle & Co., Chicago, Ill.*

Bile flow was studied in dogs anesthetized with sodium pentobarbital. The cystic duct was clamped and the common bile duct cannulated.

The volume of bile was measured at half-hour intervals and pooled hourly. Aliquots were analyzed for total solid content. No bile was returned to the animals. Basal rate of bile flow (cc/dog/hour) did not correlate with either surface area or body weight in a series of 260 dogs. Nine animals were observed for a period of 10 hours. During this interval the average rate of bile flow remained constant. The rate of total solid excretion decreased with time and tended to become asymptotic. The concentration of solids correspondingly declined. The bile output for a 30-minute period following intravenous administration of dehydrocholic acid in a dose of 20 mg/kg was studied in 88 dogs. The response was related to control level of flow and body weight, suggesting that it was determined by the total dose rather than by plasma concentration, which was presumably similar in all animals. This hypothesis was tested by comparing the bile output of the 8 largest dogs in the above series with 8 additional small dogs given approximately the same total dose. In addition, the 8 smallest dogs were compared with 8 additional large dogs. The results of these tests are in accord with the hypothesis that the response to dehydrocholic acid is determined by the total dose rather than by the dose per unit of body weight.

Some pharmacological properties of L-hydrazinophthalazine, a hypotensive agent B. N. CRAVER AND F. F. YONKMAN *Research Dept., Division of Macrobiology, Ciba Pharmaceutical Products, Inc., Summit, N. J.*

Among a series of phthalazines synthesized by Druey, the most active hypotensive agent appeared to be L-hydrazinophthalazine, an initial pharmacological study of which is to be reported (GROSS, DRUEY AND MEIER *Experientia* Inpress). The present report confirms and extends their observations. The compound has been shown to exert a hypotensive action in the following species: cat, dog, guinea pig and rat. Doses of less than 25 μ g/kg are devoid of action. Progressive increases in the dose to approximately 0.5 to 1 mg/kg have produced progressive declines in the blood pressure, but further augmentations in the dosage by many fold have not augmented the effect, although higher doses antagonized partially the action of epinephrine. The hypotensive action has developed slowly, suggesting either a metabolic alteration of the material to an active compound, or perhaps a slow accession to the structures susceptible to its effect. Rarely can any reasonable dose of the compound lower the blood pressure to below 70-80 mm Hg, at which point other hypotensive agents such as acetylcholine, histamine or the nitrites are still effective. The hypotensive effects can be counteracted by pitui-

trin, Privine, ephedrine, epinephrine and nor-epinephrine, although the antagonism exerted has been sometimes relatively brief. Preliminary experiments with the canine heart-lung preparation have suggested that doses up to 100 mg have no effect. Higher doses have been followed by decreases in the cardiac rate and output and by the appearance of irregularities. Doses up to 2 mg were almost devoid of action on the perfused feline heart. In 'spinal' dogs and cats the hypotensive effects have been minimal or absent. On various isolated tissues the compound has shown little effect. Detailed toxicological studies which will be presented have evidenced low toxicity after both acute and chronic administration.

Evaluation of simaroubidin in experimental canine amoebiasis ASHTON C. CUCKLER (introduced by HANS MOLITOR) *Merck Inst for Therapeutic Research, Rahway, N. J.*

Simaroubidin, a glycoside obtained from the plant *Simarouba amara*, is a highly potent therapeutic agent for experimental amoebiasis of dogs. Repeated oral doses of 0.5, 2 and 8 mg/kg eliminate the amoebae in severely infected dogs after 5-8 days of therapy. During a follow-up period of more than 30 days the dogs remained clinically well and it was impossible to demonstrate amoebae, microscopically or culturally, during this period. At autopsy there was no evidence of amoebic infection in any of the animals. Ten oral doses of 0.125 mg/kg were ineffective. A single dose of 5 mg/kg was ineffective, and one dose of 10 mg/kg 'cleared' the infection on the fourth day of therapy. Frequent examinations for one month were negative for amoebae, however, relapse occurred after 10 weeks. A single dose of 20 mg/kg was completely effective. Comparative studies indicate simaroubidin is more potent than many of the currently used amoebicides. The toxicity of simaroubidin varies considerably with different species. The rat, mouse, rabbit and chick tolerate much more than dogs. Daily oral administration of 2 mg/kg is tolerated by the dog for 11 weeks (55 doses) without any deleterious effects. Single doses of 8 mg/kg intravenously are likewise non-toxic. Intravenous doses of 1-50 mg/kg to anesthetized dogs had no effect on respiration, heart rate or blood pressure. Oral doses of 32 mg/kg and higher are lethal for dogs, with profuse gastro-intestinal hemorrhages developing in 2-3 days. Since simaroubidin is non-toxic in therapeutic doses for dogs, it seems justifiable to recommend clinical studies in human amoebiasis.

Failure to propagate mouse sarcoma 180 after exposure to low temperature WINDSOR C. CUTTING *Dept of Pharmacology and Therapeutics,*

Stanford Univ School of Medicine, San Francisco, Calif

Gye (*Brit M J* 1 511, 1949) and Mann (*Brit M J* 2 251, 253 and 255, 1949) recently reported that various mouse tumors could be frozen at 79°C for a month or more, and then lyophilized, and yet retain their ability to induce new growths upon implantation into mice. From 1-6 weeks were required for development of the tumor. The experiments were interpreted as supporting the thesis that a viral agent was implicated in these tumors because intact cells could not withstand this physical treatment. To test this thesis further, we removed 10 sarcoma 180 tumors aseptically from their host C₃H mice 7 days after implantation, and immediately stored them, dry, in tightly corked test tubes at -79°C. Nine weeks later, 4 tumors were removed, quickly thawed and cut into portions about 1 mm in diameter and implanted subcutaneously into 38 mixed male and female C₃H mice. Eleven weeks later, at sacrifice, no tumors could be found. A second portion of 4 tumors was removed after 14 weeks of freezing, ground in a chilled mortar, and immediately refrozen. One week later the powdered tumor was thawed, suspended in glycerin phosphate buffer, and injected subcutaneously into 15 mice. Five weeks later, at sacrifice, no tumors could be found. Attempts to transmit sarcoma 180 to mice after prolonged cooling of the tumors were unsuccessful, thus lending no support to the thesis that a virus is implicated in this tumor.

Analysis of DL-norleucine inhibition in *Achromobacter fischeri* EDWIN E. DANIEL (introduced by MARK NICKERSON) *Dept of Biology, Johns Hopkins Univ, Baltimore, Md*

DL-norleucine inhibits the growth of several microorganisms, apparently by inhibiting methionine utilization. In the luminous bacterium, *Achromobacter fischeri*, DL-norleucine inhibits growth in concentrations of 0.5 µgm/20 ml. Methionine behaved as a product of the inhibited reaction in every test. (The norleucine:methionine ratio for 50% inhibition increased with the norleucine concentration up to 5000 µgm/20 ml, with norleucine and higher sub-optimal methionine concentrations other compounds were less effective antagonists, etc.) Although tested less extensively, L-homocysteine, DL-cystathionine and DL-homoserine, but not DL-threonine and L-cysteine also antagonized norleucine as products of the inhibited reaction. DL-valine, L-leucine and DL-isoleucine behave as substrates or substrate precursors for the inhibited reaction. (The ratios of norleucine:substrate for 50% inhibition are constant and they antagonize norleucine inhibition less effectively in the presence of higher methionine concentrations.) All other common amino

acids, except threonine which was inhibitory, increase growth in the presence of norleucine and low sub-optimal concentrations of valine, leucine or isoleucine, but only L-glutamic acid, DL-phenylalanine, L-tyrosine, L-aspartic acid, DL-lysine, L-cysteine, in addition to methionine and the other products, were as effective at higher concentrations. Thiamine and pantothenic acid promote growth at both high and low substrate concentrations. Harding and Shive (*J Biol Chem* 174 743, 1948) found that many of the above compounds reversed or aided in reversing norleucine inhibition in *Escherichia coli*, but methionine, threonine and homocystine appeared to be substrates or substrate precursors while valine, leucine, and isoleucine seemed to be products of the inhibited reaction.

Technique for the screening in man of bronchodilating drugs

SIDNEY DANN* AND ROBERT C. BATTERMAN, *Dept of Therapeutics, New York Univ College of Medicine, New York City*

A battery of tests of ventilatory function has been used to determine the therapeutic response of asthmatics to the standard bronchodilating drugs. These tests include vital capacity determinations after normal and after complete expiration, maximum breathing capacity (rapid breathing) and deep breathing capacity (rapid and deep breathing), residual and complemental air values and oxygen deficit. Using two dose levels each of ephedrine, epinephrine and aminophyllin, 150 tests have been completed on 65 asthmatic patients. Twelve normal individuals and 8 asthmatics have been tested using saline. Each test consists of five 'runs', the first serving as a baseline and the battery of tests being repeated at one-half hourly intervals following parenteral administration of the drug. To better estimate the response in terms of the severity of the asthma at the time of testing, patients have been grouped as *Class I* (no obvious asthma at the time of testing), *Class II* (mild to moderate subjective distress and/or typical signs of auscultation) and *Class III* (severe attack). It has been demonstrated that the maximal breathing capacity and the deep breathing capacity which serve in effect as 'stress tests' are more sensitive indicators of response to therapy than the static vital capacity. The percentage improvement as measured by the former are larger than that of the latter and more indicative of the individual's ability to ventilate. In about 10% of the cases, especially those of *Class I* and *II* major changes in the stress tests have occurred measuring up to 100% improvement when the vital capacity showed no significant changes. The values accumulated in the testing of the three basic drugs in asthmatic therapy may serve as baselines for the screening of new drugs introduced for the treatment of asthma.

Chromatographic method for the isolation of DDT and other chlorinated insecticides from fat

BERNARD DAVIDOW (introduced by ARNOLD J. LEHMAN), *Division of Pharmacology, Food and Drug Admin., Federal Security Agency, Washington, D C*

In previous reports from this laboratory it has been demonstrated that DDT and several other chlorinated insecticides are preferentially stored in the fatty tissues of animals. Solvent methods for their extraction from these tissues always include the fat component. In many cases study of the storage and tissue distribution of these chlorinated insecticides is limited by the inability of the chemical method to determine small quantities of the insecticide in the presence of comparatively large amounts of fat. A convenient and rapid chromatographic procedure has been developed which is applicable to the isolation of DDT and several other chlorinated insecticides from fat. The procedure employs a column of Celite 545 (a diatomaceous earth), a mixture of sulfuric acid and fuming sulfuric acid (15% SO₂) as the immobile solvent, and carbon tetrachloride as the mobile solvent. A column of 30 grams Celite slurried with 9 ml of sulfuric acid, 9 ml of fuming sulfuric acid and 100 ml of carbon tetrachloride will retain approximately 5 grams of fat. It is held on the column as a brownish yellow band and the chlorinated insecticide is recovered in the eluate. Upon evaporation of the eluate, less than 1 mg of extraneous residue remains. Recoveries of microgram quantities of DDT added to 5 gm of butter oil free from DDT have ranged between 90 and 100%. Preliminary trials have indicated that, in addition to DDT, the method is effective for the separation from fat of benzene hexachloride, toxaphene and chlordane.

Pharmacology of a polymer with long acting vaso-depressor properties

PETER B. DEWS* AND EDWIN J. DE BEER, *Wellcome Research Laboratories, Tuckahoe, N Y*

Polymers prepared through the condensation of alkoxyphenylalkylamines with formaldehyde generally possess vasodepressor properties. The material resulting from the interaction of N-methylhomocanisylamine with formaldehyde is particularly active in this respect. Prolonged falls in the blood pressure of anesthetized dogs were produced by doses as small as 50 gamma per kg. Evidence was obtained that the depressor effect is due to the liberation of histamine.

Effect of salicylates on sensitization to horse serum and egg white

ROBERT H. DREISBACH, *Dept of Pharmacology and Therapeutics, Stanford Univ School of Medicine, San Francisco, Calif*

Salicylates have been alleged to act as anti-anaphylactic agents by preventing antigen-anti-

body reactions (CAMPBELL *Science* 108 478, 1949) Attempts were made to check this claim Six rabbits were fed a diet containing 1.67% sodium salicylate, the salicylate blood level being usually above 20 mg/100 ml One week later these rabbits and an additional 6 rabbits (on regular diet) were injected subcutaneously with 1 ml of a 1-10 dilution of horse serum in saline This injection was repeated weekly for 6 weeks Identical arthus type skin reactions occurred in both groups of rabbits after the third injection of serum, and continued to occur throughout the experiment Twenty-two guinea pigs were injected subcutaneously with 0.1 ml of horse serum on alternate days Four weeks later, 11 of these were given 0.5 gm/kg of sodium salicylate subcutaneously Two hours later, the 22 guinea pigs were injected intravenously with 0.1 ml of serum Of 11 salicylate treated guinea pigs, 7 died of anaphylaxis, of 11 controls, only two died Twelve rabbits were injected twice intravenously and once subcutaneously with 0.5 ml of egg white, on alternate days Four weeks later, 6 of these rabbits were given 3 injections of 0.3 gm/kg sodium salicylate gastrically at 5:00 P.M., 9:00 A.M., and 1:00 P.M. One hour after the last dose, the 12 rabbits were injected intravenously with 0.5 ml of egg white all showed cyanosis and respiratory distress, and 2 controls died Accordingly, sodium salicylate showed no demonstrable prevention of the sensitivity reactions used

Effects of 3 halogenated ethylamines on cardiac arrhythmias induced by epinephrine, nicotine and cyclopropane VICTOR A. DRILL AND HARRY W. HAYS *Dept. of Physiology and Pharmacology, Wayne Univ. College of Medicine, Detroit, Mich.*

Studies were made on the ability of 3 halogenated ethylamines to block induced cardiac arrhythmias Dogs were maintained with 16% cyclopropane anesthesia and ventricular arrhythmias induced by 1) epinephrine 2) nicotine and 3) 35% cyclopropane After the production of these control arrhythmias the animals were injected with either of the following drugs 1) N-(2-bromoethyl)-N-ethyl-1-naphthalenemethylamine HBr (SY28), 2) N-(2-(2-biphenyloxy)ethyl)-N-(2-chlorethyl)butylamine HCl (SY30), and 3) N-(2-chloroethyl)-N-ethyl-9-fluorenylamine HCl (SY21) The doses used were generally 1 mg/K, occasionally 0.5 or 2.0 mg/K Following the i.v. injection of the blocking agent arrhythmias were again induced by epinephrine, nicotine and 35% cyclopropane Epinephrine (usually 1-3 μ g/K) induced arrhythmias that were prevented by the administration of SY28, SY21 or SY30 In blocking the arrhythmias SY28 and SY21 reversed the blood pressure response to epinephrine, whereas after SY30 the blood pressure response was either normal or only

slightly reduced Nicotine (0.2-0.5 mg) produced arrhythmias that were also blocked by the drugs used, although often requiring a second dose of the blocking agent before complete block was obtained SY28 and SY21 generally prevented the arrhythmias and simultaneously reversed the blood pressure response whereas with SY30 blood pressure was not reversed when the arrhythmia was prevented All three compounds usually blocked the ectopic beats induced by 35% cyclopropane The difference in effect of the above blocking agents clearly shows that blood pressure reversal is not necessary in order to block induced arrhythmias This is further shown by the following Dogs anesthetized with 16% cyclopropane received repeated doses of nicotine at $\frac{1}{4}$ hour intervals Arrhythmias, which were induced by the first injection, were absent on repeated injections at a time when blood pressure had decreased only slightly from the original values obtained

Cholinergic action of alkyl pyrophosphoramides

KENNETH P. DuBOIS AND JOHN DOULL * *Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.*

Recent studies in this laboratory demonstrated that octamethyl pyrophosphoramidate (OMPA) exerts a cholinergic action in mammals after conversion by the liver to an anticholinesterase agent OMPA differs from other phosphorus containing cholinergic compounds in exhibiting a predominant action on peripheral cholinesterase The unique properties of OMPA stimulated our interest in searching for other phosphoramides with cholinergic activity These studies led to the development of 2 alkyl-substituted pyrophosphoramides which inhibit cholinesterase *in vitro* and *in vivo* and exert predominantly peripheral cholinergic effects These compounds are unsymmetrical diethyl di (dimethylamido) pyrophosphate and symmetrical diethyl di (dimethylamido) pyrophosphate The approximate LD_{50} of the unsymmetrical compound for rats is 2.6 mg/kg intraperitoneally and 4.4 mg/kg orally It inhibited cholinesterase 50% *in vitro* at a concentration of 2.8×10^{-7} M Twenty minutes after 1.9 mg/kg intraperitoneally the cholinesterase activity of brain, submaxillary, and serum from rats was inhibited 13%, 81%, and 99% respectively while 2 LD_{50} doses (5.2 mg/kg) produced 24%, 92%, and 97% inhibition respectively The LD_{50} of the symmetrical analog was 10.7 mg/kg intraperitoneally and 12.4 mg/kg orally for rats A concentration of 8×10^{-7} M produced 50% inhibition of rat brain cholinesterase *in vitro* Twenty minutes after 7 mg/kg intraperitoneally cholinesterase activity of brain, submaxillary, and serum of rats was inhibited 2%, 64%, and 85% respectively and 2 LD_{50} doses inhibited the activity of the tissues 30%, 89%, and 99% re

spectively These experiments demonstrated the anticholinesterase action of alkyl-substituted pyrophosphoramides *in vitro* and *in vivo*

Toxicity and anticholinesterase action of p-nitrophenyl dimethyl thionophosphate KENNETH P DUBOIS, MARCELLA MAZUR* AND KENNETH W COCHRAN* *Dept of Pharmacology, Univ of Chicago, Chicago, Ill*

The methyl analog of parathion, p-nitrophenyl dimethyl thionophosphate, is an ingredient of parathion preparations employed for insecticidal purposes in Germany Recent interest in the possible use of this compound as an insecticide in this country makes information on its toxicity and pharmacological actions desirable For toxicity studies solutions of the compound in 20% ethanol and 80% propylene glycol were employed The approximate LD₅₀ for rats was 3.6 mg/kg intraperitoneally and 12.7 mg/kg orally The sex difference in susceptibility previously observed with parathion was lacking with the methyl analog The methyl analog of parathion produced 50% inhibition of rat brain cholinesterase *in vitro* at a final concentration of 1×10^{-4} M as compared with a value of 1.2×10^{-6} M for parathion In 20 minutes after an LD₅₀ dose of the compound given intraperitoneally to female rats the cholinesterase activity of brain, submaxillary, and serum was inhibited 72%, 55%, and 25% respectively At the time of death in about 10 minutes after 7 mg/kg given intraperitoneally to female rats 68%, 57% and 30% inhibition of brain, submaxillary and serum cholinesterase respectively was observed The same dose of the compound given to male rats produced similar inhibition of brain and submaxillary cholinesterase but the serum cholinesterase which is lower in males than in females was 48% inhibited These studies indicate that the methyl analog of parathion resembles parathion in acute toxicity to rats and in anticholinesterase action *in vivo* after parenteral administration

Effect of various steroids on aerobic respiration of the brain of the castrate male rat EUGENE EISENBERG*, GILBERT S GORDAN* AND HENRY W ELLIOTT *Division of Pharmacology and Experimental Therapeutics and Division of Medicine, Univ of California School of Medicine, San Francisco*

Since steroidal hormones have been shown to have anesthetic properties (SELIE, H J *Immunol* 41 259, 1941) and to inhibit aerobic oxidation of glucose by rat brain cell suspensions (GORDAN, G S AND H W ELLIOTT *Endocrinology* 41 517, 1947) studies have been carried out to investigate possible mechanisms of these actions It has been found that deprivation of gonadal steroids by castration results in elevation of the rate of oxygen uptake by rat brain (EISEN-

BERG, E, G S GORDAN AND H W ELLIOTT *Science* 109 337, 1949) This rise is not found in tissues capable of utilizing endogenous substrates (EISENBERG, E, G S GORDAN AND H W ELLIOTT *Endocrinology* 45 113, 1949) Administration of testosterone propionate prevents this increased rate of cerebral metabolism An attempt has been made to determine whether this effect is specific for testosterone or general among the steroids Male rats, castrated at 30 days of age, were treated daily with 1 mg of steroid subcutaneously per kg of body weight for the 7 days preceding decapitation at the age of 60 days QO₂ of brain cell suspensions was determined by the direct method of Warburg using glucose as a substrate Untreated castrates and estradiol dipropionate-treated animals demonstrated elevated rates of oxygen uptake Brains from castrates treated with unesterified testosterone, testosterone propionate, methyl testosterone, testosterone cyclopentylpropionate, ethinyl testosterone, progesterone, or desoxycorticosterone acetate respired at normal rates From these studies it appears that steroids with the androstene nucleus are effective in restoring the 'braking' action normally exerted by gonadal steroids upon cerebral metabolism, as measured *in vitro*

Effect of sympathomimetic amines and sympatholytics on blood sugar and lactate of the rat SYDNEY ELLIS AND HILTON L ANDERSON* *Dept of Pharmacology, Temple Univ Sch of Med, Philadelphia, Pa and Dept of Physiology and Pharmacology, Duke Univ Sch of Med, Durham, N C*

The effects of several sympathomimetic amines on the blood sugar of the non-fasting rat were determined from tail-blood obtained before, and at 30-min intervals for 2 hours following, the intraperitoneal injection of several doses of each amine The following produce marked hyperglycemia in the amount in mg/kg indicated in parentheses after each compound Epinephrine (0.1), 1-(3,4-dihydroxyphenyl)-1-amino-2-methylaminoethane (2), Kephrene (2), Phenylephrine (2), 1-(3,4-dihydroxyphenyl)-2-methylaminopropane (30), 1-(3,4-dihydroxyphenyl)-2-aminopropane (50) The following amines did not raise blood sugar in the doses given in parentheses N-isopropyl-norepinephrine (50), 1-(4-hydroxyphenyl)-1-hydroxy-2-isopropylaminoethane (100), tyramine (100), hordenine (100), Paredrine (10), 1-(3-hydroxyphenyl)-1-amino-2-methylaminoethane (30), Ephedrine (30), mescaline (10), Forthane (50), Tuamine (10), Octin (50), Oenethyl (5), 1-cyclohexyl-2-methylaminopropane (30) Dihydroergotamine and Priscol prevent the rise in blood sugar which usually follows the administration of epinephrine Dibenzamine and SY-2S appear

to have a weak adrenergic blocking action on the rat liver. Blood lactate as well as blood glucose changes were determined after epinephrine, Keph-
rine, N-isopropyl-norepinephrine, and Octin. Ep-
inephrine and Keph-
rine elevate both glucose and
lactate of the blood. N-isopropyl-norepinephrine
raises only the blood lactate concentration. Octin
affects neither glucose nor lactate concentrations
of the blood. Following the administration of di-
hydroergotamine 3 mg/kg, epinephrine raises the
blood lactate in the usual fashion, but it does not
affect the blood sugar.

**Study of some antihistaminic drugs on the con-
vulsive threshold and on the action of anti-
convulsant drugs.** G. M. EVERETT (introduced
by R. K. RICHARDS) *Dept. of Pharmacology,
Abbott Labs., North Chicago, Ill.*

Recent clinical observations have suggested
possible dangers in the use of some antihistaminics
in epileptics (CHURCHILL AND GAMMON *J. A. M. A.*
141: 18, 1949). In the present study the effect of
Pyrabenzamine (P), Thienylene (T) and Benadryl
(B) on convulsive threshold in mice was investi-
gated. P, T, and B in subconvulsive dose (10-50
mg/kg i.p.) did not affect the electroshock thresh-
old for full tonic-clonic seizures. However, the
convulsant dose of Metrazol was lowered from
75-50 mg/kg subcutaneously. In doses of 75 to
100 mg/kg subcutaneously P, T, and B produced
clonic or running convulsions. These convulsions
were not modified by Tridione, Phenobarbital,
Phenurone or Dilantin in non-sedative doses.
The anticonvulsant action of these drugs against
Metrazol and/or electroshock was unaffected by
premedication with 10-50 mg/kg intraperitoneally
of P, T or B. Studies of possible antagonism by
P, T, or B of minimal protective doses of anti-
convulsants are in progress. Altho P, T, and B
produce sedation in clinical use, the underlying
convulsant properties of these drugs should be
considered when they are used in epileptic pa-
tients.

**Pharmacological studies of two new anticon-
vulsants 3,5-dimethyl-5-ethyl-oxazolidine-2,4-
dione (Paradione) and 3-methyl-5-phenyl-hy-
dantoin (Nuvarone).** G. M. EVERETT* AND R. K.
RICHARDS *Dept. of Pharmacology, Abbott Labs.,
North Chicago, Ill.*

Paradione is closely related to Tridione. In
mice the drug is more sedative and the ataxic dose
is 250 mg/kg. This is also the minimal protective
dose against Metrazol (100 mg/kg s.c.) convul-
sions. Duration of action is 3-5 hours. In non-
sedative doses Paradione is ineffective against
supramaximal electroshock convulsions. Chronic
toxicity studies in rats and dogs (100-200 mg/kg/
day orally) for 18 months revealed no changes in
blood count or organ damage. However the bone

marrow in dogs showed a hypoplasia which was
reversible when the drug was withdrawn. Tridi-
one acts similarly. Clinical trial for 2 years has
shown Paradione to be effective in petit mal epi-
lepsy and has a lower incidence of side effects than
Tridione. Periodical blood checks are essential
with either drug however. Nuvarone in doses of
300 mg/kg orally is effective against both electro-
shock and metrazol convulsions in mice and rab-
bits. The drug has a low toxicity, the fatal dose
being 2 gm/kg orally in mice. On high doses the
animals become ataxic and comatose. Death re-
sults from respiratory failure. Nuvarone is similar
to Mesantoin in its anticonvulsant properties.
Chronic toxicity studies in dogs and rats on doses
50-150 mg/kg/day orally for a year show normal
liver and kidney function. Blood counts and
bone marrow have also remained normal. Clinical
trial for a year has shown Nuvarone to be highly
effective in grand mal and in some cases of psy-
chomotor epilepsy. Its advantage is the low inci-
dence of side effects.

**Nonspecific factors in chemotherapy of trypanoso-
miasis.** PAUL L. EWING, HERSHAL G. TREE*
AND GEORGE A. EMERSON *Univ. of Texas Med
Branch, Galveston*

The importance of body defense mechanisms in
chemotherapy of experimental trypanosomiasis
has been stressed by several workers, e.g. the
influence of hepatectomy and splenectomy on the
course of trypanosomiasis has been described
(PFEIFFER AND TATUM *J. Pharmacol. & Exper.
Therap.* 53: 358, 1935). Treatment before or
after *T. equiperdum* infection with pentnucleotide,
desoxyribonucleic acid, reticuloendothelial im-
mune serum, malononitrile, X-ray and other
agents has no appreciable effect on the course of
infection in normal or splenectomized rats or mice.
Similar results are noted with *T. lewisi* infection
in rats. Mice infected with *T. equiperdum* die in
severe hypoglycemia, treatment with glucose has
an immediate analeptic effect on moribund ani-
mals, and alloxan diabetes prolongs survival in a
significant number of animals.

**Action of cardiac glycosides on experimental
auricular flutter.** A. FARAH AND T. LOOMIS
*Dept. of Pharmacology, School of Medicine, Univ.
of Washington, Seattle*

Self-perpetuating auricular flutter was produced
in anesthetized dogs by the method of Rosenblueth
and Garcia-Ramos (*Am. Heart J.* 33: 677, 1947).
Evidence presented by the above investigators
indicates a circus pathway in this type of flutter.
We have studied the action of cardiac glycosides
on this type of flutter. In the denervated heart
ouabain, digitoxin, Lanatoside C and tincture of
digitalis in a dosage of 50 to 60% of the toxic dose
produce a slowing of the auricular flutter rate and

a reversal to a normal rhythm. The maximum rate the auricle will follow when stimulated by repetitive stimuli is probably related to the refractory period of auricular muscle. Cardiac glycosides produce a 15-25% reduction in the maximum rate when injected in amounts which stop the auricular flutter. The excitability of auricular muscle is not changed significantly by the above amounts of glycoside though toxic doses produce a definite reduction in excitability. Experiments were also conducted on the heart with the nerve supply intact. In some of these experiments digtotoxin and Lanatoside C first produced a slowing of the auricular flutter rate followed by a sudden change to an auricular fibrillation. This change from flutter to fibrillation is mediated through the vagus nerve since cutting the vagus nerves or atropinization of the dog resulted in a prompt reversal of the auricle to a normal rhythm. These effects of vagal blockade can only be seen in dogs treated with cardiac glycosides. Furthermore, electrical stimulation of the vagus or a continuous infusion of acetylcholine converted an auricular flutter to a fibrillation. It is thus probable that the vagal effects of these glycosides result in the conversion of an auricular flutter to fibrillation.

Comparative pharmacology of khellin, visnagin and khellol glucoside EDWIN J. FELLOWS, KEITH F. KILLAM,* JOHN J. TONER,* ROBERT A. DAILEY* AND EDWARD MACKO* *Research Division, Smith, Kline and French Labs., Philadelphia, Pa.*

The present studies on the crystalline components isolated from *Ammi Visnaga* were carried out because of the controversial nature of the reports on their pharmacologic action. In isolated rabbit hearts, 1:100,000 and 1:10,000 2-methyl-5,8-dimethoxyfuranochromone (khellin) caused an increase in perfusion fluid flow of 25 to 40% and 40 to 90% respectively. In these concentrations, 2-methyl-5-methoxyfuranochromone glucoside (khellol glucoside) produced a decrease in flow. A slight but inconsistent increase in flow was observed after 1:100,000 of 2-methyl-5-methoxyfuranochromone (visnagin), however, a 35 to 80% increase was noted after 1:10,000. In anesthetized dogs, 20 to 50 mg/kg of khellin or 20 to 30 mg/kg of visnagin intravenously produced transient depressor effects. After these doses, a slight increase in cardiac rate and insignificant changes in the amplitude of cardiac contraction were recorded by means of a Cushman Myocardiograph. In 4 out of 6 experiments, 20 or 30 mg/kg of khellol glucoside produced depressor effects and slight to moderate cardiac stimulation. Khellin in 1:1,000,000 to 1:100,000 caused relaxation of the isolated rectal caecum of the fowl. In the same preparation visnagin exhibited approximately two thirds the activity of khellin but khellol

glucoside was inactive after 1:1,000,000 to 1:100,000. Oral doses of 200 to 1000 mg/kg of khellol glucoside failed to antagonize the effects of aerosolized histamine in guinea-pigs weighing 150-260 grams. Significant activity was noted in guinea-pigs after 200 to 1000 mg/kg of khellin or 500 to 1000 mg/kg of visnagin orally.

Acute pharmacology of dimethyl - 2 - chloro - 2 - phenyl-ethylamine FRANK C. FERGUSON, JR. (introduced by W. CLARKE WESCOE) *Dept. of Pharmacology, Cornell Univ. Med. College, New York City*

Dimethyl - 2 - chloro - 2 - phenyl - ethylamine (DMEA) possesses adrenergic blocking and parasympathomimetic properties. In cats, intravenous administration produces convulsions, respiratory depression, bradycardia, and secretory activity. Death occurs from respiratory failure. The intravenous LD₅₀ for the cat is 2.1 mg/kg. Poisoning in rats is similar. The slowing of the heart is abolished by atropine or vagotomy. In anesthetized cats, DMEA in small doses increases the rate and depth of respiration but in larger doses promptly abolishes it. In the cat, DMEA produces a sharp fall in blood pressure, this is annulled by atropine and replaced by a slower decline that is probably due to the removal of sympathetic tone. After small doses of DMEA the blood pressure usually returns to normal within 15-30 minutes but after large doses the pressure is lowered permanently. After adequate doses of DMEA the pressor effect of epinephrine is reversed, as are the pressor responses to asphyxia and to ganglionic stimulation. The pressor activity of norepinephrine or ephedrine is abolished, while the depressor action of Isuprel is not affected. The onset of blockade after DMEA is immediate and lasts for only two to six hours. These facts and its water solubility make DMEA unique among the adrenergic blocking agents of the beta-haloalkylamine type. In water DMEA hydrolyzes to an imine ring and finally to the ethanol derivative. The imine form possesses all the activity of the intact molecule while the completely hydrolyzed form seems inert.

Evaluation of local anesthetics for nerve block J. K. W. FERGUSON AND J. F. AIKENHEAD* *Dept. of Pharmacology, Univ. of Toronto, Toronto, Canada*

Potencies relative to procaine of several local anesthetics were determined by injecting 4 or 5 concentrations of each drug in a constant volume of 0.2 cc. behind the eyeball of the guinea pig. Loss of corneal reflex was used as the index of anesthesia. Epinephrine 1:100,000 prolonged anesthesia in this location only about 20%, 1:20,000 was no better. Relative toxicities for tissue were determined by intradermal injection in guinea

pigs Toxicity was measured by the diameter of erythema and by the occurrence of petechial hemorrhage The two criteria sometimes differed seriously The criterion of petechiae was probably better Relative Ratings, viz Relative Potency/Relative Tissue Toxicity, were calculated for piperocaine, tetracaine and dibucaine Only dibucaine was significantly better than procaine

Pharmacologic action of hydrazine E A FINE, ANNE M KUNKEL AND J H WILLS (introduced by AMEDEO S MARRAZZI) *Pharmacology Section, Med Division, Army Chemical Center, Md*

Intravenous injection of rabbits, cats and dogs with 50 mg/kg of hydrazine causes retching or emesis, tonic-clonic convulsions, dyspnea, fall in blood pressure and death Emesis and increase in tonus of the intestinal muscles of the dog were obviated by vagal section or by large doses of atropine It appears, therefore, that the effect on the smooth muscle of the gastro intestinal tract is indirect and mediated through the cholinergic fibers of the vagus nerve The fall in blood pressure is believed to be caused by both direct and indirect effects on the smooth muscle of the blood vessels Although hydrazine shows some direct action on peripheral structures, its most significant action probably is on the central nervous system A decerebrated cat exhibited tonic convulsions following injection of hydrazine Cats with spinal cord section showed tonic clonic convulsions central to the section, distal to the section there was occasional tonic activity uncorrelated with that going on above the section These findings suggest some stimulatory effect on the spinal cord as well as on the brain stem

Effect of diphenylhydantoin, temperature and phosphate concentration on P^{32} uptake by frog sciatic nerve E FINGL, D M WOODBURY, J R WARD AND J E P TOMAN (introduced by L S GOODMAN) *Depts of Pharmacology and Physiology, Univ of Utah College of Medicine, Salt Lake City*

Frog sciatic nerves in groups of four were incubated in neutral, isotonic solutions containing low (0.088 mM/l) or high (92.1 mM/l) concentrations of inorganic phosphate containing tracer P^{32} , at room (20°C) or refrigerator (4°C) temperatures, and with or without diphenylhydantoin (0.5 mM/l) for periods up to 62 hours (warm) or 160 hours (cold) After being tested for excitability, nerves were frozen and analyzed individually for P^{31} and P^{32} content of their inorganic, organic acid-soluble, lipid, and residue fractions The decreasing order of rate of P^{32} uptake was inorganic, residue, organic acid-soluble, and lipid Rate of uptake was increased 10-20 times in all fractions by the thousandfold increase in external phosphate concentration Q^{10} was 3 or greater for all organic

fractions Diphenylhydantoin usually produced a small decrease in P^{32} uptake, but probably not sufficient to explain the protection seen in all nerves against hyperexcitability induced by high phosphate concentration or excessive stimulation

Comparison of the chronic toxicities of synthetic sweetening agents O GARTH FITZHUGH AND ARTHUR A NELSON *Division of Pharmacology, Food and Drug Admin, Federal Security Agency, Washington, D C*

A comparison of the chronic toxicities of the synthetic sweetening agents dulcin (p-phenetyl urea), saccharin, P-4000 (1-n-propoxy 2-amino-4 nitrobenzene), and sodium cyclohexyl sulfamate (hereinafter called sulfamate) was made Albino rats, 21 days old, 18-24 to a group, were fed for their lifetime dosages of 1, 0.5, 0.1 and 0.01% dulcin, P-4000, saccharin and sulfamate in a stock diet Additional dosage levels of 5% saccharin and sulfamate, 0.25% dulcin and 0.001% P-4000 were used Results show that these four substances may be divided into a toxic group and a relatively nontoxic group Dulcin and P-4000 showed marked toxic effects whereas saccharin and sulfamate had only a slight effect on the animals Dulcin produced liver tumors with diameters up to 2.5 cm in animals fed dosage levels of 0.1% and above At 0.5 and 1% dulcin retarded the growth rate, increased the mortality rate and produced dark red spleens, at 1% the erythrocyte count was reduced and normoblasts occurred in the blood stream P-4000 at dosage levels of 0.5 and 1% retarded growth rate and caused kidney damage The bladders of some animals on the higher dosages of P-4000 contained much fine yellowish material Thyroid glands were colored with a brown pigment which increased with dosage level A slight retardation of growth occurred in the animals on 5% saccharin and sulfamate Also the 5% sulfamate produced marked diarrhea

Thiouracil derivatives in alloxan diabetes STERLING H FLY, JR, HARVEY HAYS, JR * AND G A EMERSON *Univ of Texas Med Branch, Galveston*
Houssay and Martinez (*Rev Soc argent de biol* 24 63, 1948) noted that several 2-thiouracil derivatives cause increased resistance to diabetic effects of alloxan when given repeatedly for 12-30 days prior to alloxan In screening a large number of pyrimidines for prophylactic efficacy vs alloxan, we (*Federation Proc* 6 238, 1947, *Proc & Trans Texas Acad Sci* 30 112, 1948) found 2-thiouracil, 6-propyl-2-thiouracil, 6-methyl-2-thiouracil, 2-thiothymine, 2-thiobarbituric acid and 2,4-dithiouracil ineffective in antagonizing an intraperitoneal dose of 1.5 mm/kg of alloxan when given intramuscularly 1 hour previously, in mice fasted 20-24 hours The thiopyrimidines were given in doses of 0.5-3 mm/kg, accord

ing to tolerance. Similarly, the 2-ethylmercapto derivatives of uracil, thymine and 5-methyleytosine were not prophylactic, and thiosinamine and alloxan-5-sulfonate were without action. Adenine thiomethylpentoside, 1 mm/kg, protected only 5/9 mice, thus being inferior to most other adenine compounds. Lack of acute effective prophylactic action of thiopyrimidines indicates that these agents do not block alloxan by competitive affinities, that their —SH groups are less effective than those of dimercaptopropanol or glutathione, and that effects obtained on repeated administration are referable to changed physiological status of the experimental animals rather than to presence of the thiopyrimidines.

Addiction liabilities of some drugs in the morphine series H F FRASER,* H G FLANARY,* R W HOEDE* and HARRIS ISBELL *Research Division, USPHS Hospital, Lexington, Ky*

The addiction liabilities of dihydrocodeinone (dicodide), 6-methyldihydromorphine ('6-methyl'), and 3-hydroxy-N-methyl morphinan (morphinan) were tested in former morphine addicts, by the single dose method for the detection of euphoria (all compounds), the single dose method for relief of abstinence from morphine (dicodide and '6-methyl') and by direct addiction (all compounds). Thirty mg of dicodide and 6-methyl or 10-15 mg of morphinan subcutaneously or intravenously induced morphine-like euphoria in these subjects. Forty-five mg of dicodide administered subcutaneously at the 30th hour of abstinence from morphine relieved withdrawal symptoms almost completely. Ninety mg and 120 mg of '6-methyl' administered at the 28th and 32nd hours of abstinence produced subjective relief but had only minor effects on objective signs. Five volunteers received the drugs for 32-38 days. The maximum daily dosages attained were 240, 180, and 60 mg for dicodide, '6-methyl' and morphinan respectively. During the experiment, behavior of the men addicted to the 3 drugs resembled that of men addicted to morphine. Complete tolerance to the sedative action was not attained. The electroencephalograms of subjects receiving dicodide and morphinan were slowed throughout addiction. The pain thresholds (modified Hardy-Wolff-Goodell) of men receiving '6-methyl' were not altered but the psychogalvanic response to thermal stimulation was reduced. The intensities of abstinence from dicodide or '6-methyl' were milder than would have been expected after withdrawal of morphine. These 2 drugs may therefore have some advantages over morphine. Abstinence from morphinan was approximately equal to abstinence from morphine.

Factors affecting tissue distribution following oral ingestion of lipid soluble substances JOHN

P FRAWLEY* AND O GARTH FITZHUGH *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D C*

Considerable discrepancies have frequently been noted in results of tissue distribution experiments performed in different laboratories and occasionally in the results of duplicate experiments in the same laboratory. A study of the various factors which may affect tissue distribution has been undertaken. The beta isomer of benzene hexachloride was selected as the first test substance because of its insolubility in water and its ready solubility in lipid-like substances. Analyses were carried out predominantly upon abdominal fat of albino rats. Marked differences in the storage of the beta isomer were noted in different sexes, with the higher storage consistently in female rats. Feeding of 200 ppm beta isomer to 2 groups of female albino rats, one group 12 weeks old and the remaining group 50 weeks old, showed, after the second week, a storage of 600 ppm in the fat of the younger rats and 225 ppm in the older rats. Increases in the percentage of fat in the diet, ranging from 0% to 8%, gave a corresponding increase in the storage of the beta isomer in abdominal fat. Substitution of an emulsifier (Tween 20) for 3% of the dietary fat gave an increase in the storage of the beta isomer.

Cutaneous absorption of phenol from intact and damaged skin MARIAN V FREEMAN,* ELSIE ALVAREZ* AND JOHN H DRAIZE *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D C*

Phenol preparations are used topically as antipruritics and as antiseptic dressings for the treatment of burns, wounds, and indolent ulcers. Fatal phenol intoxications have been reported following such use (T D CRONIN AND R O BRAUER *J A M A* 139 777, 1949, W B DEICHMANN *J Indust Hyg & Toxicol* 41 146, 1949). A preparation containing 2.35% phenol in corn oil was employed in the cutaneous exposures of the rabbit. The technique (J H DRAIZE, G WOODARD AND H O CALVERY *J Pharmacol & Exper Therap* 82 377, 1942) was altered slightly to permit 48 hours of continuous exposure. Data was obtained for a) intact skin, b) abraded skin, and c) skin with severe heat burn. Total urinary phenol values were determined daily for a 5-day period prior to exposure. Similar determinations were made during exposure and until urinary excretion figures returned to base line levels. Phenol determinations were made according to Schmidt's method (*J Biol Chem* 179 211, 1949). Normal urinary phenol excretion in the control (untreated) animal is approximately 2 mg/day.

The following urinary values were obtained during the 2-day exposure period

<i>Condition of Skin</i>	<i>Total mg of Phenol</i>
Intact	43
Abraded	65
Burned (heat)	100

Phenol is readily absorbed by intact skin as is reflected by the excretion value of 43 mg/48 hr of exposure. On the other hand, the phenol excretion values were approximately 50 and 130% higher in the subjects whose skins were damaged by simple abrasion and heat burn respectively.

Morphine-induced emesis in dogs treated with β - dimethylaminoethylbenzohydrylether - 8 - chlorotheophyllinate (Dramamine) HOMER B FREESE,* ROBERT G BIANCHI* AND W E HAMBOURGER *Pharmacology Dept, Division of Biological Research, G D Searle & Co, Chicago, Ill*

Dramamine (β - dimethylaminoethylbenzohydrylether-8-chlorotheophyllinate) has been found effective in the treatment of motion and radiation sickness. A common mode of activity might be depression of the vomiting center. The known emetic action of morphine sulfate in dogs offered a tool for testing this hypothesis. The incidence of vomiting in a group of 20 untreated animals following 0.5 mg/kg of morphine subcutaneously was found to be 80%. A total of 52 animals received pre-treatment. In each experiment half received Dramamine, either 5.0 or 10.0 mg/kg orally in capsule form, and the remainder received capsules of lactose placebo. The s.c. challenging dose of 0.5 mg/kg of morphine was given 45 minutes later and the incidence of emesis determined. In one experiment a crossover design was employed, on successive days the Dramamine and placebo treatments were reversed. No significant difference was noted between the effects of Dramamine and placebo treatment, either from the viewpoint of over-all incidence of emesis, or from that of individual experiments. The incidence of emesis in treated groups did not differ significantly from that for untreated controls.

Studies on metabolism of nicotine using spectrophotometric and radioactive techniques AARON GANZ* AND F E KELSEY *Dept of Pharmacology, Univ of Chicago, Chicago, Ill*

In the course of work on the preparation of radioactive nicotine, the use of the Beckman ultraviolet spectrophotometer was developed for the routine determination of nicotine in tobacco plant extracts (*Science* 108:558, 1948). This method was found to have considerable advantages of simplicity and sensitivity, concentrations of nicotine as low as 3-5 μ g/cc being accurately determinable. Two applications of this method to

animal work have now been made. In studies using a radioactive nicotine solution to perfuse the isolated guinea pig heart, the rate of uptake of nicotine from the perfusing fluid was studied by both isotopic and spectrophotometric techniques. Good correlation was obtained between the 2 methods. More recently the method was applied to the study of the urinary excretion of nicotine by the rat. The urine of rats injected with nicotine in amounts of 5-10 mg/kg was collected and steam distilled from successive acid and alkaline media. The final distillate was then concentrated and its absorption curve determined. A typical nicotine curve was obtained which represented 8-12% of the total amount injected. This percentage represented less than 500 μ g of nicotine excreted in the urine. The spectrophotometric method was used in these experiments along with tracer techniques to determine the proportion of changed and unchanged nicotine excreted.

Factors affecting electrogram of heart muscle

SOLOMON GARB (introduced by McKEEN CATTELL) *Dept of Pharmacology, Cornell Univ Med College, New York City*

Experiments performed on isolated cat papillary muscles indicate that the T deflection is normally in the same direction as the R deflection, i.e., upright, and that it becomes depressed and inverted following anoxia, injury and excess potassium. A lowering of calcium concentration below .216 mM results in a normal electrogram without any measurable contractile force. A marked increase in rate of stimulation produces inversion of the T deflection in normal Locke's solution but not in a solution containing less than a .216 mM concentration of calcium. Excess potassium produces inversion of the T deflection, widening of the R deflection and finally, a single, slow, upward deflection. Ammonium chloride produces marked widening of the R deflection, R-T interval, and an increase in contractile force. The theory that the T deflection is produced by membrane repolarization does not explain these observations. The need for revision of the present electrocardiographic theory is emphasized by these experiments.

Effects of mercurial compounds on saccharase

C L GEMMILL AND E M BOWMAN* *Dept of Pharmacology, Univ of Virginia Med School, Charlottesville*

A study was made of the effects of various mercurial compounds of pharmacological interest on the action of saccharase. Saccharase (Invertase) obtained from Nutritional Biochemical Corporation was diluted to a suitable concentration and placed in the side arm of a Warburg vessel. Sucrose in varying concentrations in an acetate

buffer at a pH of 4.5 was placed in the main compartment of the same vessel. When the mercurial compounds were used, they were mixed with the enzyme and were in contact with the enzyme for a period of 15 or more minutes before tipping over the enzyme and inhibitor solution into the sucrose solution. The experiments were carried out at 29.3°C and the reducing sugar was determined after 10 minutes with Nelson's method. It was found that there was a direct relationship between the concentration of the mercurial and the inhibition of activity of the saccharase. The degree of inhibition varied with the mercurial.

COMPOUND	FINAL CONC. (MOLAR)		% INHIBITION
	Drug	Sucrose	
Mercuric chloride	7.4×10^{-9}	0.08	16
Merbromin	9×10^{-8}	0.08	31
Mercurin	1.7×10^{-7}	0.16	22
Thiomerin	7.5×10^{-6}	0.16	22

Analysis of the relationship of the inhibition to concentration of substrate indicated competitive inhibition. The inhibition caused by mercuric chloride was reversed by the addition of 2,3-dimercapto-propanol (BAL).

Alleged gastric mucin deficiency in peptic ulcer

GEORGE B. JERZY GLASS AND LINN J. BOYD
(introduced by MICHAEL G. MULINOS) *Dept. of Medicine, New York Med. College, Flower and Fifth Avenue Hospitals, New York City*

To evaluate the controversial issue of a deficiency in gastric mucin as a contributing factor in the formation of peptic ulcer, 60 patients with peptic ulcer and 106 control cases were studied. Two hundred tests were performed on fasting gastric secretion: 50 after an alcohol test meal, 19 after the subcutaneous injection of 1 mg histamine, and 55 after the intravenous administration of 16U of insulin. On the 841 gastric specimens collected, we determined the total dissolved mucin or its 2 fractions of dissolved mucoprotein and mucoproteose (method of Glass and Boyd) as well as the volume and acidity of the specimens. There was found neither a deficiency of dissolved gastric mucin nor of its fractions in patients with peptic ulcer as compared to controls. The output of gastric mucoprotein which was derived from gastric glands was significantly increased in patients with duodenal ulcer, as compared to controls, both in the fasting stomach and following vagal stimulation with insulin. The concentration of gastric mucoproteose was significantly decreased in duodenal ulcer. However, this was due exclusively to dilution of this substance by the increased volume of the gastric

juice, since the total amount of gastric mucoproteose was similar to controls both under fasting conditions and after insulin. Since mucoproteose is a dissolution and split product of surface epithelium mucus, this finding does not offer any support to the theory that ulcer is due to a deficient formation or to an increased mucolytic disintegration of the protective layer of gastric mucus resulting in denudation of the gastric mucosa and formation of an ulcer. Hyperacidity in duodenal ulcer could be explained by an increased volume of HCl secretion alone and not by an alleged decrease in buffering power due to deficient secretion of gastric mucin.

Quinidine in propylene glycol by intramuscular injection in man JOSEPH GLUCK,* HARRY GOLD, WALTER MODELL, NATHANIEL KWIT,* SEYMOUR THICKMAN,* WILLIAM ZAHM* AND HYMAN BAKST* *Dept. of Pharmacology, Cornell Univ. Med. College, Cardiovascular Research Unit of Beth Israel Hospital and Cardiac Service of the Hospital for Joint Diseases, New York City*

In a previous report before this Society, a method was described for the study of quinidine action on the heart in patients by the effect on the F-F interval in the electrocardiogram. The oral route was used, but in practice many cases require the parenteral route. A preparation of quinidine sulfate in propylene glycol was made available to us, and the present report deals with its behavior by i.m. injection. It was tested in 19 patients for its local irritant action, and in 7 of these, for speed of absorption, cumulation, and duration of action. There were no troublesome local reactions in 17 patients who received only 1 dose of from 0.2 to 0.6 gm. One of the remaining 2 patients receiving repeated doses of 0.4 gm. every 6 hours for 3 days developed temporary local swelling and tenderness. The peak effect on the heart developed in from 2-4 hours after a single dose, and in about 24 hours after the repeated doses. Then cumulation ceased and the plateau persisted during the remainder of the 3 days of treatment. Approximately 75% of the maximum effect disappeared in about 8 hours. The curves of action were essentially similar to those after similar oral doses. These results showed that quinidine sulfate in propylene glycol by i.m. injection provides a satisfactory substitute in cases in which the oral route is not feasible.

Binding of barbiturates by rabbit tissue homogenates LEO R. GOLDBAUM* AND PAUL K. SMITH *Toxicology Section of Army Med. Dept., Research and Graduate School and Dept. of Pharmacology, George Washington Univ. School of Medicine, Washington, D. C.*

The extent of binding of various barbiturates

by rabbit tissue homogenates was investigated using the ultrafiltration technique of Lavietes. The tissues were homogenized with m/15 phosphate buffer pH 7.4 in an all glass tissue grinder. The final dilution of the tissue used for ultrafiltration was 1:4 and the barbiturate concentration was 0.0005 M. The barbiturate concentration was determined by means of ultraviolet spectrophotometry. The results show that liver homogenate has the greatest binding capacity for the barbiturates studied followed by kidney, plasma, brain and, red cells with muscle the least. Thio-pental was bound to the greatest extent to all the tissues studied followed by seconal, pentobarbital, phenobarbital and barbital. This order was identical to that found in ultrafiltration studies with crystalline bovine serum albumin, suggesting similar mechanisms involved in the binding of barbiturates by tissues. These results correlate well with the distribution of barbiturates in rabbits. The tissues having the highest drug concentration have the greatest binding capacity. The strongly bound barbiturates tend to concentrate in those tissues that have high binding capacities resulting in a high ratio of tissue concentration to plasma level. The weakly bound barbiturates tend to distribute themselves in the body water with a ratio of tissue concentration to plasma level near unity.

Effect of benzodioxane (933 F) on hypertension due to circulating nor-epinephrine (In man)

MARCEL GOLDENBERG AND HENRY ARANOW, JR (introduced by H. B. VAN DYKE) *Dept of Medicine, Columbia Univ College of Physicians and Surgeons, New York City*

Since nor-epinephrine as well as epinephrine has been demonstrated in pheochromocytomas, an investigation of the effect of piperidylmethylbenzodioxane on nor-epinephrine mediated hypertension in man was undertaken. An increase in systolic blood pressure averaging 44 mm Hg (range 20-93 mm) and in diastolic blood pressure averaging 25 mm Hg (range 14-40 mm) was produced in 19 normotensive subjects by the controlled intravenous infusion of nor-epinephrine. When the blood pressure became stabilized (with a constant rate of infusion of nor-epinephrine) 933 F in doses of 0.25 mg/kg was given intravenously over a period of 2 minutes. In every instance the injection was followed by a fall in systolic and diastolic blood pressure, the systolic fall averaged 22 mm Hg (range 4-46 mm) and represented 50% of the average increase produced by nor-epinephrine, the diastolic fall averaged 20 mm Hg (range 8-38 mm) and represented 68% of the nor-epinephrine mediated increase. The duration of the diminution in blood pressure produced by 933 F averaged 5½ minutes (range

190 to 570 seconds). A pheochromocytoma removed from a patient exhibiting persistent hypertension was analyzed by chemical means (partition chromatography on filter paper and von Euler's colorimetric method). More than 97% of its catechol fraction was nor-epinephrine and only a trace of epinephrine was found. Benzodioxane had been administered on 2 occasions to this patient prior to the removal of her tumor, and in each instance a significant fall in arterial tension resulted. The removal of the tumor relieved the patient of her hypertension. These data demonstrate that hypertension due to circulating nor-epinephrine in man is more susceptible to the adrenergic blocking action of 933F than animal data had suggested.

Effect of diuretics and nephrotoxic drugs on kidney ATP-ase

ANDRES GOTH, JAMES HOLMAN* AND VIRGINIA O'DELL * *Dept of Physiology and Pharmacology, Southwestern Med School, Dallas, Tex*

Certain diuretics and nephrotoxic drugs were found to inhibit adenosine triphosphatase activity of homogenized rat kidneys. The method used was that of DuBois and Potter. The ATP-ase activity of rat kidneys was of the same order of magnitude as that of the heart. The following drugs inhibited kidney ATP-ase in concentrations of 0.001 M or less: meralluride sodium, salyrgan, theophylline, mercuric chloride, caffeine and sodium benzoate, theophylline ethylenediamine, uranium acetate and aerosporin. Drugs which had no effect were urea, sodium chloride, pituitrin, phlorizin, digoxin, epinephrine, histamine, alloxan, sodium cyanide and bacitracin. Since all drugs which inhibited kidney ATP-ase are known tubular inhibitors or tubular damaging agents, it is postulated that these findings may reflect one of the mechanisms of drug induced diuresis and kidney damage. The results of *in vivo* as well as the *in vitro* experiments will be presented.

Synergism between bacitracin, polymyxin B, and streptomycin HCl *in vitro* tests with *Endamoeba histolytica* and organism *t*

MIRIAM GOULD AND EDER LINDSAY HANSEN (introduced by HAMILTON H. ANDERSON) *Division of Pharmacology and Experimental Therapeutics, Univ of California School of Medicine, San Francisco, Calif*

The activity of a combination of bacitracin, polymyxin B, and streptomycin HCl *in vitro* was determined using *E. histolytica* and organism *t*, an enteric, gram negative, spore-forming bacterium, in liver-proteose-peptone medium (Hansen). The compounds were prepared in aqueous solutions and observations made after incubation for 48 hours in cotton-stoppered and sealed cultures (HANSEN *Federation Proc* 8:299, 1949). Utilizing

the same test procedure, Bradin and Hansen found emetine hydrochloride to be amebicidal at 1 100,000-1 400,000 dilution, in both open and sealed cultures. Despite the relatively low activity levels of the 3 antibiotics, it was believed desirable to determine whether synergism might occur, and whether in combination, their potential usefulness as amebicides could be anticipated. Each agent, and particularly bacitracin, was inhibitory towards organism *t*. In combination these agents exhibited marked synergistic activity against the bacterium. Death of amebas occurred at approximately 1/16 the minimal apparent amebicidal concentrations of the separate compounds when these were added together to the open and sealed cultures. The final proof of value of the combination of bacitracin, polymyxin B and streptomycin in anti-amebic therapy rests on *in vivo* trials.

Effect of temperature on uptake of radioactive phosphate by human erythrocytes DESMOND R. H. GOURLEY* AND CHALMERS L. GEMMILL
Dept. of Pharmacology, Univ. of Virginia Med. School, Charlottesville

Radioactive phosphorus (P^{32}) has been used to determine the uptake of phosphate by human blood cells *in vitro*. The percentage P^{32} leaving the plasma in a given time is the same whether or not white cells are present. Thus, at least in the initial phase of the transfer, most of the P^{32} leaving the plasma is taken up by the erythrocytes. A study of this transfer process has been made at 9 temperatures ranging from 15°-40°C. At temperatures from 15°-22°C, the P^{32} uptake by the erythrocytes is so small that it approaches the absolute experimental error in the determination of the P^{32} remaining in the plasma (approximately $\pm 2\%$). From 22°-40°C the rate at which P^{32} disappears from the plasma is much greater and increases as the temperature rises. At 37°C approximately 50% of the added P^{32} has been taken up by the erythrocytes within 100 minutes. In the initial phase of the transfer, the reaction is of the first order. The data obtained at the various temperatures conform to the Arrhenius expression relating the transfer velocity to temperature. The activation energy, μ , calculated from the Arrhenius equation, is 16,700 calories. The marked difference in the transfer velocity over the relatively small absolute temperature range investigated indicates that the transfer process is chemical rather than physical in nature.

Chronic toxicity study of veriloid in rats and dogs JAMES T. GOURZIS, ROBERT O. BAUER AND WALTER W. JETTER (introduced by GEORGE L. MAISON) *Dept. of Pharmacology, Boston Univ. School of Medicine, Boston, Mass.*

One hundred and forty-two white Wistar rats and 12 mongrel dogs, separately housed and divided equally as to sex, were fed graded doses of Veriloid (J. W. Dart Labs' purified, reproducible extract of *Veratrum viride*) daily for 6 months. The rats received Steenbock's diet *ad libitum* whereas the dogs were presented with 15 gm/kg of horse meat 3 times daily. To these diets were added appropriate amounts of Veriloid. Food and drug consumption and weight were recorded weekly. Thirty rats and 3 dogs served as controls concomitantly with the experimental animals. At the end of 6 months each rat in the 'high' group had consumed an average of 7.44 mg/kg of Veriloid/day, while the 'medium' and 'low' groups had averaged 5.13 and 3.11 mg/kg respectively, equivalent to 70, 50 and 30 times the average daily/kg dose for humans as reported by Wilkins (*Proc. Soc. Exper. Biol. & Med.* 72:302, Nov. 1949). The weight growth curves were depressed in an amount roughly proportional to the dose of the drug. Adult rats appeared to be more resistant to the lethal effects of high dosage than young rats. The dogs averaged a daily consumption of 0.15-0.25 mg/kg. Emesis and refusal to eat the experimental diet accounted for the low dosage regimen in this species. Complete blood counts of both species and BSP tests of the dogs were unchanged from control values. Gross and microscopic pathologic changes will be discussed.

Cardiac action of certain sterols C. W. GOWDEY,* J. S. LOYNES* AND R. A. WAUD *Dept. of Pharmacology, Univ. of Western Ontario, London, Canada*

The cardiotonic effect of cholesterol, ergosterol, and certain phytosterols on the isolated frog heart was reported previously (*Federation Proc.* 8:344, 1949). This led us to investigate other compounds with closely-related chemical structures including cholesterol, stigmasterol, sitosterol, ergosterol, calciferol, cholic and desoxycholic acids, sodium dehydrocholate, glycocholate, and desoxycholate, estrone, estradiol, progesterone, pregnenolone, pregnandione, testosterone, androsterone, dehydro-isoandrosterone, androstanedione, and desoxycorticosterone. All of these with the possible exceptions of dehydro-isoandrosterone and progesterone produced a marked augmentation of the hypodynamic frog heart with little change in heart rate, and no tendency towards systolic standstill. This increased force of contraction occurred whether the compound was a) administered in fine suspension, in micro crystals, or b) dissolved first in propylene glycol or ethyl alcohol. The chief difficulty in testing sterols on the isolated rabbit Langendorff heart is their insolubility. The following compounds, however, have been investigated in concentrations varying from 2×10^{-4} to 10^{-5} sodium salts of dehydro-

cholic, desoxycholic, and glucocholic acids, the B-glucoside of desoxycorticosterone, sodium hemisuccinates of testosterone, cholesterol, dehydroisoandrosterone, pregnenolone, sodium estrone sulfate, and premarin. All of these except sodium cholesteryl succinate produced a large increase in coronary flow in the hypodynamic heart. The glucocholate, dehydrocholate, testosterone, estrone, premarin, and trans-dehydroisoandrosterone salts greatly increased the coronary flow, and markedly increased the force of contraction over a period of 5-20 minutes, but had little effect on heart rate. Desoxycholate and cholesterol salts were toxic and tended to cause systolic arrest. It is interesting that the sterols, which are chemically related to digitalis, should also have an augmenting action on the heart.

Effect of denervation on motility and response to atropine of terminal ileum and colon of the dog
 GRACE W. GRAY,* R. M. WHITROCK* AND M. H. SEEVERS *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor*

These experiments were undertaken to investigate the possibility of extending the atropine inhibition of intestinal motility by the surgical section of the extrinsic parasympathetic nerves supplying the terminal ileum and colon. Healthy trained mongrel dogs were prepared with a terminal ileostomy, ascending colostomy, and descending colostomy. Kymographic recordings of normal intestinal motility and response to atropine were obtained simultaneously in these 3 regions by means of the balloon-air-bromoform manometer method. Supra-diaphragmatic vagotomy was performed and recordings of motility and response to atropine were repeated. Next, the extrinsic parasympathetic inflow to the colon was sectioned at the sacral promontory. Recordings were again repeated. In other animals, sacral parasympathectomy preceded vagotomy. All recordings were obtained in unanesthetized animals after full recovery from surgery. A table indicates the normal and post-denervation responses to 0.5 mg atropine i.v. in terms of duration of action.

Rapidity of pituitary adrenocorticotrophic hormone (ACTH) release in response to stress
 WILLIAM D. GRAY AND PAUL L. MUNSON (introduced by D. D. BONNYCASTLE) *Dept. of Pharmacology, Yale Univ., New Haven, Conn.*

The mechanism by which certain stimuli cause release of ACTH from the pituitary is being investigated in the rat. Reduction in adrenal ascorbic acid concentration serves as the criterion indicating increased ACTH secretion. Control rats (Holtzman, 80-120 gm) under Nembutal anesthesia are unaffected by saline injections into the tail vein, while i.v. histamine, 0.3-0.4 mg/100 gm, results in a marked decrease in adrenal as-

corbic acid. Aqueous adrenal cortex extract (Wilson) (ACE), 1.0 ml/100 gm, i.v., 2-10 seconds before histamine almost completely inhibited the ACTH release. If ACE injection was begun 3 seconds after histamine, inhibition was less marked but still present. If injection of ACE was delayed until 5-10 seconds after histamine, little if any inhibition was observed. These results confirm the findings of Sayers and Sayers that high blood levels of adrenal cortex hormones tend to prevent the release of ACTH by the pituitary. They also indicate that the pituitary release of ACTH in response to histamine is very rapid.

TREATMENT	NO. OF RATS	ADRENAL ASCORBIC ACID
		mg/100 gm
Controls	22	444 ± 8
Histamine (H)	21	286 ± 7
ACE, 5-10 sec after H	15	310 ± 21
ACE, 3 sec after H	9	341 ± 24
ACE, 2-10 sec before H	10	419 ± 10

Effects of desoxycorticosterone glycoside on cardiac and renal function
 D. M. GREEN AND A. D. JOHNSON,* W. C. BRIDGES,* J. H. LEHMANN* AND F. GRAY *Dept. of Medicine, Univ. of Washington School of Medicine, Seattle*

Desoxycorticosterone glycoside (DCG) was administered to 13 human subjects by continuous intravenous infusion at the rate of 20 mg/square meter/hr. Blood pressure, glomerular filtration rate, renal plasma flow, sodium and water excretion were measured during two 20-minute control periods and three 20-minute periods of drug administration. Simultaneous measurements of cardiac output and total peripheral resistance were made in 6 of the subjects. The most pronounced action of DCG on renal function was an increase in water output. The effect on sodium excretion was variable. A net increase was observed in 7 patients and a decrease in the remainder. Urinary sodium concentration showed a consistent reduction which averaged 12% below control levels. Glomerular filtration rate and renal plasma flow rose moderately at the start of infusion and reverted toward control levels with its continuation. The filtration fraction remained essentially unchanged. No significant change in blood pressure followed DCG administration. A small net fall in cardiac output was observed which coincided with an average rise in total peripheral resistance of 15% above control values. Some correlation was noted between direction and magnitude of the changes in peripheral resistance, filtration rate and plasma flow.

Production of anesthesia in mucous membranes by procaine and physostigmine
 MARGARET E.

GREIG, W. C. HOLLAND* AND P. E. LINDVIG*
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We have previously reported that the selective permeability of living cells appears to be related to the activity of the acetyl choline—cholinesterase system. This was shown in mammalian erythrocytes (*Arch Biochem* 23:370, 1949) and in the blood brain barrier of the frog (*Science* 110:237, 1949). It is well known that procaine is not an effective surface anesthetic as it does not appear to penetrate the mucous membrane. We have found, however, that when procaine and physostigmine were together instilled into the eye (rabbit or dog) the corneal reflex was rapidly abolished (2–3 minutes) and good anesthesia was produced. Physostigmine alone had no such effect. In a series of 19 rabbits and 11 dogs it was found that the effects of physostigmine and procaine together were significantly different from those of procaine alone.

Actions of L-hydrazinophthalazine (C-5968) on vasomotor reflexes and hypertension in dogs and man. K. S. GRIMSON, J. R. CHITTUM* AND B. H. METCALF* *Dept of Surgery, Duke Univ School of Medicine, Durham, N. C.*

L-hydrazinophthalazine (C-5968) has been given to normal dogs, dogs with 'neurogenic hypertension,' and patients with 'essential hypertension.' Two mg/kg orally given dogs with hypertension several months after elimination of depressor nerves produced average reduction of blood pressure in 7 from 202 mm Hg to 114. One mg/kg i.v. in 5 caused reduction from 196–84, 2 mg/kg i.v. in 6 from 218–89, 1 mg/kg i.m. in 6 from 218–126, and 2 mg/kg i.m. in 7 from 218–90. In 8 patients 20–125 mg i.v. produced moderate reduction of pressure in 4 and definite reduction in 4. The pressor response with breath holding was blocked in 4 and with a hand in ice water, blocked in 1 and depressed in 2 of 5 tested. Toe temperature rose in 5 of 7 tested. Administration of 100–250 mg/patient orally in 6 tests variably effected reduction of pressure, block of reflexes, and warming of toes. Chronic treatment of patients with hypertension effected no persistent reduction of blood pressure. During 4 experiments in dogs 2–5 mg/kg gradually reduced blood pressure during $\frac{1}{2}$ hour, an effect lasting several hours. Pressor response with occlusion of carotid arteries was reduced or blocked, that with 1–3 μ g/kg of epinephrine was depressed, though with anoxia and central vagus stimulation pressor response was not depressed. In 4 dogs 25–27 mg/kg did not prevent normal increase of blood pressure with increased intracranial pressure.

Studies on some smooth muscle effects of 3-hydroxy - N - methylmorphinan hydrobromide

(Nu-2206) E. G. GROSS AND R. M. FEATHERSTONE *Dept of Pharmacology, State Univ of Iowa College of Medicine, Iowa City*

The effect of Nu-2206 on gastric, intestinal, and colonic motility, pyloric sphincter tone and motility, tone and capacity of the bladder, and urethral resistance to the outflow of urine were studied on unanesthetized dogs. Small intestinal activity was also observed in one human patient with a Maydl jejunostomy. Intestinal and gastric activities were studied by the water-filled balloon technique, and pyloric sphincter action by Quigley's air-filled triple balloon method. Bladder and urethral measurements were obtained by using Winter's modification of methods described by Langworthy. Dosages of 0.05–0.10 mg/kg were used for intestinal studies in the dog, with a total of 2 mg subcutaneously being used in the human subject. For the bladder measurement, 0.025, 0.05, 0.075, 0.1 mg/kg were used. Typical morphine-like reactions were obtained in the GI tract studies, namely loss of gastric motility, increased tone of the pyloric sphincter, upper and lower ileum and colon. These observations suggest that Nu-2206 might produce constipation, however, in over 300 postoperative cases in the University hospital, this has not been a noticeable feature of the drug's action. Larger doses have not been used in the dog because of the high incidence of vomiting when such doses are given. In the dose range studied, this drug has no significant effect upon the tone and capacity of the bladder or upon urethral resistance to the outflow of urine.

Effect of certain analgesics upon intestine and uterine smooth muscle. CHARLES M. GRUBER, JR., CHARLES M. GRUBER AND KWANG SOO LEE* *Dept of Pharmacology, Jefferson Med College, Philadelphia, Pa.*

An attempt was made to determine the relative activities of Nu-2206 (3-hydroxy-N-methylmorphinan), metapon, morphine, Nu-1196 (dl- α -1, 3-dimethyl-4-phenyl-4-propionyloxy piperidine hydrochloride), methadone, and demerol upon segments of excised and intact rabbit uteri, excised rabbit intestine, and the intact intestine of the unanesthetized dog. The effect of Nu-2206 and Nu-1196 on excised uterus was stimulation. The minimum concentrations for which activity could be determined were 1/200,000 for Nu-1196 and 1/100,000 for Nu-2206. For all concentrations recovery was complete. Morphine had about 1/10th the activity of Nu-2206. The intact rabbit uteri were weakly stimulated by the intravenous injections of Nu-1196 and Nu-2206. Depression of the excised rabbit intestine occurred on the application of either Nu-1196, demerol, methadone or morphine. In general, the effects of equal

concentrations of demerol and Nu-1196 were about the same. These drugs were about 5 times as active as morphine and were one fifth as active as methadone. The action on excised intestine was opposite to that of acetylcholine and barium chloride. The intact intestine of unanesthetized dogs responded with approximately similar increases in tone following the i.v. administration of 0.001 to 0.005 mg/kg Nu-2206, 0.005 mg/kg metapone, 0.01 mg/kg morphine sulfate and 0.1 mg/kg of either Nu-1196, methadone or demerol. With large doses the height of the rhythmical contractions and peristaltic activity was decreased due to the increased general tonus.

Inhibition central nervous system produced by chronic cocaine intoxication CARLOS GUTIERREZ-NORIEGA *Dept of Pharmacology, Universidad Mayor de San Marcos, Lima, Peru*

The intravenous injection of cocaine induces, in cocaine addicted to this drug, states of general inhibition of the central nervous system. The inhibitory states, accompanied by plastic rigidity of the skeletal muscles (catalepsy), were found in 30% of the addicted dogs studied. States of excitation, with complex hyperkinesia, precede and ensue the states of inhibition. In the course of the inhibition produced by cocaine, general analgesia and symptoms of parasympathetic stimulation were always present. Stimulants of the central nervous system (caffeine, harmaline, desoxyephedrine, ephedrine) and atabrine, potentiate the motor and sensitive inhibitions produced by cocaine in the addicted dogs. Alkalines, ethyl alcohol and cholinergic drugs also potentiate, in lesser degree, the cocaine inhibitory actions. Barbiturates, at subnarcotic doses, antagonize the motor inhibition produced by cocaine in the addicted dogs, and restore the voluntary movements. The general analgesia produced by cocaine in the addicted dogs is suppressed by barbiturates. A very rapid recovery of normal sensibility follows the injection of barbiturates. It was also shown that barbiturates restrain the symptoms of parasympathetic stimulation caused by cocaine in the addicted dogs. The stimulant central action of cocaine is not antagonized by barbiturates, on the contrary, it is increased in high degree in some cases. This apparent reversal of barbiturate depressive action is due to the fact that these compounds remove central inhibitions. Fatty and vascular degeneration of the liver cells was found in some of the cocaine addicted dogs.

Studies on pharmacology of dramamine LEONARD B. GUTNER,* WILBUR J. GOULD* and ROBERT C. BATTERMAN *Dept of Therapeutics, New York Univ College of Medicine, New York City*
Dramamine (beta-dimethylamino-ethyl benzo-

hydril ether γ -chlorotheophyllinate) has recently been introduced for the prevention and treatment of labyrinthine disorders, especially motion sickness. In an effort to ascertain its pharmacologic action, the cold microcaloric test and galvanic stimulation of the mastoid area were utilized, the former depending upon the integrity of the peripheral end-organ of the labyrinth and the latter upon an intact vestibular nerve tract. Sixteen patients underwent 26 trials, each of which consisted of a control period and serial measurements following the oral administration of one of these drugs, dramamine 100 mg, diphenhydramine 50 mg, hyoscine hydrobromide 0.6 mg, allyl 1-methyl-butyl barbituric acid (seconal) 100 mg, theophylline ethylenediamine (aminophylline) 500 mg, and γ -chlorotheophylline 50 and 100 mg. It was found that dramamine markedly depressed labyrinthine function as judged by its effect upon the onset, and duration and character of the induced nystagmus and the milliamperage necessary to produce tilting. Subjective vertiginous sensations were also significantly reduced. The drug produces these effects by acting both upon the vestibular end-organ and the nerve tract, peripherally or centrally. The other drugs had no significant action upon labyrinthine function although in some instances the induced dizziness (subjective) was decreased probably due to a central sedative action.

Hypothermic agents I 4-amyli N-benzohydril pyridinium bromide (B-45) and alpha naphthylmethyl imidazoline (privityne) JULINA GYLFE, ROBERT HENDRICKS, SIDNEY OCHS and H. L. WILLIAMS (introduced by C. C. PFEIFFER) *Dept of Pharmacology, Univ of Illinois College of Medicine, Chicago*

B-45 produces hypothermia in mice, rats, and guinea pigs in doses of 1-3mg/kg subcutaneously. In rats, the fall in body temperature is accompanied by hyperglycemia, glucosuria, diuresis, and a decrease of 32% in the basal metabolic rate. A dose of 2mg/kg intravenously in anesthetized dogs has a marked pressor effect which is diminished by adrenalectomy and completely blocked and reversed by adrenergic blocking drugs such as SY-28. The pressor effect is not altered by nicotine, tetraethylammonium chloride, or transection of the cervical cord. B-45 resembles epinephrine in its peripheral vasoconstricting effect. In the cat, it causes retraction of the nictitating membrane and maximal dilatation of the pupil. The hypothermic effect of Privityne is not confined to lower species of animals, but is also seen in cats and man. A table of toxicities demonstrates that it becomes more potent as animals of higher developmental levels are tested. The minimum effective dose for producing a significant fall in

body temperature of the rat is 25mg/kg subcutaneously

COMPARATIVE TOXICITIES (LD 50)

SPECIES	ROUTE	B-45 (mg/kg)	PRIVANT (mg/kg)
Mouse, 20 gm	Intravenous	1.3	170
	Subcutaneous	1.6	
	Intraperitoneal	1.5	
	Oral	35.0	
Rat, 200 gm	Subcutaneous	4.0	385
	Intraperitoneal	8.0	
	Subcutaneous	15-20	
Guinea pig			
Rabbit	Intravenous	10-12	0.8
	Subcutaneous	35-40	0.95
Dog	Intravenous	30-40	0.1
	Effective pressor dose intravenous	2-5	
Man	Effective oral dose	>750 (total dose)	5-10 (total dose)

Absorption, distribution, and excretion of sodium fluoroacetate (1080) in rats ERNEST C HAGAN, L L RAMSEY AND GEOFFREY WOODARD (introduced by A J LEHMAN) *Division of Pharmacology and Division of Food, Food and Drug Admin., Federal Security Agency, Washington, D C*

Adult rats were given single doses of aqueous sodium monofluoroacetate by stomach tube (5.8, 3.3, and 1.8 mg/kg). The rats dosed at the 5.8 mg/kg level were killed at the end of 5 hours and the organs pooled in their respective categories for analysis, (*J Assoc Off Agric Chem* 32:788, 1949). 1080 was found in concentrations of about 5 ppm in the heart, kidney, brain, skin plus hair, and carcass, and only about 1.5 ppm in the liver. Only about 1% of the ingested 1080 was found in the urine and feces. Twenty-five of the 60 rats dosed at the 3.3 mg/kg level died within 24 hours and the balance were sacrificed at the end of that period. Analysis of the organs of this group showed values for living and dead animals respectively of liver 0.5 and 0.6 ppm, kidney 0.7 and 1.6, heart 1.0 and 2.2, brain 0.9 and 2.0, carcass 0.8 and 1.8, skin 0.9 and 1.8. Of the 1080 administered, 17% remained in the stomach and small intestine of the living rats and 22% in those dying. The total 1080 accounted for was 39% in the living and 71% in the dead animals. The rats dosed at the 1.8 mg/kg level were killed at the end of 48 hours. The urine and feces collected

during this period contained only 10% of ingested 1080 and the entire bodies about 10%.

Effect of long-acting antihistaminic drugs on capillary bed THOMAS J HALEY AND MARGARET ANDERSON * *School of Medicine, Univ of California at Los Angeles*

Using the method previously described (HALEY AND HARRIS *J Pharmacol & Exper Therap* 95:293, 1949) several of the long acting antihistaminic drugs have been evaluated for their capillary effects. All of the drugs caused closure of the precapillary sphincters, a decreased flow through the capillary bed and leukocyte sticking. The degree of activity was directly related to concentration.

Antagonism of cholinergic triad by β -diethylaminoethyl xanthene-9-carboxylate methobromide (Banthine Bromide) W E HAMBOURGFR, DONALD L COOK* AND D M GREEN *Pharmacology Dept., Division of Biological Research, G D Scarle & Co., Chicago, Ill*

Acetylcholine mediates the transmission of impulses in 3 areas of the peripheral nervous system. These sites, which form the cholinergic triad are a) all autonomic ganglia, b) the neuro-effectors of all parasympathetic and some sympathetic nerves, and c) somatic neuro-muscular junctions. Antagonists are known for the action of acetylcholine at each of these 3 sites. Blockers for (a) include nicotine and tetraethyl ammonium (TEA), for (b) the atropine alkaloids and Dibutoline, and for (c) curare and bistrimethyl ammonium decane (C 10). The blocking action of each of these drugs is directed predominantly against a single cholinergic function. Evidence exists which suggests that several are capable of antagonizing more than one function. β -diethylaminoethyl xanthene-9-carboxylate methobromide (Banthine Bromide) blocks all 3 cholinergic actions at specific dosages. Small doses of Banthine approach atropine in neuro-effector anti-cholinergic action. Moderate doses equal TEA in producing superior cervical ganglion blockade and have twice the potency of TEA against pelvic nerve stimulation of the bladder. Large non-lethal doses exert a reversible curare-like effect on the sciatic-gastrocnemius system. Lethal doses produce generalized flaccid paralysis with consequent respiratory failure.

Effect of thyroxine and dinitrophenol on renal functions CARROLL A HANDLEY *Dept of Pharmacology, Baylor Univ College of Medicine, Houston, Texas*

The subcutaneous administration of 0.5 mg/kg of thyroxine daily for 5 days to 4 dogs resulted in an average increase in renal functions as follows: glomerular filtration rate 40% (range 21-56%), renal plasma flow 46% (range 36-60%), and maxi-

mal rate of dextrose absorption by the renal tubules (TmG) 49% (range 32-67%) Dinitrophenol produces similar changes in renal functions An hour after the intravenous administration of 5 mg/kg of dinitrophenol to 5 dogs, the average increases in glomerular filtration and TmG were 38% (range 19-50%) and 33% (range 20-41%) respectively In the latter experiments, renal plasma flow was not measured because metabolic products of dinitrophenol interfere with p-aminohippurate determination These observations seem to indicate that these metabolic stimulants activate additional nephrons in the kidney and that thyroxine has no specific action on enzyme systems concerned in the reabsorption of dextrose from the renal tubule as has been suggested (*Am J Physiol* 140 699, 1944)

Pathological muscle changes produced in guinea pigs by deficiency of antistiffness factors and effect of ergostanyl acetate LAWRENCE W HANLON,* N CHANDLER FOOT,* JANET TRAVELL AND SEYMOUR H RINZLER * *Depts of Pharmacology and Anatomy, Cornell Univ Med College, New York City*

Twenty-one guinea pigs were maintained on a diet deficient in antistiffness factors as described by Oleson *et al* (*J Biol Chem* 171 1, 1947) The course of deficiency alone was studied in 11 animals, in 4 the diet was supplemented by wheat germ oil, and in 2 by cod liver oil The effect of ergostanyl acetate was observed in 10 pigs The daily oral dose of ergostanyl acetate in cottonseed oil was 0.03 to 0.04 mg/kg Biopsy of the triceps muscle was done on 20 pigs and autopsy on 19 Lesions in striated muscle occurred in all deficiency animals and resembled those described by Goettsch and Pappenheimer in nutritional muscular dystrophy of guinea pigs, except that in our biopsies the earliest sign of deficiency was proliferation of myoblasts, prior to non-inflammatory degeneration Calcification was seldom seen Heart muscle was not affected Wheat germ oil did not prevent either stiffness or microscopic changes Ergostanyl acetate given to 3 guinea pigs after wrist stiffness had appeared during about 90 days on the diet, produced clinical regression of muscle shortening, and changes at post mortem were much less than in comparable controls Ergostanyl acetate given to 7 animals throughout the experiment, largely prevented stiffness and microscopic changes Cod liver oil, fed to 2 pigs for 145 and 254 days, respectively, did not accelerate stiffness and may have protected against microscopic changes

Measurement of pain threshold-raising action of aspirin, codeine, and meperidine (Demerol) JAMES D HARDY* AND McKEEN CATTELL

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During the past 3 years, 160 medical students have been studied by the thermal radiation method in an effort to measure the pain threshold raising effects of 3 common analgesics Groups of 20 students were used in each experiment and agents were administered together with placebos so that neither the subject nor the experimenter was aware of the kind or amount of agent given The technique employed for measuring the pain thresholds was essentially the same as that used in the experiments of Wolff, Hardy and Goodell Aspirin was administered by mouth and meperidine and codeine subcutaneously in the following amounts—*aspirin*—0, 0.3, 0.6, and 0.9 gm, *meperidine*—0, 20, 25, 40, 50, 60, and 100 mg, *codeine*—0, 10, 15, 20, 30 and 45 mg Comparison of the average time-course threshold curves for each agent and dose with the placebo did not reveal any significant influence on the pain threshold measurements Certain subjects receiving aspirin or the other agents exhibited a characteristic elevation of threshold, but in others it was depressed from 10-20% It is concluded that untrained subjects, even of high intelligence, cannot be used successfully to measure the threshold raising effects of aspirin, codeine, and meperidine in the amounts given

Pyrexia in rabbits following injection of filtrates of typical mold cultures WILLIAM D HARKNESS, WALKER L LOVING AND F ALLEN HODGES (introduced by BERT J Vos) *Division of Pharmacology and Division of Microbiology, Food and Drug Administration, Federal Security Agency, Washington, D C*

Rabbits were injected intravenously with filtrates of mold cultures representing 31 species which were propagated on a dextrose-peptone medium Significant elevations in body temperature were observed in the case of 16 of these species Temperature increases varied for the numerous molds employed Temperature increases also appeared to be dependent upon the chemical constituents of the media For example, when inorganic nitrogen was substituted for peptone as a nutrient source no significant temperature rises were obtained Completely negative results from injection of portions of the several lots of media used in the study indicated that the protein or other constituents of the nutrient material were not pyrogenic prior to being inoculated with mold

Comparison of rate of uptake of radioactive thyroxine by tuber cinereum, hypophysis and thyroid EDWARD O HARPER* AND PAUL A MATTIS *Dept of Psychiatry and Pharmacology,*

Western Reserve Univ School of Medicine, Cleveland, Ohio

It has been demonstrated that the iodine concentration of the tuber cinereum and pituitary is high following the administration of thyroxine. Using the method of Horeau and Sue as modified by Gross and LeBlond, thyroxine was labeled with radioactive iodine, I^{131} . Radiothyroxine was administered intravenously ($10 \mu\text{g/kg}$) to virgin female rabbits. The total amount of radioactivity injected was $2.0-3.0 \times 10^6 \text{ dpm}$. The animals were killed by rapid i.v. injection of sodium pentobarbital. Any method bringing about a disturbed state before death vitiated the results. The concentration of I^{131} in the tissue is expressed by a ratio ($I^{131}/\text{mg organ wt}$)/($I^{131}/\text{mg body wt}$). At the end of one hour the concentration in the tuber cinereum was essentially the same as in other parts of the brain (see table), but was definitely increased at the end of 2 hours. The concentration gradually decreased during the next 22 hours. Following the 2-hour determinations, the data suggest a steady increase in the I^{131} concentration in the thyroid, with a corresponding decrease in the hypophysis during the remaining 22 hours.

$$\text{Conc} = \frac{I^{131} \text{ mg organ wt}}{I^{131} \text{ mg body wt}}$$

TISSUE	30 MIN	1 HR	2 HR	4 HR
Brain (aver)	59	51	59	39
Tuber cinereum	48	46	14	13
Hypophysis	5.02	5.87	9.95	9.90
Thyroid	3.75	13.9	12.4	29.7

Blockade of epinephrine-induced hyperglycemia

STEWART C HARVEY,* MARK NICKERSON AND KATHRYN STOVER* *Dept of Pharmacology, Univ of Utah College of Medicine, Salt Lake City*

Certain of the newer adrenergic blocking agents, notably Dibenamine and Priscoline, do not effectively block the hyperglycemic response to epinephrine. This suggests that prevention of epinephrine-induced hyperglycemia may be an expression of a pharmacological property quite different from the classical 'adrenergic blocking activity' which is usually measured as an inhibition of excitatory responses of smooth muscle to adrenergic stimuli. A systematic study of the ability of various adrenergic blocking agents to prevent the hyperglycemic response to injected epinephrine in rabbits revealed little correlation between this property and adrenergic blocking potency as measured by blockade of peripheral vascular responses. Notable discrepancies between these 2 properties include: 1) Ergonovine

in large doses blocks the hyperglycemic response although this agent is completely devoid of adrenergic blocking activity as measured against peripheral vascular and other smooth muscle responses. 2) Hydrogenation fails to significantly increase the effectiveness of various ergot alkaloids in blocking the hyperglycemic response although it markedly increases their potency in blocking smooth muscle responses. 3) Fluorenyl- and α -naphthylmethyl- β -chloroethylamines are quite effective in blocking the hyperglycemic response while their benzyl and phenoxyethyl analogues are not. All of these configurations effectively block peripheral vascular responses to epinephrine; indeed, certain phenoxyethyl derivatives are the most potent. 4) Priscoline fails to block the hyperglycemic response although it inhibits peripheral vascular and most other smooth muscle responses. It is concluded that prevention of the hyperglycemic response to epinephrine is not a reliable measure of 'adrenergic blocking activity' in the sense in which this term is usually applied.

Studies on toxicity of parathion (0-0-diethyl 0-p, nitrophenyl thiophosphate) LLOYD W HAZELTON, EMIL G HOLLAND,* AND REBECCA C HELIEMAN* *Hazellon Labs, Falls Church, Va*

Upon completion of two years' chronic feeding, rats on diets containing up to 100 parts parathion/million showed normal weight, survival and food consumption. Chemical analysis revealed no tissue storage; isolated organ weights were normal, and there was no associated pathology. As previously reported, atropine antagonizes the symptoms of acute parathion toxicity and is effective in life saving against moderate excesses of the lethal dose. Following larger doses, however, death in atropinized animals is delayed but not prevented. Adequate atropinization and manual artificial respiration are effective against these large doses in dogs. Reported nicotinolytic agents such as Parpanit (1-phenyl-cyclopentane-1-carboxylic acid hydrochloride) and Diparcol (10-(2-diethylaminoethyl)-phenothiazine hydrochloride) were not effective in either atropinized or non-atropinized animals. Metrazol effectively stimulated respiration, either before or after atropine, but the combination represented no improvement over adequate atropine and artificial respiration.

Adrenergic blockade in man by a new imidazole derivative C-7337 HANS H HECHT, R CRANDALL AND A J SAMUELS (introduced by LEO T SAMUELS) *Dept of Medicine, Univ of Utah College of Medicine, Salt Lake City*

The amino-methyl imidazoline Ciba 7337 has been tested for its sympatholytic and adrenergic blocking effects in 38 subjects. The substance was

administered intravenously in doses ranging from 0.5-1.5 mg/kg. The resting arterial pressures fell briefly to subnormal values in all subjects. All developed sinus tachycardia with T wave changes and prolongation of QT reminiscent of the effects of epinephrine in dibenamidized patients although cardiac output after C-7337 remained unchanged over the control in one subject. Nasal congestion, lacrimation, and pupillary constriction in the dark were usually present. All subjects developed orthostatic hypotension and fall in pressure exceeding the control values during the Valsalva experiment and after i.v. administration of histamine. The 'overswing' of pressures after histamine was abolished (suggesting blockade of endogenous epinephrine) but the rebound after the Valsalva test appeared unaffected. Intramuscular administration of 1 mg epinephrine given at the height of the response to C-7337 demonstrated adequate blocking of changes in arterial pressure, of epinephrine induced extrasystolic disorders, of rises in blood glucose levels and in total leucocyte counts. C-7337 failed to alter the epinephrine tachycardia, the vasoconstriction of cutaneous areas, the delayed rise in polymorphonuclear cells with lympho- and eosinopenia, and the subjective vascular and central effects of epinephrine. C-7337 possesses potent adrenergic blocking properties, appears mildly sympatholytic and may be assumed to cause local vasodilatation. Its effects are largely dissipated within 1-2 hours, and minor side effects (burning sensation, nausea, sweating, abdominal cramps and diarrhea) were frequent.

Synthetic curare compounds VII N-methyl-trilobine iodide and N-methyl-isotrilobine iodide
D. A. HERRING* AND DAVID F. MARSH *Dept of Pharmacology, West Virginia Univ School of Medicine, Morgantown*

Kondo isolated trilobine and isotrilobine from *Cocculus trilobus* and *Cocculus sarmentosus*. We have converted these biscochlorine-type alkaloids to the quaternary ammonium derivatives and compared their activity with D-tubocurarine chloride. Structurally, these trilobine derivatives are related to the berbamine compounds except the isoquinoline rings are linked by 2 ether oxygen atoms instead of only one. The trilobine derivatives are less vasodepressor in the anesthetized cat and produce less salivation and lacrimation in the trained dog than D-tubocurarine. They do not appreciably influence the cardiovascular effects of epinephrine or acetylcholine. The trilobine derivatives have a relatively greater effect on the muscles of the abdomen and thorax of the rabbit than on the neck so that the apparent safety factor in the rabbit is even less than that for D-tubocurarine, if the rabbits are maintained by mechanical respiration this effect is unimpor-

tant and 30-50 times the head drop dose is well tolerated.

Distribution and excretion of bis(5-chloro-2-hydroxyphenyl) methane HERMAN C. HERRLICH (introduced by BENEDICT E. ABREU) *Research Dept., Pitman-Moore Co., Indianapolis, Ind.*

Bis(5-chloro-2-hydroxyphenyl) methane has been shown to be an effective anthelmintic against ascarids, hookworms, and tapeworms both in combination with toluene as Vermiplex in dogs (BLAIR, H. E. *N. Am. Vet.* 30: 306, 1949), in cats (BURCH, G. R. AND H. E. BLAIR *N. Am. Vet.* in press), or used alone in dogs (CRAIGE, A. H., JR. AND A. N. KLECKNER *N. Am. Vet.* 27: 26, 1946). In addition, it has also proved to be effective against the fringed tapeworm in sheep (RYFF, J. J. F., R. F. HONESS AND H. L. STODDARD *J. A. V. M. A.* 115: 179, 1949). Since the fringed tapeworm is frequently found in the bile duct and gall bladder, this suggests that the drug is at least partially absorbed and then excreted by the liver. In order to ascertain if a cycle of excretion and reabsorption similar to that of the bile salts plays a part in the effectiveness of this drug against parasites in the biliary system and intestine, a series of determinations of the concentration of the drug in various body fluids were made. The method of Gottlieb and Marsh (*Ind. and Eng. Chem. An. Ed.* 18: 16, 1946) as adapted for use with biological materials by Johnson *et al.* (*Ann. N. Y. Acad. Sc.* 52: 518, 1949) for a similar compound was used. In view of the fact that bis(5-chloro-2-hydroxyphenyl) methane may be presumed to be conjugated with either sulfuric or glucuronic acid before excretion, an extraction and colorimetric procedure is being adapted to show the extent of these reactions at the dose level studied and data on distribution will be presented.

Absorption, excretion, and tissue distribution of three mercurial diuretics R. G. HERRMANN,* G. R. KLAHM* AND H. W. WERNER *Pharmacology Dept., Wm. S. Merrell Co., Cincinnati, Ohio*

The absorption, excretion, and tissue distribution of a new thiomercorial diuretic, TMS (trisodium salt of N[2-methoxy-3(1,2-dicarboxyethylthiomercuri) propyl]-0-carboxymethyl salicylamide), mercaptomerin sodium, and meralluride sodium were studied following intramuscular and subcutaneous administration to rats, employing doses of 7.8 to 8.0 mg/kg Hg for the 3 compounds. Analysis of 1 ml injection sites 30 minutes after administration demonstrated 94, 89, and 81% absorption of administered doses for TMS, mercaptomerin sodium, and meralluride sodium respectively. Subcutaneous absorption was found to be 79, 71, and 57% at the same time interval.

Blood levels of Hg following administration of the 3 compounds by either route were low, ranging from 4-12 $\mu\text{g/gm}$ of tissue at the 30 minute period. Mercury concentrations in heart muscle were even lower than those of blood. The mercury content of the kidneys 30 minutes after i.m. injection was 24, 30, and 31% of the administered dose for TMS, mercaptomerin sodium, and meraluride sodium respectively. Four hours after injection the kidneys contained 2, 14, and 9% TMS and mercaptomerin sodium were eliminated in the urine and feces to the extent of about 35% within 4 hours and about 86% within 24 hours after injection. After administration of meraluride sodium, about 60% was excreted in the urine and feces within 4 hours and about 90% within 24 hours.

Effects of depth of anesthesia on peripheral circulation S. G. HERSHEY* AND E. A. ROVINSKY. *Dept of Anesthesiology, New York Univ - Bellevue Med Center, New York City*

Utilizing the vascular bed of the dog omentum the effects of depth of anesthesia (ethyl ether, cyclopropane, pentothal sodium) on the peripheral circulation were observed. The peripheral circulatory mechanisms employed as criteria were 1) reactivity to epinephrine (topical), 2) venous outflow from capillary bed, 3) vasomotion of metarterioles and precapillaries, 4) terminal arteriolar caliber, 5) rate of recovery to control state. *Ether*. During light and moderate degrees of anesthesia there was some decrease in the efficiency of these mechanisms. In deep and very deep anesthesia there was marked to extensive interference with these circulatory adjustments. After clinical recovery from surgical anesthesia there was a lag of at least 20 minutes before these mechanisms reverted to the control state. *Cyclopropane*. During light and moderate depths of narcosis there was no significant change in these circulatory mechanisms. In deep anesthesia there was some diminution in their efficiency. Very deep narcosis produced a marked decrease in these circulatory adjustments. Clinical recovery from surgical anesthesia was followed by a return to the control state of these mechanisms within 2 minutes. *Pentothal Sodium*. In light narcosis no appreciable changes were observed. With moderate narcosis some alterations in the peripheral mechanisms were noted. Deep and very deep anesthesia resulted in marked to severe interference. Clinical recovery was slow but when reached was followed, in approximately 10 minutes, by a return of the peripheral circulation to the control state.

Urinary chromogen formation after exposure to aniline DAVID L. HILL (introduced by AMEDEO

S. MARRAZZI) *Pharmacology Section, Med Division, Army Chemical Center, Md*

A method has been desired which is capable of detecting exposure of animals to aniline at levels well below the toxic range for this agent. In such cases the sulfonamide reaction (BRATTON AND MARSHALL *J Biol Chem* 128: 537, 1939) applied to urines has shown promise. Urines of rabbits injected intravenously with $\frac{1}{10}$ the lethal dose of aniline showed increases in both free and total urinary chromogen of as much as 2,000% over the control values. Dogs treated cutaneously gave increases in free but not total chromogen. The urines of 15 laboratory workers were analyzed with only negligible amounts of free and labile chromogen found. Four men exposed to spreading 5 ml aniline on the forearm gave no increase in free chromogen, but 100% increase in total value. No methemoglobin or other clinical symptoms were noted. A second group of 6 men received a total of 7 ml aniline in 2 doses spread upon the thorax. Total urinary chromogen increased 200-800%. Two showed significant increases in free chromogen. Methemoglobin increases averaged 50% of total hemoglobin. All developed headache and cyanosis in varying degrees.

A study of digitoxin in blood JAMES G. HILTON (introduced by C. L. GEMMILL) *Dept of Pharmacology, Univ of Virginia Med School, Charlottesville*

Studies have been carried out on the distribution and disappearance of digitoxin in the blood of dogs. To study the distribution, varying amounts of digitoxin were added to freshly drawn oxalated blood and the mixture was centrifuged at 2100 R.P.M. for 30 minutes. The plasma was separated from the cell fraction and each portion was analysed for its digitoxin content by a polarographic method (*Science* 110: 526, 1949). It was found that all of the extractable digitoxin was in the plasma both in drawn blood and in circulating blood. The cell fraction of blood took up a constant amount of digitoxin when the digitoxin was present above a certain minimal amount. This uptake occurred whether the cells were suspended in plasma or in isotonic solution. To study the disappearance of digitoxin from the circulating blood of dogs, animals weighing between 12 and 20 kg were administered 1.2 mg of digitoxin in 50% ethyl alcohol solution by i.m. injection. At periodic intervals after injection, samples of blood were withdrawn from the jugular vein and analysed for their digitoxin content. The disappearance may be seen in the table. The values reported are average values obtained on 4 dogs and are reported in $\mu\text{g}\%$ recoverable digitoxin. If the values obtained in $\mu\text{g}\%$ are plotted against time in minutes, a curve of the form

$C = nT^a$ is obtained. The value C equals the concentration in $\mu\text{g } \%$ at time T after injection and the values ' n ' and ' a ' are arbitrary constants

CONCENTRATION OF RECOVERABLE DIGITOXIN AT VARIOUS TIMES

	TIME, MIN					
	10	20	30	60	120	240
Concentration microgram per cent	0.20	0.10	0.07	0.04	0.02	0.01

A simple spectrophotometric method for determination of tetra-ethyl thuramdisulfide (antabuse) in blood and urine CHARLES H HINE AND KSHITISH J DIVATIA * *Division of Pharmacology and Experimental Therapeutics, Univ of California School of Medicine, San Francisco*

Extraction of antabuse from whole blood, plasma or urine was accomplished by shaking 1 ml of the biologic fluid and 1 ml of phosphate buffer pH 7.4 with 10 ml of ethylene dichloride (EDC) for 20 minutes in a 15 cm glass-stoppered extraction tube. After shaking, the specimen was centrifuged, the aqueous layer removed, the EDC reshaken with 2 ml of buffer and the optical density of the EDC determined at wave lengths 285 and 320 $m\mu$ using a Beckman DU spectrophotometer. Antabuse gave a characteristic absorption curve in ethylene dichloride between wave lengths 250 and 350 $m\mu$. The absorption curve falls sharply between 285 and 320 $m\mu$, hence the difference in optical densities at these 2 points was taken as a criterion of antabuse concentration. Recovery of antabuse from old (15-day) whole beef blood and plasma averaged $85 \pm 3\%$ and $95 \pm 2\%$ respectively. Recoveries from fresh blood are much lower due to enzymatic destruction of the agent. Urine recoveries averaged $100 \pm 4\%$. The method is sensitive to about 10 μg of antabuse/ml of biologic fluid. No significant level of antabuse has been found in the blood of 12 chronic alcoholics given a daily maintained dose of 0.5 gm of the drug for from 10-90 days. Antabuse has not been detected in the blood of alcoholics receiving test doses of alcohol. There is no evidence furnished by analysis of the blood to indicate whether or not antabuse accumulates in the tissues.

Rates of calcium exchange in the system, powdered bone in calcium chloride solution HAROLD CARPENTER HODGE, ELIZABETH E UNDERWOOD* AND MARLENE FALKENHEIM * *Dept of Radiation Biology, Univ of Rochester School of Medicine & Dentistry, Rochester, N Y*
Powdered bone was exposed for various times to solutions of calcium chloride containing the

radioactive isotope. With increasing time of exposure, larger percentages of the Ca_{45} originally in the solution appeared in the bone. The rates of exchange have been computed for several calcium ion concentrations. The calcium exchange is qualitatively similar to the exchange of phosphorus as shown by P_{32} but it is necessary to assume that some quantitative differences exist. Studies have been made both on glycol-ashed bone and on fresh bone.

Veratrine-like action of alkyl ethers of 3-hydroxy-2-phenylcinchoninic acid DUNCAN A HOLADAY (introduced by E K MARSHALL, JR) *Dept of Pharmacology and Experimental Therapeutics, Johns Hopkins Univ, Baltimore, Md*

The 3-alkyloxy derivatives of 2-phenylcinchoninic acid produce strong tonic 'contractures' of the somatic musculature of all species studied (mouse, rat, guinea pig, rabbit, cat, dog). The effect involves all skeletal muscles responding to centrally initiated stimuli, but is most prominent in the hind quarters. On the isolated rat diaphragm-phrenic nerve preparation the effect was demonstrated to be directly on the muscle fiber, a typical veratrine-like myogram results from direct stimulation of the fully curarized preparation. Like the contracture produced by veratrine, the effect may be relieved by exercise ('warm-up'), by drugs having quinidine-like action, and by calcium ion. The potency of the straight chain series decreases with increasing length of the alkyl chain, the methoxy compound being most potent, and the hexyloxy derivative being devoid of demonstrable activity. 3-Amino-2-phenylcinchoninic acid also possesses this property.

Studies on permeability effect of physostigmine and acetylcholine on permeability of rabbit, cat and dog erythrocytes to sodium and potassium W C HOLLAND* AND MARGARET E GREIG *Dept of Pharmacology, Vanderbilt Univ School of Medicine, Nashville, Tenn*

We have previously reported that inhibition of cholinesterase activity by physostigmine causes changes in permeability of dog erythrocytes as determined by hemolysis (*Arch Biochem* 23 370, 1949). We have also found that selective permeability of erythrocytes of dogs and other mammals to Na and K appears to depend on the activity of the acetyl choline cholinesterase system. Two groups of cells have been investigated. I) Dog, Cat, with a high Na and low K, II) Rabbit, with a high K and low Na. When washed cells of a species of group I was placed in a KCl-K bicarbonate medium, K entered the cell quite rapidly as would be expected in a non-living membrane, and the cell hemolyzed. When, however, acetyl choline was added to the medium the entrance of K was much delayed and the cell remained intact.

for a longer period of time. If physostigmine were added along with acetyl choline so that cholinesterase activity was inhibited by 40-80% the entrance of K again becomes rapid, the results being similar to those observed when no substrate was present. In the case of cells of group II the same picture was obtained when these cells were placed in a Na medium—in this case the Na ion entered the cell rapidly in absence of acetyl choline or when metabolism of acetyl choline was inhibited by physostigmine, but the integrity of the cell was maintained over a much longer period if the cells were able to metabolize acetyl choline.

Use of thermistors in twin calorimetry for enzyme reactions F C HOLLER,* D VENABLE* AND C L GEMMILL Dept of Pharmacology, Univ of Virginia Med School, Charlottesville

Thermistors, which have a sensitivity of about 10 times that of Beckmann thermometers, have been used in twin calorimeters designed for thermochemical and kinetic studies of enzyme reactions. The calorimeters were mounted in a water bath constant to $\pm 0.001^\circ \text{C}$. The calorimeters were calibrated for thermal flow effects by passing a known amount of current through a resistance mounted in each calorimeter. A special constant current electronic circuit was constructed for this purpose. This circuit was used also to obtain the heat capacities of the calorimeters and contents. Sucrose inversion with hydrochloric acid was carried out at 31.2°C . The temperature changes were observed over a period of 4 hours. The heat of reaction was calculated by Sturtevant's method (*J Am Chem Soc* 59 1528, 1937). It was found to be $-15,074$ joules/m. This agrees very well with Sturtevant's observed value of $-15,020$ joules/m. Therefore, thermistors in twin calorimeters can be used as a method of determining the heat production of chemical and enzymatic reactions.

Responses of growth of penicillia to cardiotonic glycosides JAMES P HOLLOWAY* AND JAMES C RICE Dept of Pharmacology, Univ of Mississippi School of Medicine, University, Miss

The observation that infusion of digitals increased both the rate of growth and the terminal weight of dried mycelial mats of *Penicillium notatum* and *Penicillium citrinum* growing in surface culture at 23°C in otherwise minimal solution (Clutterbuck's) led to experiments to test the hypothesis that the cardiotonic glycosides were responsible for the observed effects. Graded increasing concentrations of Deacetyldigilaid A and Deacetyldigilaid C ($1 \mu\text{g/ml}$ to $10 \mu\text{g/ml}$) not only failed to accelerate growth of *Penicillium notatum* but actually retarded it. The 3 digilaidins in graded increasing concentrations ($1 \mu\text{g}$ to 10

$\mu\text{g/ml}$) produced similar results. When ample time was permitted for completed growth the 5 glycosides tested had no effect on terminal weight of dried mycelial mats. Fragmentary evidence gained with *Penicillium citrinum* followed much the same pattern as above. None of the agents employed conduced to pH relations of the cultures or antibiotic titers (Oxford Cup Method) differing impressively from those expected for minimal media. Rate of growth was determined by killing, drying, and weighing mycelial mats on each of 5 consecutive days beginning on the 4th or 5th day after inoculation with homogenized spore suspensions, by plotting the average weights of the mats as ordinates against abscissa, time, and by comparison of slopes of curves, and heights of ordinates at fixed time, with those for controls. For effects upon total weight the mats were dried, weighed, and the weights averaged terminally. Results with alcohol-water dispersates of ouabain, digitoxin, and strophanthin were ambiguous.

Curarimimetic activity of mono- and bis-(amino and ammonium-alkylamino)-benzoquinones J O HOPPE, A E SORIA AND C J CAYALLITO (introduced by A M LANDS) Sterling-Winthrop Research Inst, Rensselaer, N Y

The curarimimetic activity of a series of 2,5-bis-(ammoniumalkylamino)-benzoquinones in which the distance between quaternary nitrogen atoms ranged from 11\AA to 20\AA was found to be similar to that of D-tubocurarine chloride in the mouse but from one-half to five-fold that in the rabbit. The more active compounds were examined by means of the nerve-muscle preparation in the dog 2-(Ammoniumalkylamino)-benzoquinones were as active as the bis derivatives. The activity depends on the presence of the iminoquinone structure as well as the onium group. Considerable activity was observed in mice with the corresponding 2- and 2,5-aminoalkylamino-benzoquinones. Distances between basic nitrogen atoms in the bis series and between basic nitrogen and iminoquinone structure in the mono series were critical factors determining activity. With these amines, activity increases with increase in basicity of the terminal nitrogen atoms. One of the most active compounds in the series was 2,5-bis-(3-diethylaminopropylamino)benzoquinone bis-benzylchloride (WIN 2747) which was 5 times as active as D-tubocurarine chloride in the rabbit, one-half as active in the mouse and twice as active in the dog nerve-muscle preparation. WIN 2747 had little or no effect on blood pressure or heart rate and had no severe cardiotoxic effect by continuous infusion into anesthetized dogs at dosages up to 1500 times the curarizing dose.

Antibiotics against natural amebiasis in macaques ARSENY K HRENOFF* AND HAMILTON

H ANDERSON *Division of Pharmacology and Experimental Therapeutics, Univ of California School of Medicine, San Francisco*

A combination of 3 antibiotics, polymyxin B, streptomycin and bacitracin exhibited synergism when tested *in vitro* against *E histolytica* (see M GOULD AND E L HANSEN, current *Proceedings*) Because of possible value in the chemotherapy of amebiasis 10 monkeys were given varying oral doses of the combination over 5-6 day periods. The drug was administered in tablets which contained polymyxin B, 20 mgm, streptomycin (as base), 250 mg, and bacitracin, 5,000 units each. Two animals received only $\frac{1}{2}$ of these doses/kg. They failed to clear of amebas. One of these animals, given twice this dose, was ameba free for 2 weeks. Five animals were given from $\frac{1}{2}$ to $\frac{3}{4}$ these doses/kg. One monkey was cleared for 4 weeks. The doses were doubled and the animal remained free of amebas for another 4 weeks (to date). Another animal was freed of amebas for 10 weeks (to date). The remaining 3 monkeys were not cleared until 10 days elapsed after therapy. They have stayed free of parasites for 3 weeks (to date). Of the remaining 3 macaques, receiving $\frac{1}{2}$ to $\frac{3}{4}$ the initial doses/kg, one was freed of amebas for 11 weeks (to date), the other was freed for 5 weeks (to date), and the third failed to clear. Without exception all animals tolerated this regime, all gained up to 10-18% their initial weight, their appetites increased, and stools changed from liquid or semi-liquid to solid. The hemoglobin, leucocytes, erythrocytes, bromsulphalein and blood urea tests and electrocardiograms exhibited no toxic effects. The stools have been studied almost daily by iron-hematoxylin technic. Thus, 7 of 10 animals have been cleared of pathogenic parasites and signs of amebiasis over 3-11 weeks.

Cause of increasing intravenous toxicity of hydrogen peroxide with progressive dilution M CAROLINE HRUBETZ, LILLIAN W CONN, HYMAN R GITTES AND JAMES K MACNAMEE (introduced by AMEDEO S MARRAZZI) *Toxicology and Pathology Sections, Med Division, Army Chemical Center, Md*

Studies of the intravenous and percutaneous toxicity of 90% hydrogen peroxide have shown that death is associated with gas embolism and that this is probably the immediate cause. With *iv* injection, the toxicity in rabbits is predictably increased with the dilution. For example, the LD_{50} for undiluted was 15 cu mm, for 40%, 8.9 cu mm, and for 4%, 3.2 cu mm. In casting about for a plausible explanation for this finding our studies with percutaneous administration suggested that, grossly, the greater the local reaction, the lower the toxicity. The effect is initially due

to the liberation *ic*, *sc*, and *im*, of oxygen. This suggests that less of the administered material is therefore available for the production of gas emboli in the blood stream and would account for the lower toxicity on an embolic basis. In fact, when the agent is injected *iv* in the rabbit's ear, it is apparent that the more concentrated the form, the greater the breakdown within the immediate area as evidenced by the peculiar and characteristic blanching due to gas bubbles entrapped in the surrounding tributary vessels and adjacent tissue.

Experimental addiction to barbiturates (motion picture) HARRIS ISBELL, SOL ALTSCHUL,* C H KORNETSKY,* H F FRASER,* H G FLANARY,* A J EISENMAN, A WIKLER AND HARRIS HILL* *Research Division, USPHS Hospital, Lexington, Ky*

Five former morphine-addict volunteers received barbiturates for 92-144 days. The highest daily dosages attained were 1.8, 1.8, and 3.8 gm daily of seconal, pentobarbital, and amytal respectively. While chronically intoxicated, all patients showed impairment of mental ability, increased emotional lability, infantile behavior and confusion. No toxic psychoses were observed while patients were taking the drug. Neurological signs included nystagmus, dysarthria, ataxia in gait and station, and depression of superficial abdominal reflexes. The effects of the same dose of the drug varied widely from day to day and was partially dependent on food intake. Partial tolerance was observed. In the electroencephalogram the percentage of β waves was increased. Following withdrawal of barbiturates, neurological signs disappeared and weakness, anxiety, anorexia, nausea, vomiting, rapid weight loss, fever, elevation of NPN's, convulsions (4 of 5 patients) and a psychosis (4 of 5 patients) resembling alcoholic delirium tremens were observed. During withdrawal, the percentage of β waves was reduced in the electroencephalogram and paroxysmal bursts of slow waves appeared. Recovery was complete.

Influence of age on metabolic rate and phosphate compounds of cardiac muscle JO'ANN JEHL AND ALBERT WOLLENBERGER (introduced by OTTO KRAYER) *Dept of Pharmacology, Harvard Med School, Boston, Mass*

On incubation in Krebs-Ringer-phosphate glucose solution, slices of cardiac ventricular muscle of new-born guinea pigs respire at a more or less constant rate, whereas the rate of respiration of ventricular muscle slices of guinea pigs 3 weeks to 30 months old declines 30-50% over a period of 30-45 minutes before reaching constancy. The Q_{O_2} characteristic of the steady state of activity diminishes with advancing age. The following

values, based on the combined dry weight of slices and trichloroacetic acid precipitate in the medium, were obtained 4-5 at birth, 3-4 at 3 weeks, 2.5-3 at 5 months, and ~2 at 30 months of age. The Q_{O_2} is usually maintained for several hours at or near these levels in the heart slices of the younger animals, but in the tissue of the 30 month old animals it falls to below 1 after 2 hours. 10^{-6} M ouabain, which in cardiac muscle of 5 and of 30 month old guinea pigs causes marked acceleration of respiration followed by severe inhibition, produces only moderate changes in the myocardium at the age of 3 weeks, and exerts no significant effect on the respiration of myocardium of new-born guinea pigs. Analyses of some acid-soluble phosphates, including ATP, and of creatine in the cardiac ventricles of guinea pigs ranging in age from a few hours to 30 months revealed two pronounced changes related to age: a 200% increase in phosphocreatine from the time of birth to the age of 11 months, half of which occurs during the first 3 weeks, and a 100% increase in total creatine during the same 11 month period. At birth ~1/3 of the total creatine in the ventricles is phosphorylated, in the ventricles of animals 3 weeks to 30 months old the phosphorylated fraction amounts to almost 1/2 of the total creatine. The possible significance of these findings is discussed.

Water and electrolyte equilibria factors in fluoroacetate poisoning LINARES B. JOHNSON,* ALEXANDER KANDEL* AND MAYNARD B. CHENOWETH
Dept of Pharmacology, Univ of Michigan, Ann Arbor

Disturbances of certain physiological and biochemical balances result in an altered response to sodium fluoroacetate (FA). Shifts in intracellular water and electrolytes can either increase or decrease the sensitivity of animals to FA depending on the direction of movement. This was first observed in experiments with albino Sprague-Dawley rats. Male animals were dehydrated for seven days. One group was given 20 mg/kg of FA intraperitoneally, the remaining group was allowed water for a period of 4-5 hours and then injected with the same dose of FA. Sixty-four control rats receiving 20 mg/kg showed an average time to death of $231 \pm S.E. 22$ minutes as compared to $78 \pm S.E. 11$ minutes for 60 dehydrated rats. Eighty-nine dehydrated animals which subsequently received water manifested an increased resistance to FA. As compared to no survivals in the control group beyond 15 hours, 27 (30%) of these rats lived for more than 24 hours after injection. In contrast, 66 rats following 7 days of fasting, but allowed water *ad libitum* responded in the same manner as controls to 20 mg/kg of FA and died in an average time of 255

$\pm S.E. 26$ minutes. Preliminary experiments indicate that these phenomena are also demonstrable in rabbits, in which FA produces fibrillation, and to a lesser extent in dogs, in which, like rats, FA has a convulsant action. These and other experiments related to electrolyte balance will be reported.

Effects of certain enzyme inhibitors on breakdown of pentobarbital *in vitro* J. B. KAHN, JR (introduced by J. M. COON) *Dept of Pharmacology, Univ of Chicago, Chicago, Ill*

Following studies on effects of ions on destruction of pentobarbital by rat liver slices (KAHN, J. B. *Federation Proc.* 8: 305, 1949), it was decided to investigate effects of certain factors known to influence enzymatic equilibria on *in vitro* destruction of pentobarbital. Slices of rat liver approximately 0.5 mm thick were placed in a cylinder containing 49.0 ml of oxygenated Krebs-Ringer phosphate with glucose and maintained at 37°C. Three mg of pentobarbital was added in 1.0 ml Krebs-Ringer phosphate, and the concentration of pentobarbital determined spectrophotometrically at 0, 60, and 120 minutes. Control tests were performed similarly, using boiled tissue slices. Effects of the following compounds were studied: cyanide, fluoride, arsenite, iodoacetamide, glutathione, and cysteine. Cyanide and fluorid ($10^{-3}M$) had no effect. In the absence of a -SH inhibitor, destruction in the boiled slices was approximately 10% of that in the unboiled. Iodoacetamide or arsenite ($10^{-3}M$) permitted normal destruction in the unboiled slices, but also caused destruction in boiled slices which was 75% of that in the unboiled. When the same experiment was performed without added tissue there was no removal of barbiturate, so results noted are not due to direct combination of barbiturate and inhibitor. When -SH groups as reduced glutathione or cysteine were added to the system containing unboiled slices, destruction of pentobarbital was inhibited approximately 60%. From this evidence the hypothesis is advanced that -SH groups inhibit the *in vitro* destruction of pentobarbital by rat liver slices.

Effects of methyl bis (β -chloroethyl) amine (nitrogen mustard) on regeneration and alkaline phosphatase activity of urodele larvae forelimbs ALEXANDER G. KARCZMAR AND GEORGE G. BERG (introduced by CHARLES F. MORGAN) *Dept of Pharmacology and Materia Medica, Georgetown Univ School of Medicine, Washington, D. C.*

Inhibitory effect on regeneration of amputated larval urodele forelimbs can be used to test anti-mitotic activity of chemical agents (KARCZMAR *Anal. Rec.* 101: 712, 1948). With effective anti-mitotics regeneration blastema is never formed and complete regression of amputated limbs en-

sues Accordingly, a test was devised to measure drug activity by means of photographing periodically the regressing limb and computing the regression rates Histology or mitotic counts are thus unnecessary for screening these agents The test shows also the regressive effect of denervation of amputated limbs which can be used as reference unit, with regression rates of 3μ and $1 \text{ cu } \mu/\text{hr}$ Nitrogen mustard causes a much slower regression with chronic exposures to concentrations of 50% of LD_{50} (1 150000) and similar regression ($3 \mu/\text{hr}$) recorded over 2-weeks period after single exposures for less than 1 hour to strong (1 5000) nitrogen mustard solutions Combination of denervation and chronic exposure to nitrogen mustard solutions (1 200000) caused regression of amputated limbs twice as rapid as that following denervation alone Preliminary histochemical investigation showed that nitrogen mustard blocks the formation of epidermal alkaline phosphatase which precedes normal regeneration However, it enhances phosphatase activity in regressing mesodermal tissues The effect of nitrogen mustard on phosphatase activity is specific and differs from that of denervation Since mitotic inhibition by nitrogen mustard or by denervation alone is almost complete, their synergism indicates a more complex causation of regression, mitotic activity being only one of the contributing factors

The effect of adrenal steroids on growth of chick embryo D A KARNOFSKY, C C STOCK AND C P RHOADS (introduced by F S PHILIPS)
Division of Experimental Chemotherapy, Sloan-Kettering Inst for Cancer Research, New York City

It had been previously shown that an extract of the adrenal cortex would retard the growth of the chick embryo (LANDAUER, *Endocrinology* 41 489, 1947) In the course of our cancer chemotherapy studies, the effect of various pure steroids in aqueous solution or in propylene glycol were examined by injecting them into the yolk sac of the 4-day-old chick embryo Compound E, at a critical dose of 1-2 mg/egg, produced a marked decrease in the growth of the chick embryo This was manifested by reduction in the formation of the chorioallantoic membrane and a stunted embryo, which was often eviscerated, and showed scanty and abnormal feather formation These embryos usually died 6-8 days after injection, but some survived to 18 days, and weighed 4-6 gm as compared to 18-21 gm for the controls The stunted embryo was well-proportioned, and the egg contained a large amount of unused yolk and albumen as compared to the controls Compound F had a similar effect, but, in preliminary studies, it appeared to be 20-40 times more active than Compound E in the chick embryo A number of

other steroids is being assayed for their 'compound E' activity in the chick embryo, in order to elucidate the chemical structures necessary for this effect

Clinical evaluation of two new analgesics, heptazone and WIN 1161-2 as a test of a new method of measuring analgesic power ARTHUR S KEATS* AND HENRY K BEECHER
Anesthesia Lab of Harvard Med School at Massachusetts General Hospital, Boston

Two new synthetic analgesics of the methadone series, DL-4,4-diphenyl - 6 - morpholino - heptanone-3 hydrochloride (Heptazone) and levo 3-dimethylamino-1,1-diphenylbutyl ethyl sulfone (WIN 1161-2) were tested for analgesic potency in postoperative pain of man The analgesic activity of different dose levels was compared to that of 10 mg of morphine/150 lb of body weight in the same group of patients This was accomplished by alternating doses of morphine between doses of the drug to be tested in the same patients Neither patients nor observers were aware of which drugs were given Heptazone when given as 50-60 mg/150 lb of body weight relieved pain as frequently as 10 mg of morphine WIN 1161-2 was found to be erratic in action and unsuitable for clinical use The method used in this evaluation is presented in detail elsewhere Its accuracy and consistency were demonstrated by evaluating morphine itself when treated as an unknown The method's use in determining the analgesic potency of new drugs is demonstrated here

Pain relief with hypnotic doses of barbiturates ARTHUR S KEATS* AND HENRY K BEECHER
Anesthesia Lab of Harvard Med School at Massachusetts General Hospital, Boston

It was observed (BEECHER) in badly burned patients and later in men suffering from war wounds that small doses of barbiturates appeared to be useful in relieving pain These findings led to the present controlled study in which the data obtained demonstrate the analgesic power of hypnotic doses (60 and 90 mg/150 lbs body weight) of pentobarbital sodium This analgesic power has been compared with that of a placebo (1 cc normal saline) and a standard dose of morphine (8 mg/150 lbs body weight) in the same patients The order of administration was randomized in accordance with sound statistical practice The agents were administered intravenously All drugs were dealt with as unknowns both to the patient and the observer The subjects were 175 postoperative patients in pain They were studied during the 18th to the 30th hour following surgery The pain experience of man consists of both perception of painful stimuli and the psychic modification of these stimuli We wish to emphasize the importance of utilizing subjects in pain

from disease or from surgery rather than experimentally produced pain in the assay of analgesic power. Hypnotic doses of pentobarbital sodium administered intravenously relieves postoperative pain in 50% of patients in contrast to 20% with saline and about 80% by morphine.

Pharmacology of khellin KEITH F. KILLAM* AND EDWIN J. FELLOWS *Research Division, Smith, Kline and French Labs., Philadelphia, Pa.*

Khellin (2-methyl-5,8-dimethoxyfuranochromone), one of the major crystalline components of *Annu Visnaga Lam.* has been reported (ANDEREF, *et al.*) to exhibit smooth muscle relaxing activity with some degree of specificity for the coronary vessels. In the present experiments a modified Langendorff technique was used to determine the effect of khellin on isolated rabbit hearts perfused with oxygenated Locke's solution. Khellin was administered over a 2-minute interval in order to simulate recirculation which would occur in an intact animal and also to obtain reproducible results. The rate of injection was proportional to the rate of perfusion fluid flow. The 2-minute period immediately prior to each test period served as a control. The percentage change in perfusion flow was calculated by the following formula: $\text{Change in flow (cc) during test} / \text{Flow in cc during control period} \times 100$. In 157 experiments concentrations of 1, 100,000 khellin produced an average increase in perfusion fluid flow of 32%. An average of 67% was noted after 1, 10,000 khellin in 67 experiments. In anesthetized, heparinized dogs, normal and changing blood flow was determined by inserting a glass cannula into a convenient superficial coronary vein and the outflow measured by a circuit-breaking graduated tilt cup or drop recorder. In 8 of 11 experiments, 2.0, 3.0 and 5.0 mg/kg of khellin intravenously produced a significant increase in coronary flow. After 2.0 mg/kg khellin caused an increase of 20-88% for 2-20 minutes, after 3.0 mg/kg, an increase of 49-100% for 28 minutes to one hour, and after 5.0 mg/kg an increase of 75-230% for periods of 1½ hours which was the duration of the experiments. In all experiments, khellin produced a moderate fall in blood pressure which returned to normal in 2-4 minutes.

Rapid technique for statistical evaluation of 3, 4 or 5 dose assays LILA F. KNUDSEN (introduced by BERT J. Vos) *Division of Food, Food and Drug Administration, Federal Security Agency, Washington, D. C.*

Assays involving percentage responses with equal numbers of animals on 3 to 5 or more doses on each of the standard and the unknown can be rapidly evaluated using the angular transformation to obtain estimates of potency, and standard error of the assay. Where routine labora-

tory assays are conducted on a relatively large scale, it is possible to plot the slopes of the individual dosage-response curves over a period of time. If these slopes are in 'statistical control' (relatively constant within sampling errors), the best estimate of the slope is the average. When the control chart technique is thus used on the slopes, it is possible to construct one graph from which can be read estimates of potency, upper and lower confidence limits for the assay from the simple calculation of 2 values V and W . Assays involving graded responses with equal numbers of animals on each of 3 to 5 (or more) doses on each of the standard and unknown can be evaluated by relatively the same technique by substituting the 'range' for the standard deviation in the proper formulas.

Role of specific and non-specific cholinesterase in spontaneous contractions of isolated ileum

GEORGE B. KOELLE, ETHOL S. KOELLE* AND JONAS S. FRIEDENWALD* *Wilmer Ophthalmological Institute, Johns Hopkins Univ. and Hospital, Baltimore, Md.*

Histochemical studies have revealed that both specific and nonspecific cholinesterase (ChE) are widely distributed throughout the ileum of the cat, the latter was found in both the muscle fibers and in the ganglion cells of the plexus. These observations suggested that nonspecific ChE might play a role in the transmission of impulses at these sites similar to that generally ascribed to specific ChE. To investigate this possibility further, the effects of varying concentrations of di-isopropyl fluorophosphate (DFP) have been tested on the spontaneous activity of isolated ileum preparations. Afterwards, the preparations were washed in saline and homogenates and frozen sections were prepared to permit quantitative determinations of the degrees of inhibition of both types of ChE and microscopic studies of their residual distributions. It was found that concentrations of DFP producing slight inhibition of specific ChE but relatively marked inhibition of the non-specific esterase caused a definite increase in both the tonus and amplitude of spontaneous contractions.

Antioxidant and stabilizing action of propylene glycol THEODORE KOPFANYI *Dept. of Pharmacology and Materia Medica, Georgetown Univ. School of Medicine, Washington, D. C.*

Solutions of epinephrine hydrochloride in pure propylene glycol or in from 60 to 70% aqueous solutions of propylene glycol are protected from oxidation. In accelerated tests where epinephrine hydrochloride (1:1000) is treated with 1 cc. of 3% hydrogen peroxide, the glycol prevents to a great extent the red discoloration of epinephrine, even when the mixture is heated for 10 minutes at a

temperature of 70°C Commercial samples of epinephrine (1 10,000) exposed to air when kept at a temperature of 15°C develop red color after 67 hours, whereas when kept in propylene glycol, the reddish tinge appears only after 23 days Other glycols are less effective Propylene glycol also protects epinephrine solutions in the presence of liver slices Propylene glycol inhibits the decomposition of other drugs such as apomorphine, physostigmine, "Clorarsen" and arsphenamines In a number of dogs, it was found that the i.v. injection of from 0.5 to 1 cc. of propylene glycol/kg. in diluted form potentiates the pressor effects of epinephrine both as to height and duration This glycol in similar doses, may also produce a slow, gradual rise of blood pressure in dogs with arterial pressures at shock levels

Action of epinephrine and veratramine upon heart rate and oxygen consumption in heart-lung preparation of dog KURT KRAMER,* ULRICH LUFT* AND OTTO KRAYER *Dept of Pharmacology, Harvard Med School, Boston, Mass., and School of Aviation Medicine, Randolph Field, Tex*

In 9 heart lung preparations on dogs, the influence of epinephrine and veratramine upon oxygen consumption and heart rate under constant conditions of work were studied After a control period of 30 minutes when the oxygen consumption, heart rate and coronary blood flow had reached constant values, L-epinephrine in doses of 1.5 to 7 $\mu\text{gm}/\text{min}$ were infused over a period of 1-2 hours While the heart rate rose to 95% of its maximum within 4-10 minutes the increase in oxygen consumption of the heart progressed much more slowly, in some cases reaching its maximal value only after one hour During the subsequent steady state each heart beat consumed 30-40% more oxygen than during the previous control period Regardless of the inhibition of epinephrine cardioacceleration, veratramine, injected in successive doses of 100, 200 and 700 μgm , did not change the oxygen demand of each beat Only after eliminating the epinephrine by flushing the system with fresh blood could the oxygen consumption per beat be reduced to its initial value or below Repetition of the epinephrine infusion augmented the oxygen consumption as before but without cardioacceleration due to the persistence of the veratramine effect Previous investigations indicated that the positive chronotropic action of epinephrine can be separated from its positive inotropic action by use of veratramine According to results of the present study cardiac metabolism under epinephrine appears to be as little affected by veratramine as the force of contraction Since it is thus possible to discriminate between accelerator and metabolic action

of epinephrine, further investigations may throw light on the relationship between force of contraction and cardiac metabolism

Solanum alkaloids with antiaccelerator cardiac activity OTTO KRAYER *Dept of Pharmacology, Harvard Med School, Boston, Mass*

Solasodine and several of its hydrogenation products, notable β -dihydrosolasodine and tetrahydrosolasodine, were found to possess an antagonistic action to the positive chronotropic effect of epinephrine which is obtainable also in the isolated mammalian heart under atropine These solanum alkaloids are similar in this regard to the veratrum alkaloids, veratramine and jervine Like these veratrum alkaloids, they are secondary amine bases of steroid nature β -dihydrosolasodine and tetrahydrosolasodine are intermediate in potency to veratramine and jervine, while solasodine is weaker than jervine The substances used in this study were made available by Prof. L. H. Briggs of Auckland Univ. College, New Zealand

Inhibition of succinic dehydrogenase by methylglyoxal A regulatory mechanism of sulfhydryl enzymes ERNEST KUN *Dept of Medicine, Division of Infectious Disease and Dept of Biochemistry, Tulane Univ., New Orleans, La*

The succinate system of isolated rat liver mitochondria was measured by 3 methods: 1) using the cytochrome system as electron carrier to molecular oxygen, 2) with ferricyanide and 3) with triphenyltetrazolium chloride as electron acceptors Since enzyme activity in each system was equally inhibited in the presence of methylglyoxal, it is concluded that the dehydrogenase is inhibited Cytochrome oxidase is unaffected The inhibition rapidly increases with length of incubation, approaching 100% after 30 minutes The presence of substrate somewhat delays inhibition, however does not protect the enzyme The type of inhibition is non-competitive, and the mechanism of inhibition consists of the reaction of methylglyoxal with the -SH of the dehydrogenase This was shown by direct determination of -SH groups of mitochondria The decrease in -SH, after incubation with methylglyoxal, corresponds with the decrease in enzyme activity Incubation of the inhibited enzyme with glutathione, or repeated washing of inhibited mitochondria does not reverse the methylglyoxal effect Glyoxalase (present in total liver homogenates) does not counteract the inhibition by methylglyoxal, but glyoxalase plus glutathione has a protective effect against the inhibitor Since other -SH enzymes (malic-, glutamic-, triose dehydrogenases, and ATP-ase) are also inhibited by methylglyoxal, it is suggested that the methylglyoxal-glyoxalase-

glutathione system plays an important role in the regulation of -SH enzymes

Storage of methoxychlor in the fat of the rat

FRIEDA M. KUNZE,* EDWIN P. LAUG AND CLAUDIA S. PRICKETT* *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.*

Comparative acute and chronic studies have indicated that methoxychlor is considerably less toxic to rats than DDT. These studies were made to determine to what extent methoxychlor is stored in the fat, liver and kidney. Following exposure of weanling rats to diets containing 25, 100 and 500 p.p.m. methoxychlor for from 4 to 18 weeks, there was found 1) at the 500 p.p.m. level of methoxychlor in the diet, storage of the order of 30 p.p.m. in the fat, but essentially no storage at 25 and 100 p.p.m. dietary levels, 2) in contrast to DDT, no clearly defined tendency of the female to store more methoxychlor in the fat than the male, 3) minimal storage of methoxychlor in liver and kidney tissues, 4) no increase of storage of methoxychlor in fat after the first 4 weeks of exposure, 5) disappearance of stored methoxychlor from fatty tissue within 4 weeks after cessation of exposure.

Effect of hypoadrenalcorticoidism and excessive doses of desoxycorticosterone acetate and thyroid upon response of rat to cardiac glycosides

HERBERT S. KUPPERMAN* AND ARTHUR C. DEGRAFF *Dept. of Therapeutics, New York Univ. College of Medicine, New York City*

The string galvanometer (Cambridge) with suitable modifications has been employed in obtaining electrocardiograms in the rat. The modifications include acceleration of the rate of speed of passage of photographic paper through the camera and use of electrodes comprised of 30-gauge copper wire. Animals were anesthetized with sodium pentobarbital. Hypoadrenalcorticoidism, induced by adrenalectomy, was associated with decreased voltage in the electrocardiogram and increased sensitivity of the rat to i.v. injections of ouabain or digitoxin. Doses of the glycosides that produced minimal effects in intact control rats caused A-V dissociation, bundle branch block, marked slowing of the ventricular rate and cardiac arrest in some of the adrenalectomized animals. Desoxycorticosterone (DCA) administration on the other hand resulted in increased resistance of the rat to administration of doses of the cardiac glycosides which produced marked effects in normal untreated control rats. Ascites and anasarca associated with cardiac hypertrophy induced by chronic and simultaneous administration of high doses of DCA, 0.1% thyroid in the food, 0.9% saline in the drinking water, was ameliorated by

the i.v. injection of a rapid acting glycoside into these rats. A marked diuresis occurred following intravenous administration of ouabain. This was accompanied by a decrease in extent of ascites and venous engorgement.

Local vs systemic emetic action of digitalis

glycosides in man. NATHANIEL T. KWIT,* WALTER MODELL AND HARRY GOID *Dept. of Pharmacology, Cornell Univ. Med. College, Cardiovascular Research Unit of the Beth Israel Hospital, and the Cardiac Service of the Hospital for Joint Diseases, New York City*

When digitalis or its glycosides are administered orally in repeated doses in man until marked action on the heart is in evidence, the nausea and vomiting which may follow are almost invariably ascribed to a systemic action. In a report of animal experiments presented here last year, doubt was cast on the foregoing assumption, since small oral doses of glycosides in cats often produced vomiting 8 to 10 hours later, when assay showed none of the glycosides in the body. If this were to apply to man, the vomiting which usually occurs would constitute not only a protection against serious poisoning, but also a bar against the most effective therapeutic results. An unusual opportunity to test this formulation presented itself in a patient with an extremely resistant auricular flutter. This report concerns the observations in this case during a period of 3 months. During the first month (oral doses), the ectopic rhythm was abolished 3 times, but each time nausea and vomiting preceded the cardiac effect by 2 to 4 days. In the remaining period (intramuscular), the ectopic rhythm was abolished 5 times, each time without gastrointestinal symptoms. These results indicate that a higher degree of cardiac action is possible by parenteral than by oral administration, without gastrointestinal symptoms. Results support the position that vomiting after digitalis materials, which is usually ascribed to a systemic action, may be a local gastrointestinal action. In view of the importance of this observation, a more extensive project to test this question further is in progress.

Potentiating effect of glucose and its metabolic products on barbiturate anesthesia

P. D. LAMSON, MARGARET E. GREIG AND B. H. ROBBINS *Dept. of Pharmacology, Vanderbilt Univ. School of Medicine, Nashville, Tenn.*

We have found that glucose has a very definite potentiating effect on barbiturate anesthesia in certain species of animals. This varies in intensity in dogs, is not present in rats, but is very marked in guinea pigs and definite in hamsters and rabbits. Certain of the decomposition products of glucose metabolism as hexose diphosphate, lac-

tate, pyruvate, succinate and fumarate, as well as malonate and the water extract of both brewers' and bakers' yeast, produce the same effect, some more strongly than glucose. No such effect is obtained with sucrose. This potentiation seems confined to barbiturates. It was absent in ether, chloral and chloralose anesthesia. A simple way in which to observe this potentiation is to give a guinea pig 0.25 ml/100 gm of a 2% hexobarbital solution intraperitoneally. The pig will sleep for about 45 minutes. When definitely awake, but before it can walk, inject 2 ml or less of 50% sodium lactate (Mallinckrodt) and the pig will immediately go into deep sleep and anesthesia. Neither glucose nor its decomposition products alone have any anesthetic effect.

Effects of alterations of the pH of the blood on dynamics of the circulation KURT LANGE, FRANK GRAIG, DAVID WEINER AND VICTOR TCHERTKOFF (introduced by M. G. MULINOS), *Dept of Medicine, New York Med College, New York City*

Intravenous infusions of gluconic acid or acid sodium phosphate solutions were given to dogs on artificial respiration. The resultant lowering of the pH of the blood leads to a marked reduction in peripheral resistance without lowering of the blood pressure until very low pH values are reached. Up to this point a compensatory increase in minute output occurs, accomplished by a marked increase in stroke volume and in spite of a slowing of the pulse rate. Generalized vasoconstriction with resultant increase in peripheral resistance induced by hypothermia can be effectively reduced by acidification. The shock-like picture in diabetic or uremic coma is probably explained by this mechanism.

Effect of certain compounds on biological inactivation of epinephrine EDWARD LARSON AND JOSEPH P. ADAMS, JR * *Dept of Physiology and Pharmacology, Univ of Miami, Medical Research Unit at the Veterans' Hospital, Coral Gables, Fla*

Since the biological inactivation of epinephrine might be influenced either by compounds having a similar structure or certain groups in common, hydroxy compounds such as catechol, phenol, resorcinol, hydroquinone etc were injected simultaneously with definite doses of epinephrine into anesthetized dogs. The effects of these compounds on the actions of epinephrine considered, i.e. rise of the arterial blood pressure, duration of this rise and heart rate, were compared with the effects produced by a control dose of epinephrine administered previously. Addition of catechol or pyrogallol caused a slight increase in the blood pressure response in some instances and in nearly

every case a marked increase in the duration. Several other compounds such as phenol, resorcinol, phloroglucinol etc were without effect. Since inactivation of epinephrine may proceed along several lines (BACQ, *J Pharmacol & Exper Therap* 95, part 2, 1, 1949) various compounds such as glycine, oxophenarsine hydrochloride, ascorbic acid, and nicotinamide were substituted for the hydroxy compounds. None of these compounds affected the epinephrine actions studied.

Glomerular filtration— Tm_g relationships in the dog JULES H. LAST, ISADORE PITESKY,* PAUL JORDAN, JR * AND EPPERSON BOND * *Dept of Pharmacology and Surgery, Univ of Illinois College of Medicine, Chicago, Ill*

In the course of routine Tm_g determinations on normal, unanesthetized dogs, it was observed that changes in Tm_g values seemed to be accompanied by similar changes in glomerular filtration rates (GFR). These observations are at variance with accepted concepts that Tm_g is independent of GFR. Computation of coefficients of correlation (r) based on 246 clearance periods performed on 7 dogs yielded a mean r of 0.561 which was highly significant ($P = < .01$). In 5 out of 7 animals the r values ranged from 0.569–0.879. In these experiments the dogs were not dehydrated by the infusion of 16–17% glucose at 6 cc/min. The data indicate that in the dog the Tm_g is not independent of GFR. To explain these observations in terms of the theory that all nephron units are simultaneously active in the dog kidney, one must postulate that any factor altering the physical process of glomerular filtration must in similar manner alter an enzymatic process of tubular resorption. A more plausible explanation is found in the theory of intermittency of nephron activity, where activation or inactivation of nephron units would directly influence both GFR and Tm_g . Alterations in mean filtration pressure would fail to explain the phenomenon as long as the glucose load exceeds the Tm_g .

Survey analyses of human milk and fat for DDT content EDWIN P. LAUG, CLAUDIA S. PRICKETT,* AND FRIEDA M. KUNZE * *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.*

Twenty-four samples of human milk and 35 samples of human fat were analyzed for DDT. The milk samples were obtained from subjects, some of which occasionally used the common DDT-containing household sprays. There was no occupational exposure to DDT. Abdominal fat was obtained at autopsy or biopsy from subjects, some of which had occupational exposure, but the survey is too preliminary to report any exact correlation between exposure and DDT.

content of the fat at this time. Analyses for DDT were made by a modified Schechter-Haller procedure, sufficiently sensitive to detect and quantitate 5 μ gm DDT in 100 ml milk or 3 μ gm DDT/gm of fat. The DDT concentration in human milk ranged from 0 to 0.77 p p m, with 'high' values of 0.18, 0.28 and 0.77 and 'low' values of 0, 0.04, 0.04, 0.05, 0.06, 0.06, 0.06. The average for all samples was 0.14 p p m. The DDT concentration in human fat ranged from 0 to 34 p p m with high values of 10, 11, 15, 17 and 34. In 12 samples no DDT was found, or only in traces (0.5 p p m). The average for all samples was 5.1 p p m.

Systemic versus nonabsorbable sulfonamide in the treatment of acute bacillary dysentery
DAVID LEHR, JOHN T. LUETTERS,* ARNOLD J. CAPUTE,* HAROLD ABRAMSON,* AND LAWRENCE B. SLOBODY * *Depts. of Pharmacology and Pediatrics, New York Med. College, Flower and Fifth Avenue Hospitals, New York City*

The therapeutic effectiveness of sulfadiazine was compared with that of a triple mixture of sulfonamides (sulfadiazine, sulfamerazine and sulfacetimide in equal amounts) and that of the nonabsorbable sulfonamide—phthalylsulfacetimide, in an outbreak of bacillary dysentery (*Shigella sonnei*) among 330 children ranging in age from early infancy to 14 years. A total of 79 affected children received oral sulfonamide therapy in weekly courses. Of these 27 were treated with sulfadiazine (0.2 gm/kg daily plus double the amount of sodium bicarbonate), 24 children received the triple mixture in the same dosage but without alkali, and 28 received phthalylsulfacetimide in amounts increasing from a daily dosage of 1.5 gm for the ages of 2-6 years, to 2 gm for 6-10 years and 4 gm for those above this age group. Consecutive negative cultures from rectal swabs on the 4th to 7th day after discontinuation of the sulfonamides were considered indicative of cure. The triple mixture demonstrated the highest therapeutic efficacy. It effected the cure of 20 patients after one course of treatment, as compared to only 11 each in the sulfadiazine and phthalylsulfacetimide groups. This difference between the triple mixture and the other 2 sulfonamides is statistically significant (chi square method). The fact that the triple mixture produced substantially higher blood levels than sulfadiazine could not adequately explain the greater therapeutic efficacy of the former, since phthalylsulfacetimide proved as good as sulfadiazine without having entered the bloodstream to any significant extent. It would seem, therefore, that the triple mixture exerts a substantially higher antibacterial effect within the gastrointestinal tract. The curative efficacy of phthalylsulfacetimide

appears highly satisfactory since it compares favorably with sulfadiazine.

Effects on anaphylactic shock of salicylates, aminopyrine and other chemically and pharmacologically related compounds
MARK H. LEPPER,* BENJAMIN F. MILLER,* ESTON R. CALDWELL, Jr,* AND PAUL K. SMITH *Depts. of Medicine and Pharmacology, George Washington Univ. School of Medicine, Washington, D. C.*

The effects of salicylates on anaphylactic shock in rabbits were compared with that of certain compounds chemically and pharmacologically related to the salicylates. The compounds studied were acetanilid, neocinchophen, aminopyrine in the group with similar pharmacologic properties and para-aminobenzoic acid, sulfanilamide, and sodium gentisate in the chemically related group. All compounds were used in equivalent amounts, based on aspirin, 0.64 gm. In addition, for special control purposes, the following were employed: sodium bicarbonate, 0.32 gm; Nembutal, 30 mg; and codeine phosphate, 5 mg. Rabbits weighing approximately 2 kg were sensitized by repeated injections of egg white. After 2 weeks had been allowed for sensitivity to develop, the animals were divided into experimental and control groups. In the former animals, the drug was given suspended in water by pharyngeal tube, while the latter received an equal dose of water by tube one hour before a shocking dose of egg white was given. Aspirin, sodium salicylate, and aminopyrine were found to give significant protection against this type of shock (Aspirin: 8 deaths in 57 animals, controls: 39 deaths in 74 animals; Sodium salicylate: 9 deaths in 43 animals, controls: 32 deaths in 60 animals; Aminopyrine: 1 death in 22 animals, controls: 19 deaths in 34 animals). None of the other compounds was definitely effective, although some may have demonstrable effect in more animals or by different techniques. The activity of the salicylates and aminopyrine did not correlate with their other pharmacologic properties. They exhibited no antihistaminic action in rabbits.

Factors influencing toxicity of fortified red squill powder
E. WILLIAM LIGON, JR., AND ROBERT R. SPROWELL * *Pharmacology Laboratory, Insecticide Division, Livestock Branch, Production and Marketing Administration, U. S. Dept. of Agriculture, Beltsville, Md.*

A fortified red squill powder was bioassayed on 6 strains of male albino rats. After being fasted for 24 hours, rats weighing between 150 and 250 gm were offered bait consisting of 10% squill in bread crumbs. After 16 hours they were returned to normal diet for an 8-day observation period. Bioassays were similarly performed, by administering through a stomach tube squill suspended in

tragacanth These animals were returned immediately to normal diet

STRAIN	LD ₅₀ , MG VOL. FEEDING	SQUILL/KG RAT STOMACH TUBE
I	210 ± 27 0	165 ± 14 2
II	127 ± 11 0	125 ± 13 5
III	147 ± 9 2	125 ± 9 7
IV	154 ± 10 3	127 ± 16 2
V	148 ± 15 7	140 ± 10 6
VI	170 ± 15 0	175 ± 24 3

Bioassays were also performed with two variations in technique In one case, different concentrations of squill were used for voluntary feeding In the other, food was withheld for 16 hours after dosage by stomach tube The LD₅₀ for 10% bait was 125 ± 15.5 mg/kg, for 3%, 200 ± 29 mg/kg, and for 1%, 200 ± 20 mg/kg The LD₅₀ for controls fed immediately after stomach tube administration was 177 ± 19 mg/kg and for those denied food for an additional 16 hours was 108 ± 11 mg/kg

Bioassay method for diuretic substances J MAXWELL LITTLE AND CARLOS COOPER, JR * *Dept of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest College, Winston-Salem, N C*

Female dogs were prepared by episiotomy for easier catheterization The dogs were given 35 cc of distilled water per kg by stomach tube, and the food and water were removed Approximately 16 hours later the animals were catheterized, the bladder was emptied, and the urinary specific gravity was determined If the specific gravity was 1.030 or greater the animal was discarded The animals were given 25 cc of distilled water per kg by stomach tube and the diuretic substance was administered The animals were put in metabolism cages in a room maintained at 22°C At the end of 6 hours the animals were catheterized, and the total urine volume was measured Results were expressed as diuretic ratio $\left(\frac{6 \text{ hour urine volume}}{25 \text{ cc} \times \text{kg body weight}} \right)$ Ninety control experiments on 12 dogs gave a mean ratio of 1.04 ± 0.172 In arriving at this method several hydration procedures were tested removing food and water 16 hours before the experiment, omitting the first hydration procedure, and collecting for 5 hours at room temperature yielded a mean ratio of 0.20 ± 0.149 in 14 experiments, repeating this experiment with the exception of leaving water available until 1 hour before the experiment gave a mean ratio of 0.37 ± 0.274 in 12 experiments repeating this experiment with the exception of collecting for 6 hours at 22°C gave a mean ratio of 0.82 ± 0.299 in 111 experiments The mean ratios following the iv injection of 2-2.5 cc of 3 pooled dialyzed concentrated human urine samples are 1.98 ± 0.355 , 1.79 ± 0.396 and 1.59 ± 0.314

Effect of epinephrine on intestinal volume A E LIVINGSTON *Dept of Pharmacology, Temple Univ School of Medicine, Philadelphia, Pa*

When epinephrine is injected into a systemic vein of an anesthetized dog, an increase in volume of an intestinal loop in a plethysmograph takes place The portal vein pressure increases and the liver volume usually decreases These effects begin when the systemic blood pressure is near the maximum When the injections are made into a mesenteric vein a prompt and similar effect is produced on intestinal volume and portal pressure, though the effect on the systemic blood pressure may be very slight If the injection is made into a branch of the mesenteric artery leading to the loop of intestine, a marked decrease in volume of the loop takes place and an increase in portal pressure follows When the portal blood is shunted to an external jugular vein and injection is made into a femoral vein a similar increase in volume of intestinal loop and portal pressure takes place, and an increase in blood volume flows through the shunt during the increase in portal pressure When injections are made into a femoral vein and arterial pressure is held constant, or nearly constant, an increase in intestinal volume and portal pressure usually is produced though the magnitude of effect is not so great as when the blood pressure is allowed to rise An increase in intestinal volume and portal pressure is observed after the administration of atropine, section of both vagi, or the section of the splanchnic nerves

Sympathetic ganglionic stimulant drugs S LOEWE, L S GOODMAN AND SUSANNE L PUTTUCK * *Dept of Pharmacology, Univ of Utah College of Medicine, Salt Lake City, Utah*

Potent and selective ganglionic vasopressor agents were found in the new class of o-cresyl choline ethers ($R-CH_2-C_6H_4-O-C_2H_4-N(CH_3)_2-OH$) Greatest potency was found in those representatives of the class in which R is phenyl, p-fluorophenyl or 2-thienyl, these 3 substances are approximately equal in potency Like nicotine, these drugs are sympathetic and parasympathetic ganglionic depressants at high dosage levels, their depressant doses are similar to those of nicotine However, the new choline ethers differ from nicotine in the following respects 1) They lack the muscarinic and curare-like actions of nicotine, its central nervous actions and its activity on smooth muscle and carotid sinus chemoreceptors 2) The threshold dose for sympathetic ganglionic stimulation is smaller than that of nicotine 3) They gave no indications of parasympathetic ganglionic stimulation in a wide range of doses inducing stimulation of sympathetic effector functions The degree of selectivity of sympathetic as against parasympathetic stimulation is undergoing con-

tinued study —An interesting feature of the new choline ethers is that they also possess considerable antihistaminic activity, being approximately equal in potency to diphenhydramine

Mechanism of vasomotor actions of sparteine Go LU *Dept of Pharmacology and Therapeutics, Stanford Univ School of Medicine, San Francisco, Calif*

Sparteine sulfate (5-10 mg/kg) injected i v in anesthetized (chloralose, pentobarbital, or morphine-ether- α -tubocurarine) dogs and cats produced bradycardia and fall of blood pressure. After injecting 40 mg/kg within 30 minutes, an additional 5-10 mg/kg i v caused slight pressor effect (mostly under artificial respiration). The depressor phase was accompanied by increase in leg volume and decrease in kidney, spleen or intestine volume and was due to peripheral vasodilatation, since the cardiac output (Fick's principle) increased slightly or remained unchanged. Cardiometric record showed ventricular dilatation with increase in pulse volume. The peripheral vasodilatation was not significantly altered by section of vagi, atropinization, pyribenzaminization or carotid denervation. However, dilatation was prevented by pithing, denervating a leg, nicotine or ergotoxinization. Cisternal injection revealed no central vasomotor depression. Femoral artery injection revealed no effect or slight vasoconstriction. Sparteine prevented the pressor effects of carotic occlusion, or of sciatic stimulation, and hyperpneic responses of nicotine and acetylcholine (atropinized animals), and potentiated the pressor effect of epinephrine. It also interrupted the contraction of the nictitating membrane produced by electrical stimulation of the preganglionic fibers, whereas postganglionic stimulation remained unaffected. Thus, sparteine produces vasodilatation by depression of sympathetic ganglia. The later pressor effect accompanied by decreases in kidney, intestine and leg volumes is due to peripheral vasoconstriction resulting from direct smooth muscle stimulation.

Diethylaminoethyl esters of 2-hydroxy- and 2-alkoxy-4-aminobenzoic acid as local anesthetics

F P LUDUENA AND JAMES O HOPPE * *Sterling-Winthrop Research Inst, Rensselaer, N Y*

The local anesthetic activity of 2-hydroxy and various 2-alkoxy analogs of procaine, synthesized by Clinton and co-workers, was determined by various methods. A comparison of the duration/log concentration curves obtained by the sciatic nerve block method in guinea pigs disclosed an increase in activity with an increase in length of the alkoxy side chain from methoxy through the butoxy substituent. Procaine is less active than the 2-hydroxy and more active than the 2-methoxy

analog. The butoxy analog (WIN 3706) is about as active as tetracaine. By intraspinal injection in rabbits, both the propoxy analog (WIN 3459) and WIN 3706 produce anesthesia of rapid onset and relatively short duration. Spinal anesthesia was obtained with a concentration as low as 0.03% of WIN 3706 which is below the threshold concentration for tetracaine. In these rabbits, the signs of local injury were minimal, no paralysis was observed with WIN 3706 and, of the 30 rabbits injected with sublethal doses of WIN 3459, only one showed a residual hind leg paralysis. Topical anesthetic activity was determined by corneal irrigation in rabbits. Both WIN 3459 and WIN 3706 were more active than cocaine but less active than tetracaine. The toxicity, as determined by i v injection in mice, parallels the local anesthetic potency, as determined by the nerve block method. The LD_{50} of WIN 3459 is 9 ± 0.4 mg/kg, and that of WIN 3706, 4 ± 0.3 . When the local irritation produced by intradermal injection in rabbits was determined by the trypan blue method, WIN 3459 and WIN 3706 were found $\frac{1}{2}$ and $\frac{1}{3}$ as irritating as tetracaine, respectively.

Sodium catechol disulphonate protection in experimental uranium nitrate poisoning L M LUSKY* AND H A BRAUN * *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D C*

When an aqueous solution of disodium catechol disulphonate (DCD) is mixed with an aqueous solution of uranium nitrate, an amber colored complex is formed. Protection against the systemic effects of uranium nitrate in rabbits occurs when DCD is administered by intramuscular injections. For example, the LD_{50} of uranium nitrate administered s c is increased more than 4-fold if intramuscular treatment with DCD is started immediately after injection of the uranium salt. Protection is also obtained, though to a lesser extent, when treatment with DCD is delayed one hour after administration of uranium salt. Combined treatment with DCD and sodium citrate was more effective in protecting rabbits from systemic effects of uranium nitrate than treatment with either DCD or sodium citrate alone given under the same schedule.

Vasomotor and gastrointestinal studies using Diparcol C KEITH LYONS, AND BOYD H METCALF (introduced by KEITH S GRIMSON) *Dept of Surgery, Duke Univ School of Medicine, Durham, N C*

Diparcol, diethylamino, 2, ethyl-N, dibenzopara-thiazine hydrochloride, has been tested in dogs and man. In 4 dogs with vagi cut Diparcol was given using initial doses of 0.5 mg/kg and adding larger increments until during 3 or 4 hours total amount was 81.5 to 130 mg/kg. With the

final dose death occurred suddenly. Results achieved when 5 to 10 mg/kg had been given were not much altered by later larger amounts. Though increments of 5 to 20 mg/kg effected reduction of pressure lasting several minutes, pressure generally maintained at normal values to the lethal dose. Increase of blood pressure with carotid artery occlusion occurred throughout the experiment, as did rise of pressure with stimulation of the central end of one vagus. Marked slowing of pulse and drop of pressure with stimulation of the peripheral end of a vagus were partially blocked after 3.0 to 23 mg/kg total accumulative dose and completely blocked after 5, 10, and 31 mg/kg in 3 animals. In the 4th animal even to the lethal dose block remained partial. Pressor response with epinephrine, 1 to 5 μ gm/kg, was blocked by 5, 6.5, 20, and 44 mg/kg total additive dose of Diparcol. After large doses anoxia produced decrease of blood pressure in 2 dogs, no change in 1 and slight decrease followed by an increase just after the anoxia in the 4th. Five to 15 mg/kg i.v. effected temporary rise of blood pressure and pulse in 3 unanesthetized dogs. Gastric emptying and ileal transit time determined by roentgenologic methods in 9 dogs after 5 to 30 mg/kg were essentially normal. Observations in patients are similar.

Comparative effects of repeated doses of curarizing agents in man DAVID W. MACFARLANE* AND KLAUS R. UNNA. *Dept. of Pharmacology, Univ. of Illinois, College of Medicine, Chicago, Ill.*

In 4 normal male subjects, the effects of a first and a second dose of D-tubocurarine chloride (DTC), dimethyl ether of D-tubocurarine chloride (dimdTC), and decamethylene-bis(trimethyl-ammonium bromide) (C10) were compared. Criteria for degree of effect were depression of grip strength as measured by a dynamometer, recovery time measured as time in minutes for 75% recovery of grip strength (t_{75}), and depression of vital capacity. The interval between i.v. injections was chosen to be 45 minutes for DTC and dimdTC and 30 minutes for C10. It has been shown, previously, that at the time intervals chosen, effects of the first injection could no longer be objectively measured. It was found that after 45 minutes, only $\frac{1}{2}$ of the initial dose of DTC or dimdTC was required to produce effects similar to those of the original dose. This was unlike C10, which if reinjected in the full initial dosage after 30 minutes, failed to produce effects comparable to those of the first injection. In all subjects, the effect of the second dose of C10 on grip strength and vital capacity was significantly smaller. In an attempt to obtain data on animals under comparable conditions, head drop doses of DTC and C10 were determined on both cats and rabbits and redetermined at

suitable intervals. With both drugs and in each animal species, the second head drop dose was significantly smaller than the first. Thus, the tachyphylaxis to C10 observed in man was not seen in cats or rabbits.

Influence of penicillin-penicillinase combinations and PABA sulfonamide mixtures on the growth of higher plants compared with bacteria DAVID I. MACHT. *Dept. of Pharmacology, Sinai Hospital, Baltimore, Md.*

Inasmuch as many botanists regard bacteria as microscopic plants, experiments were made in regard to the action of sulfonamides and PABA separately and in combination with each other on the root growth of *Lupinus albus* seedlings. The drugs studied were 3 aminobenzoic acids and various sulfa compounds. Seedlings of *Lupinus albus* were grown in Shive's solution with and without the above drugs. It was found that the ortho-aminobenzoic acid was the most toxic for root growth, and para-aminobenzoic acid the least toxic. All the sulfa compounds exerted a biphasic effect, dilute solutions stimulating the root growth, while greater concentrations inhibited it. Combinations of PABA and sulfonamide always produced a synergistic effect. That is, instead of PABA inhibiting the action of sulfa drugs, it actually potentiated their toxicity. The action of penicillin and penicillinase was studied in 2 ways: the effect on the root growth of *Lupinus albus* seedlings and the effect on respiration of *Lupinus* seed emulsions. Penicillin, itself, is toxic for root growth, and the amorphous variety is more toxic than the crystalline. Penicillinase (Schenley) was employed, obtained from cultures of *B. cereus* strain NRRL-B569. This enzyme is very little toxic for *Lupinus* root growth. Combinations of penicillinase and penicillin produced a synergistic effect. The reductases of *Lupinus* seeds were studied by Thunberg's method. Penicillin retarded the reducing process. Penicillinase alone exerted little effect, a combination of the 2 acted synergistically.

Toxicity of penicillin for previously treated animals DAVID I. MACHT AND THOMAS HOFFMASTER*. *Dept. of Pharmacology, Sinai Hospital, Baltimore, Md.*

Because of the very low toxicity of penicillin, it was deemed desirable to test its effect on animals previously conditioned, either physiologically or pharmacologically. The animals employed were mice, rats, guinea pigs, rabbits and cats. Physiologically conditioned were hypophysectomized rats and rabbits unilaterally adrenalectomized. No difference in the toxicity of either crystalline or amorphous penicillin was found between these and normal animals. In pharmacological experiments large doses of various drugs chosen for

their selective action for different physiological functions were given to different animals and the effect of subsequent injections of penicillin in large doses was investigated. The following drugs were studied: digitalis, Cobra neurotoxin, mercuric chloride, alloxan, sulfonamides dicumarol, heparin, salicylates, antipyrin, dinitrophenol and pentothal. None of these increased the toxicity of penicillin on subsequent injections even in very large doses (100,000 to 500,000 units). A potentiation in toxicity was noticed in animals treated with phosphorus and with camphor. The mortality from these poisons was greater when penicillin was combined with them. Next to the invaluable chemotherapeutic properties of penicillin the authors find the most important pharmacological properties to be its thromboplastic effect. This is efficiently antagonized by dicumarol and heparin. The salicylates and sulfadiazine do not effectively prolong coagulation time except after their repeated administration for a long period of time.

Pharmacologic data on DL-2-amino-L-(p-methylphenyl)-propane EDWARD MACKO,* EDWIN J. FELLOWS AND A. JAMES FENDRICK * *Research Division, Smith, Kline and French Laboratories, Philadelphia, Pa.*

In the present studies the hyperkinesthetic activity of DL-2-amino-L-(p-methylphenyl)-propane (No. 42) in rats was found to be $\frac{1}{2}$ intraperitoneally and $\frac{1}{8}$ orally that of D-2-amino-L-phenylpropane (No. 2). Orally, No. 2 was more than 10 times as active as No. 42 in antagonizing the depressant effect of phenobarbital. The analeptic potency of No. 42 therefore does not parallel its effectiveness in producing an increase in motor activity. Subcutaneously in dogs, 5 mg/kg exhibited the same antiappetite potency as 1 mg/kg of No. 2. The foregoing data suggested that No. 42 may be a useful anorexigenic agent with a low order of antisleep effect. No. 42 also possesses an analgetic action as indicated by the fact that doses of 5.0 to 20.0 mg/kg intraperitoneally markedly elevated pain thresholds in cats. In subacute and chronic toxicity studies no evidence of changes due to administration of No. 42 was observed by histological examination of tissues removed from rats, rabbits, guinea-pigs and dogs which received massive daily doses (up to 30 mg/kg) of this compound for periods of one to 3 months. The data obtained in the present experiments indicate that No. 42 should be evaluated clinically as an antiappetite and as an analgetic agent.

Bioassay method for derivatives of Veratrum Viride GEORGE L. MAISON AND J. W. STUTZMAN *Dept. of Pharmacology, Boston Univ. School of Medicine, Boston, Mass.*

Hypotensive action in dogs under pentobarbital anesthesia has been utilized as bioassay method for Veratrum Viride derivatives. Great quantitative variability of response (not tachyphylaxis) on repeated administration to the same dog as well as to different dogs resulted. Most satisfactory route of administration proved to be i.v. infusion at constant rate (in $\mu\text{g/kg/min}$) for 10 minutes. Variability was reduced by bracketing the infusion of unknown between two infusions of a standard reference powder. The fall of mean femoral pressure at its nadir expressed as per cent of pre-existing pressure was used as the criterion of response. Statistical analysis showed that units of 6 dogs were required for a detection level (± 2 standard deviations) of 20% difference in potency. Units of 9 or 12 dogs did not improve the detection level. The reference standard utilized was Veriloid (J. W. Dart Labs). In 232 trials the mean response to standard infusion was $29.9 \pm 12\%$ fall of mean arterial pressure. The following substances had less than 10% of the potency of the reference standard (mg for mg): jervine, rubijervine, isorubijervine, n-methyl jervine*, o-acetyl n-methyl jervine*. * Not derived from Veratrum viride.

Studies on renal function during various stages of anesthesia produced by thiopental in dogs ARNOLD H. MALONEY, WALTER M. BOOKER, JAMES R. TUREMAN* AND CLAUDIA M. RATLIFF* *Dept. of Pharmacology, Howard Univ. Med. School, Washington, D. C.*

In connection with studies on the course of pentothal anesthesia as related to nutritional states, it was deemed important to make some evaluation of the effects of various states of anesthesia on kidney function in dogs. Accordingly, the following experiments have been designed to study glomerular filtration rate and renal plasma flow as related to the plasma pentothal levels. The periods of anesthesia were divided into light, moderate, and deep as determined by the Draper-Whithead reflex response. During these periods, the glomerular filtration rate is determined by the creatinine clearance method and the effective renal plasma flow by the diodrast plasma clearance. Also, during these periods, the plasma level of pentothal is determined. Preliminary experiments at this time show that the highest values for the glomerular filtration rate are obtained in the periods of light anesthesia and the lowest values during the periods of deepest anesthesia. The results of the effective renal plasma at the time are not definitive. The plasma levels of pentothal may be correlated with degree of anesthesia. Experiments are now under way to extend these observations.

In vitro antihistamine and anti-acetylcholine activity of several antihistamines S MARGOLIN, A MAKOVSKY AND R TISLOW (introduced by H B HAAG) *Biological Research Laboratories, Schering Corporation, Bloomfield, N J*

Isolated portions of guinea pig ileum suspended in Tyrode solution were used to determine the *in vitro* activity of several antihistamines. The end-point in these tests was inhibition of the spasmogenic effect of histamine dihydrochloride (1 5, 000,000) or acetylcholine chloride (1 50,000,000) following a 3-minute exposure of the tissue to the antihistamine. Dilutions which would inhibit the spasmogenic contraction by 50% and confidence limits were calculated from responses to graded concentrations of the drugs. While the data are of interest in differentiating between antihistamine and anti-acetylcholine activity under the conditions of an *in vitro* test, they do not give an index of antihistamine efficacy when compared with tests *in vivo* and with clinical experience.

Effect of localization of pentothal in fat on its duration of action LESTER C MARK,* ELEANORE BERNSTEIN* AND BERNARD B BRODIE *Depts of Anesthesiology and Biochemistry, New York Univ College of Medicine and New York Univ Research Service, Goldwater Memorial Hospital, New York City*

Previously reported studies indicated that pentothal is slowly metabolized *in vivo* and that the rapid decline in plasma levels and quick recovery following a single *i v* dose of the drug are due to its localization in body fat. The interrelationship of plasma, tissue and fat concentrations has been further studied in dogs. After the *i v* administration of pentothal, concentrations in liver and plasma were high at first but decreased rapidly, while those in fat were negligible at first but increased steadily, and finally accounted for most of the drug in the body. Concentrations in the central nervous system appeared to decline with those of liver and plasma as evidenced by cerebrospinal fluid levels. Different fat depots varied in the rate of pentothal uptake but within 24 hours the pentothal concentrations of all fat depots were similar. The clinical effects of a given dose of pentothal appear therefore to depend upon the plasma level at which equilibrium is established between fat and the rest of the body. After a small dose, this level is subhypnotic and the drug is ultrashort acting. After a large dose, the equilibrium level is above the hypnotic level and is maintained there for considerable periods by the pentothal reservoir in fat. Preliminary investigation with the oxygen analogue, pentobarbital, indicated that its localization in fat was less extensive.

Synthetic curare compounds VI Isomers of N-methyl-tetrandine iodide DAVID F MARSH AND D A HERRING * *Dept of Pharmacology, West Virginia Univ School of Medicine, Morgantown, W Va*

Of the 8 possible isomers of the tetrandine type biscochlorine alkaloids, 5 have been isolated and characterized. We have converted these to the quaternary ammonium derivatives and compared their activity with *D*-tubocurarine chloride. As well as apparently unrelated side-effects, *D*-tubocurarine and, *O,N*-methyl-repandine have some histaminic activity, and all the agents in high dose in cats, except the *N*-methyl-tetrandine, produce non-specific falls in blood pressure. An attempt has been made to correlate these observations with the probable structural models of the compounds.

Studies with low vapor concentrations of carbon tetrachloride labeled with carbon 14 I Absorption, distribution and elimination upon inhalation by monkeys D D MCCOLLISTER, W H BEAMER, G J ATCHISON AND H C SPENCER (introduced by D D IRISH) *Biochemical Research Laboratory and Spectroscopy Laboratory, Dow Chemical Company, Midland, Mich*

Monkeys inhaled radioactive carbon tetrachloride through a mask and respiratory valve system at a 'contact' concentration of 46 p p m (0.289 mg/l) for periods of 139 to 344 minutes. Absorption occurred at an average rate of 1.34 mg/kg/hour, or 30% of the total weight of carbon tetrachloride inhaled, the blood concentration of carbon-14 did not reach saturation within the time of the experiments. Tissue analyses for radioactive carbon gave distribution ratios (blood = 1) for depot fat = 7.86, liver = 3.00 and bone marrow = 2.97. Bone, lung, muscle, spleen, heart, kidney and brain had distribution ratios ranging from 0.14 to 0.96. Carbon tetrachloride was metabolized by the monkeys. Ten to 20% of the total radioactivity in the blood and expired air was present in the form of $C^{14}O_2$. Carbon-14 was also identified in urinary urea and carbonate but the chief metabolic product in urine was an unidentified non-volatile material found to have attributes suggestive of a conjugate. Twenty percent of the absorbed carbon tetrachloride was eliminated by exhalation in the form of $C^{14}O_2$ and volatile 'carbon tetrachloride' during the first 18 hours following the end of an exposure. Carbon-14 was measurable in samples of expired air over a 4-week period. It was estimated that a total of 50% or more of the carbon tetrachloride absorbed by the monkey was eliminated in this manner. The remainder appeared to be excreted in the feces and urine.

Electrical and chemical activities of fibrillating denervated and innervated muscle deprived of Ca^{++} A R MCINTYRE, A L DUNN,* AND FENWORTH DOWNING * *Dept of Physiology and Pharmacology, Univ of Nebraska College of Medicine, Omaha, Nebr*

Intravenous perfusion *in vivo* of fibrillating denervated dogs' muscles with the following solutions (at pH 7.0, 37°C) was without immediate effect on the electrical activity of the fibrillating muscles, Tyrode solution, Tyrode solution containing eserine 1:100,000, Ca^{++} free Tyrode solution and Ca^{++} free Tyrode solution containing 1 mM of procaine hydrochloride. In contrast the electrical activity of innervated muscle aroused by perfusion with Ca^{++} free Tyrode solution was promptly interrupted when procaine was added to the Ca^{++} free solution. Muscle electrical activity was recorded throughout the experiment. The perfusing solutions were collected and rendered protein free by trichloroacetic acid precipitation. The protein free filtrates were each divided into 2 fractions. One of each pair was heated at pH 9, cooled and neutralized with CO_2 . All the filtrates were then tested for ACh on the isolated heart of *Venus mercenaria*. All filtrates obtained from perfusates collected from both innervated and denervated muscles while electrically active contained a heat labile cardio-inhibitor (ACh) in concentration of approximately 1.5 to 3.2 μgm of ACh per ml. No ACh was detected in any perfusate collected from muscles at rest. Examination of single spike potentials from innervated and denervated muscles revealed no consistent differences. Very rapid spikes (0.5 msec) were obtained from both. The increased sensitivity to ACh in the denervated and innervated muscle, deprived of calcium, is of the same order (circa 1,000-fold). These findings are discussed and indicate that the activity of cholinesterase at the junction is decreased by denervation and, in innervated muscle, by loss of normal resting polarity at the nerve endings.

Effect of colchicine on certain synthetic reactions *in vitro* GORDON R. MCKINNEY (introduced by FREDERICK BERNHEIM) *Dept of Physiology and Pharmacology, Duke Univ School of Medicine, Durham, N C*

Rat liver slices or homogenates were incubated with appropriate substrates in 20-ml beakers in a Dubnoff Metabolic Shaking Incubator at 38°C. The tissue was suspended in Krebs-Ringer's bicarbonate buffer, saturated with 95% O_2 -5% CO_2 mixture, which was also the gas phase. 1×10^{-3} M colchicine inhibited the formation of creatine from guanidoacetic acid and L-methionine by 65%. It was as effective as methyl-bis-(beta-chloroethyl)-amine HCl (MBA), and more effective than ure-

thane. Colchicine and MBA blocked the methylation of nicotinamide by methionine to a greater degree than did urethane. Colchicine, however, inhibited the formation of p-aminohippuric acid (PAH) only by 15%, whereas MBA inhibited by 70%. Urethane did not affect PAH synthesis. Neither colchicine nor urethane affected the conjugation of morphine which was inhibited by 85% by MBA. □

Plasma levels and urinary excretion of cocaine in dog and rabbit F G McMAHON,* L A WOODS AND M H SEEVERS *Dept of Pharmacology, Univ of Michigan, Ann Arbor, Mich*

Using an original and specific colorimetric method, the details of which will be published elsewhere, capable of estimating cocaine in concentrations above 0.4 $\mu\text{gm}/\text{cc}$ of plasma and 1.0 $\mu\text{gm}/\text{cc}$ of urine, a study of the plasma levels and urinary excretion of cocaine has been completed in the dog and rabbit. Intravenous administration of 5 mg/kg (base equivalent) of cocaine hydrochloride to the dog is followed by disappearance of the drug from venous plasma in about 30 minutes. Subcutaneous injection of 10 mg/kg and 15 mg/kg (base equivalent) of cocaine hydrochloride to the dog results in slowly increasing plasma levels of cocaine to a maximum of 1.1 to 1.8 $\mu\text{gm}/\text{cc}$, 1½ to 3 hours after the dose. Seven to 10% of the cocaine can be recovered from the urine of dog after s.c. and i.v. administration. The plasma concentration of cocaine in the rabbit, 60 to 90 minutes after s.c. administration of 30 mg/kg, is about 1.5 $\mu\text{gm}/\text{cc}$. Most workers have been unable to recover cocaine in rabbit urine. We can find none in cage-collected urine but cocaine is detectable in small amounts (less than one per cent of dose) if catheter-collected samples are analyzed.

Silicone fluids as vehicles for the intramuscular administration of drugs BERNARD P MCNAMARA, ELIZABETH A MCKAY* AND MAGGIE M QUILLE * *Pharmacology Section, Medical Division, Army Chemical Center, Md*

Silicone fluids offer possibilities as non-aqueous vehicles for intramuscular administration of drugs. Certain drugs injected intramuscularly in such fluids exert systemic effects within one minute. Animals injected (0.1 cc/kg) in a single area, 3 times per week for a period of 2 months showed no clinical signs of pain or muscle injury. The R.B.C., W.B.C., hemoglobin, hematocrit and body weight of these animals were not adversely influenced. The intravenous administration of such fluids in rabbits sometimes results in death, possibly because of 'fluid embolism'.

Sensitivity to digitals at the altitude of Bogotá K MEZEY AND R BAQUERO (introduced by

McKEEN CATTELL) *Cesar Uribe-Piedrahita Research Labs, Bogotá, Colombia*

In a previous report (*Am J Pharm* 115 326, 1943) it was shown that the cat unit of the same digitalis preparation under identical experimental conditions (weight, sex of animals, anesthesia, rate of infusion) was 17.6% less in Bogotá (altitude 2640 meters, temp 14.5°C) than in Girardot (altitude 326 meters, temp 29°C). In order to find out whether this difference is due to the diminished partial pressure of oxygen in Bogotá (110 mm Hg) or to the lower environmental temperature, experiments were carried out in the following manner. In the first series of 20 cats the cat unit of Digitaline Nativelle in a 1:100,000 solution was determined. The second series of 10 experiments was made in the same manner as the first except that the right nostril of each cat was connected with an oxygen bottle supplying 0.6 liter of oxygen per minute, started 15 minutes before beginning the infusion. The third series of 10 experiments was performed as the second except that a room-temperature of 29°C was maintained. Healthy cats of both sexes were used weighing from 1.7 to 3.6 kg and fasted 24 hours. Thiopental, 30 mg/kg intraperitoneally, was used for anesthesia. We used the technique of continuous injection or infusion since we did not find any advantage in the modification described in the USP XII. The following average lethal dose values were obtained: Control, 0.335 ± 0.001 mg, Oxygen, 14.6°C, 0.365 ± 0.0012 , Oxygen, 29.0°C, 0.378 ± 0.0009 . We conclude that the average lethal dose of a given digitalis preparation is lower in the natural environmental conditions of Bogotá than in an artificially oxygenated atmosphere and that the higher toxicity of digitalis in Bogotá is due mainly to the partial lack of oxygen and only to a slight extent to the lower environmental temperature.

In vivo conjugation by rats of parenterally administered histamine R. CARL MILLICAN (introduced by SANFORD M. ROSENTHAL *National Insts of Health, Bethesda, Md*)

It has been shown that an acetone powder extract of liver, in the presence of Coenzyme A, can conjugate histamine (*J Pharmacol & Exper Therap* 97 4, 1949). Conjugation *in vivo* was investigated by subcutaneous administration of histamine to normal and enterectomized rats. A colorimetric method was employed for the determination of free and conjugated histamine in the urine. The determination of conjugated histamine was confirmed by the addition of histaminase. In normal rats total urinary excretion of free and conjugate during the 48 hours following administration ranged from 21 to 43% of administered histamine. Twenty to 40% of the administered histamine was excreted as free histamine within

17 hours. Conjugated histamine was demonstrated in the urines of normal rats 17 to 24 hours following subcutaneous administration of 500 mg histamine base per kilo, in amounts ranging from 14-51% of total present. Following 100 to 200 mg of histamine base per kilo subcutaneously to enterectomized rats, the percentage conjugate excreted 17-40 hours following administration varied from 24-55% of the total present. Employing a modified metaphosphoric acid-cotton acid succinate method for demonstrating conjugated histamine, the viscera of several species of animals were analyzed for the conjugate. No significant amounts were demonstrable except following oral or parenteral administration.

Incidence of local reactions to mercurhydrin and thiomerin by subcutaneous injection in patients with congestive failure WALTER MODELL, HARRY GOLD, NATHANIEL KWIT,* LEON WARSHAW,* HAROLD OTTO,* AND WILLIAM ZAHM* *Dept of Pharmacology, Cornell Univ Med College, Cardiovascular Research Unit, Beth Israel Hospital, and the Cardiac Service of the Hospital for Joint Diseases, New York City*

In a recent paper from our clinics, attention was called to the fact that the control of congestive failure most effectively, requires self-injection of the diuretic as in the case of insulin for diabetes. An effective mercurial diuretic tolerated by the subcutaneous route would therefore constitute an important advance. Several recent reports declared that thiomerin by the subcutaneous route was substantially free of local reactions, and during this period one report stated that mercurhydrin, which is commonly used by intramuscular injection, is also relatively free of local reactions when given subcutaneously. The present study was carried out to secure more evidence concerning the local reactions to the two materials by subcutaneous injection. Severe pain, inflammatory induration, ecchymosis, slough, and fibrous nodules. In the case of thiomerin, 601 injections of 0.25 to 2 cc in 187 patients, yielded one or more of the above reactions in 28% of patients (26% of injections in those with only 1 injection). In 43 patients with about 10 injections each, reactions increased to 44% of patients (27% of injections). In the case of mercurhydrin, 1132 injections of 1 to 2 cc in 200 patients (average of 6 per patient), yielded one or more reactions in 27% of patients (9% of injections). The relative merits of thiomerin and mercurhydrin by subcutaneous injection will be discussed.

Antagonism between acetylcholine and tetraethylammonium in normal and denervated ganglia G. K. MOE AND H. KONZETT* *Sandoz Labs, Basel, Switzerland*

Addition of tetraethylammonium chloride (TEA) in a concentration of 1 100,000 to the fluid perfusing the normal superior cervical ganglion of the cat causes significant reduction of the response of the mediating membrane to previously maximal doses of acetylcholine injected into the ganglion. TEA in a concentration of 1 20,000 may completely block the response to control doses of acetylcholine, though larger doses of the mediator will again excite the ganglion. The ganglion sensitized to acetylcholine by previous section of the preganglionic fibers is much more resistant to the blocking agent TEA in a concentration of 1 100,000 causes no measurable depression of the response to acetylcholine, and in a concentration of 1 20,000 causes only moderate inhibition. It might be argued that a procedure which potentiates acetylcholine would correspondingly reduce the effectiveness of an acetylcholine antagonist, but if sensitization following denervation is due solely to reduction of the cholinesterase concentration in the ganglion, then the effective doses of acetylcholine reaching the ganglion cells must be the same in both normal and denervated structures. Reduction of the effectiveness of TEA by denervation implies a more profound change in the characteristics of the denervated ganglion cells.

Comparison of certain ganglionic blocking agents with tetraethylammonium bromide JAMES L MORRISON, CORNEILLE HEYMANS,* HARRY A WALKER AND ARTHUR P RICHARDSON *Dept of Pharmacology, Emory Univ School of Medicine, Emory University, Ga*

Dosage-effect-time relationships of tetraethylammonium bromide (TEA), bis-(α, α' -trimethyl ammonium propyl ether) dichloride-hydrate (MC-2444), 10-(2-diethylaminoethyl)-phenothiazine (Diparcol), and diethylaminoethyl ester of phenyl-cyclopentane-carboxylic acid (Parpanit) have been quantitated using depression of the carotid sinus pressor reflex and the nicotinic action of acetylcholine as the test responses. Dogs were anesthetized with chloralose and morphine, vagotomized and atropinized. Drugs, in progressively increasing doses, were given intravenously and tests made for blockade of the carotid sinus reflex and pressor response to acetylcholine 5 minutes after each injection of the test drug. This procedure was repeated until one or both of the responses were apparently blocked. Tests were made at frequent intervals for a period of two hours after the last dose of the drug. Using averages of the per cent change in blood pressure of 5 or 6 dogs, the results were plotted on graph paper and the ED_{50} 's were approximated and compared to TEA. In terms of dosage required to block the carotid sinus pressor effect Parpanit was $\frac{1}{4}$, Di-

parcol $\frac{1}{3}$, and MC-2444 5 times as active as TEA. In terms of dosage required to block the nicotinic action of acetylcholine Parpanit was 2 times, Diparcol 3 times, and MC-2444 10 times as active as TEA.

Studies on alterations of renal function following sciatic nerve stimulation and metabolic stimulants JOHN H MOYER* AND CARROLL A HANDLEY *Depts of Medicine and Pharmacology, Baylor Univ College of Medicine, Houston, Texas*

An attempt has been made to further evaluate the concept of functional renal bypasses as reported by Trueta *et al*. Changes in renal blood flow (measured with a bubble flow meter), systemic blood pressure, and renal arteriovenous oxygen differences following sciatic nerve stimulation were determined. After 2 hours the mean renal blood flow in the stimulated animals decreased 40% as compared to 6% in the control animals. This was accompanied by an increase of mean renal A-VO₂ of over 100% in the former as compared to 4% in the latter. The increase in A-VO₂ was consistently due to a fall in oxygen content of the blood coming from the kidney. Arterialization was never observed. Renal O₂ uptake remained more or less constant. Decreasing renal blood flow occurred irrespective of blood pressure changes although the latter increased in about $\frac{1}{4}$ of the animals receiving sciatic stimulation. Concurrently with these studies, we have observed by clearance methods that urinary output, glomerular filtration and glucose Tm increase in direct proportion to the renal plasma flow as a result of thyroxin or dinitrophenol administration. At the same time, the filtration fraction remained constant. Following these observations an experiment was set up during which renal blood flow was altered by sciatic nerve stimulation, adrenalin, and dinitrophenol administration. Direct measurement of renal blood flow and A-VO₂ differences were made concurrently with clearance studies.

On the biological antagonism between aspartic and glutamic acids controlling the acid produced by *Streptococcus fecalis*-R MICHAEL G MULINOS, A MARINARO* AND NELDA I RICCI* *Interchemical Corporation and Dept of Pharmacology, New York Med College, New York City*

Competitive inhibition among the various amino acids is known but its importance is microbiological and as a possible therapeutic approach has not been fully appreciated. Using the L-forms of aspartic and glutamic acids, the acid producing powers of *S. fecalis*-R were investigated at 37°C for from 24-120 hours. Biotin has been shown to substitute for aspartic acid but glutamic acid is essential for the growth of *S. fecalis*. Concentrations of L-glutamic acid below 0.15 mg per tube

as in the microbiological assay for this acid do not support growth in the presence of the usual amount of 20 mg of aspartic acid. Growth occurred when L-glutamic acid was raised or L-aspartic acid was lowered to 0.5 mg with optimal growth at an aspartic-glutamic ratio of 1:6. The data show that excessive concentrations of either L-glutamic or L-aspartic acid in relation to each other become inhibitory to acid production by *S. fecalis*. This fact may explain the difficulties encountered in the use of *S. fecalis* in the microbiassay of glutamic acid. The data present another example of a biological inhibition between two metabolically important compounds.

Liberation of histamine from carnosine and histidine JOE NASH* AND G. A. EMERSON *Univ of Texas Medical Branch, Galveston*

Treatment of carnosine and histidine solutions *in vitro* in various ways designed to resemble *in vivo* conditions of histamine release resulted in essentially negative findings. Tests for histamine were made with the Code modification of the Barsoum Gaddum technique. Solutions were treated with normal and sensitized tissue homogenates both in the presence and absence of antigen, with toxoid and toxoid plus tissue, and with UV light. These results are discussed in relation to evidence that carnosine acts upon histamine receptors, and in regard to the weak antihistamine activity of carnosine and histidine.

Actions of certain sympathomimetic amines on the excised, perfused guinea pig lung ENID A. NEIDLE,* CHARLES M. GRUBER AND JOAN E. COPELAND* *Dept of Pharmacology, Jefferson Med College, Philadelphia, Pa*

Sixty-six guinea pig lungs were perfused with Tainter's solution according to the method of Sollmann and von Oettingen and the responses of the bronchial musculature to various sympathomimetic drugs were recorded. One hundred and sixty-five injections of epinephrine in dilutions of 1:10,000 and 1:1,000 caused significant dilation of the bronchioles in every case and this response was taken as indicative of an active lung preparation. Of 108 injections of 1:1,000 ephedrine, 4 caused dilation of the bronchioles, 13 produced no change, and 91 caused marked constriction or spasm of the bronchial musculature. The constriction evoked by ephedrine was effectively antagonized by epinephrine and occasionally by epinine, kephrine, cobefrine, octin and isuprel. Amphetamine and tyramine, in 33 and 23 experiments respectively, caused marked constriction or bronchospasm which could be antagonized by epinephrine. Isuprel in a dilution of 1:10,000 was without effect. In 26 experiments 1:1,000 isuprel caused bronchodilation in 6, no significant change in 17, and marked bronchoconstriction in 3 cases.

Furthermore, in many experiments, epinephrine administered to an isuprel-treated lung either had no effect or caused slight constriction. It is possible that isuprel has a general toxic effect, rendering the lung unreactive to epinephrine and other active compounds. Neosynephrine, parendrine, tuamine, and desoxyephedrine evoked constriction in the isolated lung, while kephrine, cobefrine, and epinine and sympatol caused dilation or were ineffective. Octin and aranthol caused initial dilation followed by marked bronchospasm. Further experiments with these drugs will be performed on the histamine-treated guinea pig and the intact dog.

Studies on the surface chemistry of bone I. Recrystallization W. F. NEUMAN AND B. J. MULRYAN* *Dept of Radiation Biology, School of Medicine and Dentistry, Univ of Rochester, Rochester, N. Y.*

The problem of the skeletal deposition of toxic metals has received considerable attention. One common observation has been the localization of the foreign mineral in areas of active calcification. This phenomenon has led to an association of the calcification process with the mechanism of deposition of unphysiological minerals in the skeleton. Since the phenomenon of bone growth is very poorly understood, the situation did not lend itself to further exploitation. With the finding that uranium, radiophosphate, and fluoride are all taken up by bone by a physico-chemical process unrelated to cellular activity, a new emphasis has been placed on the surface chemistry of bone. It was hoped that the concentration of these minerals in the regions of active calcification could be explained in part by the greater surface activity of bone taken from these areas. The experiments performed did not critically test this hypothesis. Rather, they demonstrated that fresh bone undergoes recrystallization at a surprising rate. Nonetheless, a means of testing bone preparations for surface activity, even in the presence of active recrystallization, was devised. The problem may now be reinvestigated.

A comparative study of pharmacological properties of procaine and procaine amide PEGGY JO NEWMAN* AND BYRON B. CLARK *Dept of Pharmacology, Tufts College Med School, Boston, Mass.*

While procaine has been used for years as a local anesthetic, only recently have some of its other pharmacological properties been utilized. Some of these properties are shared by procaine amide which is metabolized differently than procaine. There are no extensive studies available on the pharmacological properties of either, and comparative studies are also lacking. Some re-

sults of such an undertaking are hereby presented

- 1) Duration of local anesthesia is the same for both at 0.5% without epinephrine, twice as long for procaine as for procaine amide with epinephrine. At 2% procaine amide anesthesia lasts twice as long as procaine.
- 2) Neither is effective on rabbit cornea at 2%. At 10% both produce anesthesia for 20 minutes.
- 3) Procaine (2%) produces a nerve block (dog sciatic), procaine amide does not.
- 4) Nerve action potential is abolished by procaine twice as rapidly as by procaine amide.
- 5) Acetylcholine induced motility in isolated rabbit ileum is antagonized by both. Procaine is about 1/13,000, and procaine amide 1/140,000 as effective as atropine.
- 6) In prolongation of refractory period of isolated rabbit auricle, procaine is nearly as potent as quinidine, procaine amide is only 25% as potent.
- 7) Both block superior cervical ganglionic transmission and nicotinic pressor response.
- 8) Systemically, procaine is a more potent respiratory depressant than procaine amide. Both produce hypotension in large doses (40 mg/kg), and procaine may raise blood pressure slightly in lower doses. Both cause bradycardia, T wave and QRS depression. Both are vasodilators, procaine being about five times more potent than procaine amide.

Blockade of epinephrine-induced cardioacceleration in the frog MARK NICKERSON AND GEORGE M. NOMAGUCHI * *Dept. of Pharmacology, Univ. of Utah College of Medicine, Salt Lake City*

Although many investigators have confirmed the inability of adrenergic blocking agents to prevent stimulation of the mammalian myocardium by epinephrine, reported results employing the frog heart have been highly variable. Study of the conditions required for blockade of stimulation of the isolated frog heart by epinephrine has revealed the following:

- 1) The inotropic response is not blocked under any of the conditions employed.
- 2) The chronotropic response of the winter-frog heart is effectively blocked by β -haloalkylamines and ergot alkaloids.
- 3) The chronotropic response of the summer-frog heart is unaltered by the β -haloalkylamines, and the ergot alkaloids have much less effect than on the winter-frog heart.
- 4) Anterior-pituitary extract injected into the intact animal decreases the sensitivity of the winter-frog heart to blockade so that it is comparable to the summer-frog heart.
- 5) Although not effective alone, fluoroacetate or iodoacetate combined with a β -haloalkylamine produce a blockade of the summer-frog heart comparable to that produced by the β -haloalkylamine alone in the winter-frog heart. Many other inhibitors were tested and all were ineffective alone or combined with β -haloalkylamines.
- 6) Glycerolmonoacetate antagonized the blockade produced by

β -haloalkylamines, alone or combined with fluoroacetate, in a non-competitive manner. Glycerol and sodium acetate are ineffective. It is concluded that epinephrine releases energy for the production of tachycardia in the winter-frog heart by only one mechanism, while at least two mechanisms are involved in the summer-frog heart. The difference is under anterior pituitary control. Both mechanisms involve the production of utilizable acetate and are independent of the energy sources for the inotropic response.

Studies on the interaction of ureas and barbiturates in potentiation of hypnosis STATA NORTON* AND EDWIN J. DE BEER *The Wellcome Research Laboratories, Tuckahoe, N. Y.*

Sub-hypnotic doses of unsymmetrical alkyl aryl ureas were found to potentiate barbiturate hypnosis in the mouse. Some of the urea derivatives possessed little or no hypnotic action in themselves. The degree of potentiation was also related to the size of the dose. Evidence was obtained which indicated that the potentiation could not be regarded as a simple summation of the hypnotic action of each of the two compounds injected. For some ureas there was an inverse relationship between the potentiation of hypnosis and the rate of respiration.

Electrical mechanism of brain waves HYMAN OLSEN (introduced by F. M. BERGER) *Training Division, Navy Dept., Washington, D. C.*

The current trend in brain wave research is to consider brain waves the result of large masses of neurons oscillating electrically and falling into step or synchronism with each other, thereby creating a large oscillatory-current area in the cortex. A theory is proposed for this oscillatory action of individual neurons and for their falling into step with one another. The theory is based on considering the neuron equivalent to a multi-vibrator circuit which is widely known in the electronic field. Such a circuit will shift readily from one of several types of electrical operation to another with slight changes of its battery voltages or circuit parameters (resistance, capacitance, etc.). It is suggested that, correspondingly, the neuron could shift from one to another of the several types of electrical operation necessary to produce brain waves with slight changes of its metabolic conditions.

Subacute toxicity of 'Thiomerin' compared to other mercurial diuretics O. S. ORTH, F. L. KOZELKA AND R. T. CAPPS * *Dept. of Pharmacology and Toxicology, Univ. of Wisconsin Med. School, Madison*

Clinical reports that N(gamma-carboxymethyl-mercaptomercuri-beta-methoxy)-propyl camphoric acid (Thiomerin) is but 1/100 as toxic as

Mercuhydrin are based on the well established acute cardiac ratio for these compounds when tested on anesthetized cats. Other tests such as intravenous toxicity in mice and actual demonstration of renal impairment, as determined by creatinine clearance in dogs, have indicated a relatively higher toxicity for Thiomerin than other common mercurial diuretics. In attempting to determine the effect of thioglycollic acid on toxicity of mercurial diuretics it was found that rather than a reduction there actually was an increase in toxicity when it was combined with Mercurophylline. This fact initiated a determination of the subacute toxicity of Mercurhydrin, Mercurophylline, Mercurophylline plus Sodium Thioglycollate, and Thiomerin, each administered subcutaneously or intravenously as a single injection. Deaths caused during or within the first 50 minutes after intravenous administration were definitely greater with Mercurophylline. In the table below is given the MLD₅₀ of a subacute type (deaths between 1 and 7 days) as determined in Sprague-Dawley rats, 200 of which were injected subcutaneously with various dosages of the mercurial expressed as total mercury, and 203 tested with mg/kg dosages given intravenously.

	SUBCUTANEOUS TOTAL MG OF HG	INTRAVENOUS HG IN MG/KG
Mercuhydrin	4 0	8 0
Mercurophylline	6 0	24 0 to 32 0
Mercurophylline + Na Thioglycollate	3 0	6 0
Thiomerin	1 0	6 0

While the combination of thioglycollic acid in organic mercurial diuretic compounds reduces the acute cardiac toxicity, it apparently *markedly increases* the subacute to chronic toxicity of such compounds whether given subcutaneously or intravenously.

Effects of ouabain on the coronary circulation and cardiac energetics

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The effect of ouabain on cardiac energetics was studied in the intact normal dog under morphine, nembutal-dial-urethane anesthesia. The following determinations were made: coronary circulation (by the N₂O-catheterization technique), cardiac O₂ consumption (coronary circulation times arterio-coronary sinus oxygen difference), cardiac output (direct Fick-oxygen method), cardiac work in KgM (cardiac output times mean arterial blood pressure $\times 13.5 \times 10^{-6}$), rate (by electrocardiogram), total peripheral resistance (mean arterial blood pressure times 1334 divided by cardiac out-

put per second), coronary resistance (mean arterial blood pressure divided by coronary flow), and mechanical efficiency (cardiac work divided by KgM supplied by O₂ consumed). Following a series of determinations, a 'therapeutic dose' of ouabain was given intravenously and after thirty minutes a second series of determinations was made. Comparing the results with a series of dogs to which no drug was given, it was found that with low doses (0.026 mg/kg) the only significant change in the above list of determinations was an increase in the cardiac output. When larger doses (0.037 mg/kg) were given, there was a tendency for a rise in arterial blood pressure, an increase in total peripheral resistance and a decrease in pulse rate and there was no appreciable change in the other factors listed above.

Oral toxicity of bacitracin

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The oral administration of bacitracin has been found to produce beneficial effects in amebiasis (Most, *N Y Acad of Med* 25 717, 1949), diarrhea in dogs (KONDE, *North Am Vet* 30 776, 1949), and in hemorrhagic dysentery in swine (GRAHAM Unpublished results). Acute and chronic toxicity studies following the oral administration of bacitracin have been conducted in mice, rabbits, and dogs. The following LD₅₀ values were obtained: mice, 510,000 units/kg; rabbits, greater than 200,000 units/kg; and dogs, greater than 400,000 units/kg. Mice tolerated the oral administration of 62,000 units of bacitracin/kg daily for 30 days. The chronic studies in rabbits were complicated by the presence of coccidiosis in the colony. Dogs were treated orally with 5,000, 10,000, and 25,000 units/kg daily for 30 days. All of the dogs survived the above treatment. There was no evidence of albumin or sugar in the dogs' urine. Microscopic examination of the urine revealed no abnormalities except in one dog. This dog received the lowest dose (5,000 units/kg) and granular and hyaline casts were observed at the end of the experimental period. In dogs, bacitracin was present in the blood following the oral administration of the above doses. Histopathological findings will be reported.

In vivo microscopic studies of intramuscular repository penicillin preparations

HAROLD M PECK (introduced by KARL H BEYER) *Dept of Pharmacology, Med Research Division, Sharp & Dohme, Inc, Glenolden, Pa*

It has been demonstrated clinically and in the laboratory that small particle crystalline procaine penicillin suspended in peanut oil and 2% aluminum monostearate, injected intramuscularly, maintains a therapeutic blood level for a

longer period than does large particle crystalline procaine penicillin suspended in the same vehicle. It has been demonstrated also that sodium penicillin suspended in peanut oil and 5% aluminum monostearate protects mice against lethal doses of pneumococcus for as long periods as does large or small particle procaine penicillin in peanut oil and 2%, or 5%, aluminum monostearate. This is contrary to the usual observation that the therapeutic activity is of much shorter duration when sodium, rather than procaine, penicillin is used. Several penicillin preparations were injected into the abdominal muscles of anesthetized mice and observed microscopically. The following observations were made: 1) As the oil vehicle is dispersed through the tissue many of the large crystals of penicillin become incompletely surrounded by the oil, while the small crystals of penicillin remain completely surrounded by oil. 2) Sodium penicillin in peanut oil and 5% aluminum monostearate formed a double emulsion consisting of the following phases: water (penicillin solution)/peanut oil/water (body fluids). The double emulsion did not form when sodium penicillin was suspended in peanut oil and 2% aluminum monostearate, or when procaine penicillin was suspended in peanut oil and 5%, or 2%, aluminum monostearate.

Analysis of regression equations for curarizing agents in man E. W. PELIKAN* AND K. R. UNNA *Dept of Pharmacology, Univ of Illinois College of Medicine, Chicago*

Analysis of calculated regression equations confirm and extend conclusions reported earlier on the effects of curarizing agents: decamethylenbis(trimethylammonium bromide) (C10), d-tubocurarine (dTC), and the dimethyl ether of d-tubocurarine (dimdTC) in four unanesthetized human subjects. Mean rates of regression (slope) of grip strength on log dose were greatest with C10, last with dTC; these equations also confirmed the observation that of the 3 drugs C10 is the most, dTC the least, potent. Variability of responses in any subject and among all subjects was greatest with C10 and least with dimdTC. Mean regression rates of decrease of vital capacity on decrease of grip strength differed insignificantly from each other and from a slope of 1.0. The thresholds of the respiratory musculature differed for each drug (lowest to C10, highest to dimdTC) and differed from the thresholds of the muscles determining grip strength for any given drug. However, once their threshold had been reached, muscles of respiration—as measured by vital capacity—became curarized at a rate equal to that of the peripheral muscles. A 'coefficient of safety' was calculated for each drug by comparing the dose required to decrease vital capacity 50% to the

dose required to decrease grip strength 95%; this coefficient for C10 was less than one and significantly lower than those for dTC (1.5) and dimdTC (3.0).

Prolongation of thiopental anesthesia by anoxia R. C. PETERSON,* F. E. SHIDEMAN AND LINARES B. JOHNSON * *Dept of Pharmacology, Univ of Michigan, Ann Arbor*

Wooster and Sunderman (*J Pharmacol & Exper Therap* 97:140, 1949) demonstrated that certain nitrites and nitrates are capable of prolonging the action of pentobarbital. They postulated that this might be due to an increased amount of drug reaching the central nervous system as a result of the vasodilating properties of the nitrites. The following experiments were performed to determine whether reduced atmospheric oxygen and methemoglobin formation would effect a similar result since anoxia appeared to be a more likely explanation of their results. Dogs were anesthetized with a standard intravenous dose of sodium thiopental (20 mg/kg). Duration of action was determined as the time required to regain the righting reflex, plasma levels of thiopental were obtained at various intervals after administration of the drug. An agent (p-aminoacetophenone) capable of producing methemoglobin (46-78%) was administered orally after a lapse of not less than 48 hours or the animal was forced to inhale an atmosphere containing 6-7% oxygen and the procedure repeated. The results demonstrate that the duration of anesthesia is prolonged markedly, the righting reflex returning at plasma levels much below (19-44%) controls. The resultant anoxia does not appear to alter the rate of detoxication of the drug as evidenced by plasma thiopental decay curves. These results demonstrate that the potentiation of barbiturate anesthesia can be accomplished by any means whereby central anoxia is produced and indicate that it is a more important factor than central vasodilatation.

Hypothermic agents. III. Effect in man CARL C. PFEIFFER, LOUIS SCHLANN* AND L. MEDUNA * *Depts of Pharmacology and Psychiatry, Univ of Illinois College of Medicine, and Manton State Hospital, Chicago*

4-amyl N-benzohydril pyridinium bromide (B-45) is completely ineffective in lowering the body temperature of the rabbit, dog and man. Doses as high as 80 mg given slowly intravenously, 160 mg intramuscularly, or 720 mg orally were ineffective in man. In contrast, Privityne (alpha naphthyl methyl imidazoline HCl) is relatively impotent in lowering the body temperature of rats and mice, but is extremely effective in higher mammals, including adult man where a 10 mg oral dose will decrease the body temperature 1

to 3°F with a mean drop of 1.3°F. This occurs at the 4th to 6th hour. Infants are more resistant to this hypothermic effect. Objective findings were slowing of the heart rate, spiking of the T wave, shortening of the P-R interval with the P wave occasionally disappearing in the QRS wave of the EKG. (These changes suggest hyperkalemia.) No changes occurred in the EEG. A rise in blood pressure of only 10 mm Hg was seen. Persistent piloerection obtained. Subjective symptoms included a feeling of coldness without shivering, persistent painful tingling of the scalp, urgency for micturition, and in some cases extreme lethargy. SAR studies suggest that this effect is produced by blocking the oxygen or muscarinic pharmacophore group of acetylcholine and the imidazoline pharmacophore group of histamine. The data suggest that lower mammals maintain body temperature through an acetylcholine-like substance, but in higher mammals this function is served by the imidazoline group of either histamine, histadine, or an unknown compound.

Adenine intoxication in relation to *in vivo* formation and deposition of 2,8-dioxyadenine in renal tubules. F. S. PHILIPS, J. B. THIERSCH* AND A. BENDICH*. *Pharmacology Section, Division of Exper. Chemotherapy, and Division of Protein Chemistry, Sloan-Kettering Inst. for Cancer Research, New York City.*

Early descriptions of extensive deposits of 2,8-dioxyadenine (2,8-DOA) in renal tubules following administration of adenine were recently confirmed (BENDICH, *et al* *J. Biol. Chem.* In press). These observations suggested that renal damage might account for stimulation of myelopoiesis as seen by others. Also a syndrome resembling B vitamin-deficiency has been reported to follow adenine-intoxication. In the present experiments the response of rats to adenine given in single or repeated doses has been studied and related to the extent of renal deposition of 2,8-DOA. Deposition has been measured by quantitative, spectrophotometric analysis and by histological study of crystal-aggregations in tubules. Daily doses of adenine eliciting little or no tubular deposition of 2,8-DOA are well tolerated without evidence of untoward histopathological changes. However, daily doses giving rise to extensive deposits in the kidney may be associated with debilitation and death. Large intratubular aggregations of crystals with inflammation and hydronephrosis occur. Moderate granulocytosis, depletion of nucleated erythroid cells in bone marrow and moderate anemia may result. Hematological changes are considered secondary to renal damage. No other lesions of significance have been found. It is, therefore, reasonable to attribute effects of prolonged administration of adenine to *in vivo* forma-

tion and tubular deposition of 2,8-DOA. No evidence has been found to support contentions that adenine stimulates hematopoiesis or acts as a vitamin-antagonist. To the contrary, administration of adenine may lead to renal impairment.

Neutralization of pit viper venom by king snake serum. V. B. PHILPOT* AND RALPH G. SMITH. *Dept. of Pharmacology, Tulane Univ. School of Medicine, New Orleans, La.*

The king snake (*Lampropeltis getulus*) is highly tolerant to water moccasin (*Agkistrodon piscivorus*) venom. By intramuscular injection one snake (wt., 470 gm.) received 140 mg. of venom in 4 doses within 5 days. Another snake (wt., 770 gm.) received 1035 mg. in 5 injections within 21 days, the largest single injection being 320 mg. The only adverse effect observed was slight swelling at the site of injection with the larger doses. Serum of the king snake detoxified by heating at 56° for 30 minutes was mixed in varying proportions with moccasin venom. On intraperitoneal injection of 15 mg./gm. of venom (approximately 2.5 LD₅₀) mixed with serum, 95, 66 and 23% of white mice were saved respectively by 0.03, 0.01 and 0.0075 cc./gm. of serum. Total doses of 1 cc. of serum (0.034–0.040 cc./gm.) counteracted 7 lethal doses of venom. In terms of lethal doses the serum was approximately as active against the more toxic venom of the rattlesnake (*Crotalus adamanteus*). Commercial antivenin used by the same techniques had a lower degree of protective action against both venoms and was more toxic than king snake serum. Heat detoxified king snake serum is well tolerated by mice in doses (0.15 cc./gm. intraperitoneally) much larger than those used above. The following intravenous doses were also well tolerated: guinea pig (399 gm.) 5 cc., rabbit (1.55 kg.) 5 cc., dog (13 kg.) 20 cc., man (91 kg.) 16.5 cc. Heating king snake serum at 56° for 30 minutes destroys its hemolyzing action on human erythrocytes.

Distribution of C¹⁴ labeled meperidine in the albino rat. NIKOLAS P. PLOTNIKOFF (introduced by HENRY W. ELLIOTT). *Division of Pharmacology and Experimental Therapeutics, Univ. of California School of Medicine, San Francisco.*

N-methyl labeled meperidine HCl has been administered by the subcutaneous route to the rat in a dose of 125 mg./kg. (1.2 × 10⁶ counts) and the distribution determined after 2 hours. The expired air contained 16.6% of the injected radioactivity indicating demethylation of the meperidine molecule. Urinary excretion amounted to 9.9%. Other organs which contained large amounts of activity were: injection site (left hind leg) 15.9%, carcass 11.0%, skin and hair 4.9%, small intestine and contents 4.5%, liver 3.7%, stomach 2.9%, and

kidney 15% Organs which contained concentrations of radioactivity appreciably above carcass level included liver, small intestine, stomach, lungs and spleen There was no concentration of activity in the brain or adrenals These data indicate that meperidine is degraded in the rat and that either meperidine or its degradation products are excreted in the urine and via the gastrointestinal route

Study of the fate of sodium-5-iodo-2-thiouracil

ALBERT I PUMMER (introduced by F F YONKMAN) *Research Dept, Division of Microbiology, Ciba Pharmaceutical Products, Inc, Summit, N J*

A study has been made of the excretion of sodium-5-iodo-2-thiouracil (originally supplied to us by Dr Frank Gassner of Colorado A & M College and reported on clinically by Dr R Williams *et al*, *J Endocrinol* 9: 801, 1949) to determine whether the iodine was removed from the compound in its metabolism Qualitative experiments in the rat, and preliminary quantitative experiments in the dog indicate that iodine is removed from the compound in both species After the administration of sodium-5-iodo-2-thiouracil to the dog orally (400-600 mg TID, 8-10 kg dogs) and intravenously (200 mg), the excretory products were quantitatively determined by the paper-chromatographic technique of Shabica and Solook (this issue) Within an hour after intravenous injection the unchanged drug appeared in the urine to be followed at the end of two hours by the appearance of 2-thiouracil The excretion of each reached a maximum within 6 hours, fell sharply at 8 hours, and had disappeared by 24 hours About 12% of the drug was recovered in 24 hours One-third of this had been metabolized to 2-thiouracil After oral dosage, excretion was more prolonged, and there was more conversion to 2-thiouracil From 11% to 41% of the administered drug was found in the urine Of this from one third to one half was converted to 2-thiouracil As after oral administration the maximum excretion occurred at 6 hours, but there was a slower drop in the excretion rate during the remainder of the 24-hour period After both oral and intravenous administration the presence of inorganic iodine in the urine gave confirmation of the splitting of iodine from the administered compound

A rapid micro method for determination of DDT and methoxychlor in animal tissues and fluids

CLAUDIA S PRICKETT,* FRIEDA M KUNZE* AND EDWIN P LAUG *Division of Pharmacology, Food and Drug Admin, Federal Security Agency, Washington, D C*

The presence of residues consisting chiefly of fatty material has been a serious restriction to the

application of the Schechter-Haller method for the determination of DDT and methoxychlor in the range of concentration of 0.1 to 10 ppm DDT Excessive amounts of fat interfere with nitration of DDT and methoxychlor, and in addition contribute nitration residues which produce yellow 'off' colors which are difficult to remove This method consists in the production of the dehydrochlorides of DDT and methoxychlor by refluxing for 30 minutes with 2% KOH in ethyl alcohol Simultaneously the fats are saponified, and the dehydrochlorides of DDT and methoxychlor are then easily separated by extraction with petroleum ether It is possible by this means to reduce 5 grams of fat to a 10-milligram residue, consisting chiefly of nonsaponifiable material which does not interfere with subsequent nitration Upon nitration the dehydrochlorides of DDT and methoxychlor are converted to the respective nitro benzophenones, which upon treatment with methylvale reagent give red colors having absorption maxima at 520 m μ for DDT and at 530 m μ for methoxychlor It is not possible by this method to separate the two insecticides, but work in this direction is in progress

Production of auricular fibrillation in the dog by dipropylene glycol methyl ether (Dowanol 50B)

LEONARD PROCITA* AND F E SHIDEMAN *Dept of Pharmacology, Univ of Michigan, Ann Arbor*

Dipropylene glycol methyl ether (Dowanol 50B)

is a central nervous system depressant of low toxicity, the degree of depression produced being proportional to the dose When administered to the unanesthetized dog in amounts as large as 5 cc/kg orally or intravenously, loss of righting reflexes and first plane anesthesia occur, with uniform recovery in 24 hours or less and without any evidence of delayed toxicity In the anesthetized dog intravenous administration produces cardiac slowing, transient hypotension and a Biot type of respiration Complete cessation of respiration will occur when doses of 0.35-0.5 cc/kg are administered The lethal intravenous dose in the artificially respired anesthetized animal is approximately 1.3 cc/kg The most interesting pharmacological action of this compound occurs in the artificially respired dog at a dose level of 0.65-0.75 cc/kg At this dosage auricular fibrillation occurs quite consistently Prior to the onset of fibrillation there is a gradual ventricular slowing followed by ventricular asystole Auricular fibrillation then ensues and within a short time an occasional ventricular beat occurs with eventual return of regular beats as the fibrillation continues The auricles may fibrillate for 4 or 5 minutes and ventricular asystole has been observed for as long as 3 minutes Fibrillation has been produced as many as 4 times in the same

animal Neither atropine nor bilateral vagotomy prior to administration of the compound will prevent the occurrence of the fibrillation

Toxicity and effects on electrolyte excretion of lithium chloride administered to dogs JACK L. RADOMSKI,* HENRY N. FUYAT,* ARTHUR A. NELSON AND PAUL K. SMITH *Division of Pharmacology, Food and Drug Admin., Federal Security Agency, and George Washington Univ. School of Medicine, Washington, D. C.*

Lithium chloride in daily doses of 50 mg/kg killed after 7 months one of two dogs on a normal diet, but 20 mg/kg daily showed no evidence of toxicity. Lithium did not accumulate in the surviving animals. The same doses given to dogs on a low salt diet produced marked evidence of toxicity and accumulation of lithium in the blood. There was loss of body weight, anorexia, salivation, weakness, muscle tremors and polyuria. Terminally there was a high blood nonprotein nitrogen and oliguria with damage of the distal convoluted renal tubules and other terminal portions of the nephrons. Lymphocytopenia developed gradually and became pronounced before death. Most of the lithium was recovered in the urine. There was an excessive sodium loss and a slight potassium retention. The serum lithium rose gradually, then more rapidly to 4 to 5 mEq/liter before death. Usually the serum sodium fell, while the serum potassium rose to 8 to 9 mEq before death. There were marked electrocardiographic changes with depressed S-T segments, inverted T waves and widening of the QRS complex. Administration of desoxycorticosterone acetate did not alter this toxicity or pattern of electrolyte excretion. After a single intravenous injection to normal dogs the apparent distribution of lithium was equivalent to about two thirds of the body weight and the renal clearances were appreciably below those of creatinine. It is apparent that in dogs the administration of lithium salts results in renal damage and consequent alterations in electrolyte metabolism.

Effect of diethylstilbestrol on respiration of cat heart tissue JOSEPH F. REILLY (introduced by OSCAR BODANSKY) *Dept. of Pharmacology, Cornell Univ. Med. College, New York City*

Diethylstilbestrol in a concentration of 2×10^{-4} M decreased significantly the respiration of slices of cat heart tissue in the absence of added glucose. In this concentration, diethylstilbestrol also inhibited the utilization of succinate, fumarate, malate and oxaloacetate. The effect of diethylstilbestrol at various concentrations on the oxidation of malate, fumarate, succinate, and oxaloacetate by homogenates was studied. In the presence of cytochrome C these oxidations were completely inhibited by diethylstilbestrol

(2×10^{-4} M). In the presence of brilliant cresyl blue, the oxidations of malate and succinate were inhibited from 0 to 50% dependent on the time of addition of the substrate and drug. Diethylstilbestrol (2×10^{-5} M) and (1×10^{-6} M) inhibited oxaloacetate oxidation less than the complete malate, fumarate or succinic oxidizing systems or a cytochrome C reductase system utilizing malate (0.02 M). The cytochrome oxidase system of cat heart was inhibited approximately 20% by diethylstilbestrol (2×10^{-1}) and added cytochrome C did not prevent this inhibition. At higher concentrations of homogenate the inhibitory effects of diethylstilbestrol in the cytochrome oxidase and on the complete succinic and malic oxidizing systems was somewhat decreased.

Metabolic action of veratrum alkaloids upon tissue of the rat heart MELCHIOR REITER (introduced by OTTO KRAYER) *Dept. of Pharmacology, Harvard Med. School, Boston, Mass.*

The right atrium of the rat heart, slit open, and homogenates of ventricle muscle were used and metabolic effects were studied using the Warburg technique. The metabolic action of veratridine differs from that of veratramine, veratrosine and pseudojervine. Veratridine, the veratric acid ester of the tertiary amine cevine, in a concentration range of 1 to 8×10^{-4} causes an increase in oxygen consumption followed by inhibition of respiration which is marked with the higher concentrations. This effect resembles that of ouabain with which veratridine shares a positive inotropic action upon the intact heart. The metabolic action of veratridine is largely dependent upon the integrity of the cell, in homogenates only a slight inhibition or no effect can be observed. In the active concentration range the secondary amine bases, veratramine, veratrosine and pseudojervine, inhibit the respiration of the atrial tissue without preliminary stimulation. These substances have no positive inotropic action in the intact heart, they antagonize the positive chronotropic effect of epinephrine. The inhibitory effect upon respiration occurs in the homogenate as well as in the 'intact' atrial tissue. Oxidation of lactic acid, pyruvic acid and fumaric acid is inhibited while anaerobic glycolysis is not influenced. The cytochrome oxidase system and succinic dehydrogenase likewise are not influenced by the secondary amines.

Kidney function and ultra short acting barbiturates R. K. RICHARDS, K. E. KUETER* AND J. D. TAYLOR* *Dept. of Pharmacology, Abbott Labs., North Chicago, Ill.*

It is generally assumed that kidney function is not important for the duration of action of ultra short acting barbiturates. This conception is largely based upon the small amount excreted

in the urine and a limited direct evidence. Rats and rabbits were used in the following experiments. Doses of Pentothal (Thiopental) and Evipal (Hexobarbital) producing similar sleeping time were injected IV. This was repeated on the same or comparable groups of animals at various intervals after bilateral nephrectomy under local or light ether anesthesia. Pentothal or Evipal injections immediately after the operation produced sleep of about the same duration as in normals or sham operated controls. However, when 3 hours had elapsed after nephrectomy rabbits showed a definite (50%) and rats a marked (100%) prolonged sleeping time with Pentothal. This became more pronounced with longer intervals. Evipal did not show this phenomenon in rats after 3 hours and only moderately so after 20 hours, it was even less evident in rabbits. An artificial NPN solution calculated to raise the blood NPN to several times the normal value was injected iv into rats immediately after bilateral nephrectomy. This caused the prompt appearance of an about 100% prolonged sleeping time after Pentothal. With Evipal such action was absent or slight. Preliminary experiments indicate that cats and especially dogs are less prone to show this change of sensitivity to Pentothal after nephrectomy.

Pharmacologic significance of m-hydroxyphenyl trimethylammonium ion WALTER F. RIKER, JR. AND W. CLARKE WESCOE *Dept of Pharmacology, Cornell Univ Med College, New York City*

m-Hydroxyphenyl trimethylammonium bromide (Nu-2561) possesses cholinergic properties. In the cat, its effects on the cardiovascular system, smooth muscle and glandular structures are weak in comparison with those of acetylcholine. However, its action on the blood pressure is characteristic in that it produces a biphasic response. This consists of an initial sharp rise terminating in an abrupt fall below the control level. The magnitude of this response is proportional to the dose, the duration of the response is fleeting. The administration of large doses accentuates and prolongs the sharp rise in blood pressure but a fall may not occur. In the atropinized cat the pronounced sharp pressor response to Nu-2561 reveals the potent action of this compound on the sympathetic vasoconstrictors. The action of Nu-2561 on neuromuscular function in the cat is most prominent and comparable to that of its acetyl ester (Nu-2017), acetylcholine and neostigmine. The close intra-arterial injection of Nu-2561 (5-10 $\mu\text{g/kg}$) in the gastrocnemius-soleus preparation, produces a rapid contractile response. A similar administration during indirect stimulation of the muscle with single maximal shocks, potentiates

the response of the muscle. The duration of this effect is brief. Doses of 50-100 $\mu\text{g/kg}$ of Nu-2561 produce a profound depression of the contractile response to nerve stimulation. The anti-curare action of Nu-2561 is equivalent to that of Nu-2017 and neostigmine. The potent stimulatory effect of Nu-2561 at the neuromuscular junction and the prominence of its pressor action in the unatropinized cat emphasize the significance of the basic structural component that endows the many derivatives of this base with their direct 'nicotinic' actions.

Anhidrotic action of agaric acid in humans MORTON L. RODMAN (introduced by THEODORE KORFANIS) *Dept of Pharmacology and Materia Medica, Georgetown Univ School of Medicine, Washington, D C*

The relative efficiency of various doses of agaric acid in thermal sweating was determined by comparing mean sweating rates during the fifth and eighth hours after ingestion of the drug with control readings of the amount of sweat collected by a gravimetric technique during one hour of exposure to heat at 104°F Dry Bulb/87°F Wet Bulb. Agaric acid in doses below 100 mg did not appear to reduce sweating significantly. Doses of 150 mg-500 mg reduced sweating from 40% to 60% below normal rates. Daily doses of 150 mg for one week suppressed sweating to the same extent, and this was followed by somewhat lower rates of sweating for a day or two after administration had been discontinued. Sweating then increased progressively to normal levels by the 4th or 5th day. In no case did these large doses produce atropine-like effects on the heart rate, eye or salivary glands or any of the symptoms of gastrointestinal irritation frequently encountered with doses as low as 30 mg of the impure resin-containing extract, agaricin, of which agaric acid is the active principle. Atropine (0.6 mg and 1.2 mg) administered alone or in addition to agaric acid (300 mg and 500 mg) resulted in inhibition of sweating not significantly greater than when effective doses of agaric acid alone were used. These preliminary observations indicate that agaric acid may prove a safe and effective anhidrotic.

Vasopressor action of pilocarpine MARY A. ROOT (introduced by OTTO KRAYER) *Dept of Pharmacology, Harvard Med School, Boston, Mass*

Although pilocarpine is generally considered to be a parasympathomimetic drug, several phenomena not explainable on this basis have been observed by various workers. We have studied the vasopressor activity in detail in an attempt to elucidate the mechanism of this action of pilocarpine. When given intravenously to rabbits, cats, or dogs under dial-urethane anesthesia pilo-

carpine produced a marked fall in blood pressure with a rapid return to normal and occasionally a secondary rise which might be quite large. After pretreatment with nicotine, curare, or tetraethylammonium ion in amounts presumed to block autonomic ganglia, an injection of pilocarpine produced a marked rise of blood pressure which was proportional to the dose of pilocarpine. The blood pressure rise was not reduced by adrenalectomy, by evisceration, by vagotomy, nor by denervation of the carotid sinus. During the blood pressure rise the heart rate either increased or decreased in cats, whereas in rabbits it always decreased. When blood flow was measured in the femoral artery of the dog using the differential manometer technique of Moe, it indicated a peripheral vasoconstriction during the blood pressure rise. After the blood pressure had returned to normal a marked vasodilatation followed. The vasopressor action of pilocarpine could be abolished completely by atropine and greatly decreased, abolished, or even reversed by ergotoxine, dibenamine, or 2-(N, p-tolyl-N(m' oxyphenyl) amino-methyl)-imidazoline (C-7337).

Relation between chemical constitution and bronchodilator activity of sympathomimetic amines

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The bronchodilator activities of 27 dihydric, sympathomimetic amines have been determined by the guinea pig tracheal chain method. The following observations have been made from an analysis relating structure to activity: 1) Increasing the length of the phenylalkyl chain increased bronchodilator activity (arterenol < cobe-frine < butanefrine). 2) N-isopropyl substitution markedly increased activity in each of the 3 types. 3) Increasing the length of the phenylalkyl chain on N-isopropyl derivatives progressively decreased activity. 4) Substitution of ketone for alcoholic hydroxyl on N-isopropyl derivatives decreased bronchodilator activity. 5) With N-isopropyl ketones peak activity was observed with dihydroxy- α -aminopropiophenone. 6) In general, N-substitution of groups larger than isopropyl on the cobe-frine nucleus tended to decrease bronchodilator activity. Relative bronchodilator activities as determined by the tracheal chain and the perfused guinea pig lung methods were in fair agreement.

Effects of steroids on the isolated frog's heart

BERNARD RUBIN (introduced by WILLIAM T. SALTER) *Labs. of Pharmacology and Toxicology, Yale Univ. School of Medicine, New Haven, Conn.*

Conjugated and unconjugated steroids of the estrogen and pregnane series have been studied

on 38 isolated frog-hearts with constant perfusion (3-5 cc/min). The hearts were made hypodynamic by reducing the calcium concentration to 50% of that present in the usual Clark's solution. The steroid was added in the presence of low calcium and changes in rate and amplitude observed for 120 minutes. The pH of the solutions was 7.6-7.8 and the temperature varied between 21 and 25°C. Sodium estrone sulfate, in concentrations ranging from 25-400 μ g %, produced augmentation of amplitude. The response appeared to follow a log-concentration curve, as did the reaction time for peak response. The latter varied from about 110-40 minutes for the concentration-range indicated. A cardiotonic response in the presence of normal calcium concentration was also observed, but this was apparent only when compared with control hearts similarly perfused in the absence of the steroid. Estrone in concentrations of 100 and 200 μ g % produced little or no augmentation. Other steroids were tested similarly. For example, progesterone in a concentration of 25 μ g % produces augmentation. With 50 and 400 μ g %, however, a bradycardia and some increase in amplitude are observed. Pregnandiol glucuronide, in concentrations below 25 μ g % appeared to produce a considerable augmentation without bradycardia. Concentrations of 25 to 200 μ g %, however, produced only slight increases in amplitude with an almost immediate bradycardia.

Studies on carbohydrate metabolism of the rabbit ileum

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An attempt is being made to correlate changes in the functional capacity of the isolated rabbit ileum with alterations in its carbohydrate metabolism. Analyses of phosphorylated intermediates were made on ileum of rabbits starved for 48 hours. Although the glycogen content did not differ significantly from the normally fed animals, the inorganic phosphorus content remained high, suggesting a high rate of glycolysis. This was further indicated by a high lactic acid content and by *in vitro* studies on glycolysis from glucose of ileum homogenates. Phosphocreatine and ATP were high, whereas fructose phosphates were low. The ability of washed ileum homogenates to oxidize several substrates was determined manometrically. Malate, pyruvate and fumarate were oxidized readily (QO₂ 10-16) even after the homogenate was stored at 0 degrees for 3 days. Succinate and ketoglutarate were oxidized only slightly (QO₂ 3-5) the first day and not at all on the second day. Oxidative phosphorylation of the isolated ileum proceeds much more rapidly with pyruvate as a substrate than with glucose. With pyruvate as a substrate most of the pyrophos-

phate was in the form of ATP. It is conceivable that the immediate increase in tone elicited upon the addition of pyruvate to starved isolated rabbit ileum is associated with the rapid formation of ATP. The phosphocreatine content increased over 100% above that of the normal starved ileum when these substrates were added to the bath.

Effects of beryllium chloride in Locke-Ringer solution upon the isolated rabbit ileum and heart

P. R. SALLERNO*, L. E. ELLIWOOD*, L. G. ARON* and J. M. COON. *Univ. of Chicago Toxicity Lab. and Dept. of Pharmacology, Chicago, Ill.*

Beryllium chloride ($1 \times 10^{-3}M$) caused a fleeting decrease in tone and decreased survival time of isolated rabbit intestine. A concentration of $5 \times 10^{-3}M$ abolished all activity in 1 minute. The isolated heart showed a temporary decrease in amplitude following single injections of 1 to 2 mg $BeCl_2$. It was noted that a precipitate, probably $Be(OH)_2$, varying in quantity with the amount of $BeCl_2$ added, was formed in the Locke-Ringer solution. If the concentration of sodium bicarbonate was decreased below the normal level of 50 mg %, less precipitate was formed and the sensitivity of the isolated ileum to $BeCl_2$ was increased. The effects of $BeCl_2$ could be reversed by flushing the bath with fresh Locke-Ringer solution or by the addition of sodium bicarbonate. These observations suggested that these actions of $BeCl_2$ were due to removal of bicarbonate from the Locke-Ringer solution, with consequent destruction of its buffering power. Titration studies revealed that 1.6 moles of sodium hydroxide were necessary to neutralize a solution of one mole of $BeCl_2$ to pH 7. It was found that all of the effects of $BeCl_2$ described above were comparable to the effects of HCl of 1.6 times the molar concentration of $BeCl_2$. Since concentrations of $BeCl_2$ which do not materially affect the pH of Locke-Ringer solution lack any influence on the behavior of the isolated intestine and heart, and since the pH of solutions of greater concentrations of $BeCl_2$ are not compatible with normal function, it has not been found possible to demonstrate any direct pharmacologic action of $BeCl_2$ in these systems.

Nomogram for cardiac synergism of calcium potassium and digitalis bodies (in "therapeutic" concentrations) WILLIAM T. SALTER, BERNARD RUBIN* and ELIZABETH A. RUNELS*. *Labs. of Pharmacology and Toxicology, Yale Univ. School of Medicine, New Haven, Conn.*

Positive and negative synergisms of calcium and potassium ions have been measured concomitantly on the frog heart under the influence of digitalis bodies in 'therapeutic' concentrations. The resulting four-dimensional diagram is conveniently epitomized at the 50 percentile level

of maximal contractile force. The relationship is described approximately by the following equation: $1/b \left(\frac{R}{100R} - 1 \right) = k[G] + C_1[Ca^{++}] - C_2[K^+] - a$, where R is the contractile response in percentage of maximum, and $[G]$, $[Ca^{++}]$, and $[K^+]$ represent the respective concentrations of glucoside and ions. The constant a is characteristic for each heart at physiological acidity and the temperature designated. This nomogram can be used to standardize the potency of cardiac glucosides in terms of molar equivalents of inorganic ions. With the help of buffered sodium citrate it can also be applied to serum. The simultaneous control of $[Ca^{++}]$ and $[K^+]$ increases the flexibility of the procedure. The results emphasize the analogy of digitalis bodies to cardiac vitamins or hormones.

Effect of tetraethylthiuram disulfide (Antabuse) on the metabolism of isopropyl alcohol. R. W. SCHAFFERZICK (introduced by P. J. HANZLIK). *Dept. of Pharmacology and Therapeutics, Stanford Univ. School of Medicine, San Francisco, Calif.*

The effect of tetraethylthiuram disulfide on blood alcohol was studied in rabbits. Tetraethylthiuram disulfide was given gastrically, 0.5 gm/kg twice daily to 2 rabbits, and 0.5 gm/kg 3 times daily for 2 days to 2 other rabbits. Three hours after the last dose, all 4 rabbits and 2 untreated controls were given isopropyl alcohol, 1250 mg/kg. Blood alcohol and blood acetone were determined at intervals to 20 hours. While the control rabbits remained alert and responsive, the tetraethylthiuram disulfide treated animals showed marked motor depression within 10 minutes after giving the isopropyl alcohol, and 5 of the 4 tetraethylthiuram disulfide treated rabbits died within 48 hours. There were no significant differences between the curves of blood alcohol of the control and premedicated rabbits and the combination of tetraethylthiuram disulfide and isopropyl alcohol did not increase the blood acetone above that of the animals receiving isopropyl alcohol alone. Thus, while the combination of Antabuse and isopropyl alcohol is more toxic than is this secondary alcohol alone, the rate of oxidation of this alcohol is not demonstrably altered, contrary to recent claims (Larsen *Acta Pharmacol.* 4:321, 1948) for ethyl alcohol (primary) given under similar conditions.

Cardiac action of nitrogenous lactones. F. W. SCHUELER and C. C. HANNA*. *Dept. of Pharmacology, College of Medicine, State Univ. of Iowa, Iowa City*

Using a frog heart perfusion technique similar to that described by Giarman (*J. Pharmacol. &*

Exper Therap 96 119, 1949) we have tested four series of unsaturated lactones (I-IV) synthesized in our laboratory under a grant from the USPHS R_1 = 2-fluorenyl-, 3-diphenylene oxide-yl, 2-(5,6,7,8-tetrahydronaphthyl)-, 2-pyrenyl-, phenyl-, and substituted phenyl derivatives as follows p-chloro-, p-bromo-, p-acetyl-amino-, p-phenoxy-, 2,4 dimethyl-, 2,5-dimethyl-, p-methyl- R_2 = furyl-, β -furylvinyl-, phthalyl-, phenyl-, β -phenylvinyl-, α -methyl- β -phenylvinyl-, and substituted phenyl derivatives as follows p-dimethyl-amino-, 3,4-methylenedioxy-, 2,3 dimethoxy-, 4-methoxy-, 3-chloro-4,5-dimethoxy-, p-acetoxy-, 2,6-dichloro-, 2-nitro-, 3-nitro-, 4-nitro-, 2-chloro-, 4-methyl-, 4-isopropyl-, 3-acetoxy-, 3,4-diethoxy- R_3 = phenyl-, 2-hydroxyphenyl-, 4-methoxyphenyl-, β -phenylvinyl- Group II was synthesized as a control series in the study of III Cardiac digitals like activity followed a virtually identical pattern in series II and III Thus, the 3,4 methylenedioxyphenyl-, 3-nitrophenyl-, and furyl derivatives of both II and III were active materials All other derivatives in II and III showed either no or very low activity All compounds termed 'active' were capable of restoring the ventricular amplitude of 3 out of 4 hypodynamic hearts to 75% or more of their normal amplitude in 20 minutes at a concentration of 1 mg % in the low calcium solution In series I only the phenyl-, p-chlorophenyl-, and p-bromophenyl-, derivatives showed activity None of the derivatives of IV possessed activity, though this series is very incomplete As a result of this study it appears that the cardiac 'digitals' like action of the nitrogen analogues III of the lactones II are qualitatively and quantitatively similar And all the carbon-unsaturated lactone ring, therefore, does not appear essential, at least, when the nitrogen atom is not linked directly with the ester-oxygen as in IV

Comparison of seven vaso-pressor compounds

LLOYD D SEAGER AND WILLIAM WEISS * *Dept of Physiology and Pharmacology, Univ of Arkansas, Little Rock, and the Dept of Anesthesiology, Woman's Med College of Pennsylvania, Philadelphia*

The study was made on vagotomized dogs under phenobarbital and nembutal anesthesia Forced artificial respiration was maintained to nullify respiratory effects of the drugs Epinephrine, Neosynephrine, Nu1683, Aramine, Oenethyl, Ve-250 and Desoxyephephrine were the drugs studied The latter 3 compounds show cross tachyphylaxis and the comparisons on these were made chiefly on litter mate pups using the result of first injection only Comparisons of drugs not showing tachyphylaxis was made in part by several injections in the same animal, and rotating the order

of injection in different animals Aramine and Nu1683 were found not to exhibit tachyphylaxis and to give more prolonged effects than neosynephrine Using approximately equivalent epinephrine pressor ratios the order of duration of response were epinephrine, neosynephrine, Nu-1683, Oenethyl, Aramine, Desoxyephephrine and Ve250

Method of screening steroids on synovial membrane for possible anti-arthritic action JOSEPH SEIFTER, DAVID H BAEDER,* ALBERT J BEGANY,* GEORGE ROSENKRANZ,* CARL DJERASSI,* JOHN PATAKI* AND STEPHEN KAUFMANN * *Wyeth Inst of Applied Biochemistry, Philadelphia, Pa, and Syntex, S A, Mexico*

Hyaluronidase and steroids alter the permeability of certain membranes, particularly the synovial membrane, by acting upon the hyaluronate of the ground substance (SEIFTER, BAEDER AND DERVINIS *Proc Soc Exper Biol and Med* 72 136, 1949, SEIFTER, BAEDER AND BEGANY *Ibid* 72 277, 1949) Of the physiological steroids cortisone is the most efficient in decreasing permeability Adrenocorticotrophic hormone (ACTH) has the same effect as cortisone Desoxycorticosterone (DCA), on the other hand, increases permeability, as does hyaluronidase Both simulate the initial lesion of rheumatoid arthritis Cortisone and ACTH are effective against the action of DCA and hyaluronidase Forty steroids (kindly supplied by Dr I V Sollins of the Chemical Specialties Co, Inc) and some of their esters have been screened on the rabbit synovial membrane for 1) effect on permeability, 2) anti-hyaluronidase effect and 3) anti-DCA effect These steroids have a pharmacological rather than physiological effect on the synovial membrane Several steroids were as effective as cortisone in their ability to decrease permeability and in their anti-hyaluronidase and anti-DCA action The most interesting of these were related to DCA by chemical structure, suggesting a basis for competitive inhibition Δ^4 Pregnene-3 β , 21-diol, 20-one, 21-monoacetate had a cortisone ratio of 130% and was 5 times as effective against DCA as against hyaluronidase In clinical trials it was a useful anti-arthritic DCA structure is not essential either for anti-hyaluronidase or anti-DCA action, or for clinical efficiency

Quantitative chromatographic method, applicable to body fluids, for the determination of 2-thiouracil and 5-iodo-2-thiouracil singly or in mixtures ANTHONY C SHABICA AND EDWARD SOLOOK (introduced by E OPPENHEIMER) *Research Dept, Division of Development Ciba, Pharmaceutical Products, Inc, Summit, New Jersey*
Although the 'Grote' spectrophotometric procedure for the determination of total thiouracil in body fluids has been reported by McGinty et

al and Williams *et al*, no method has been available for a quantitative analysis of the individual components of a mixture. The method described has been devised in order to permit an elucidation of the metabolism of 5-iodo-2-thiouracil. Since the spectrophotometric method does not differentiate between 2-thiouracil and 5-iodo-2-thiouracil, a 'paper chromatographic' method has been developed. Using the ascending technique and developing with aqueous butanol, the above compounds ascend to spots sufficiently apart to effect an excellent and reproducible separation. The ratio of distance travelled and solvent front, R_f values, are 0.50-0.53 and 0.67-0.70 for 2-thiouracil and 5-iodo-2-thiouracil, respectively. The method of detection of the separated compounds is accomplished by spraying the paper chromatogram with 'Grote's' reagent, a blue color appearing for 2-thiouracil and blue-green for 5-iodo-2-thiouracil. An alternative method is based on the observation of Holiday and Johnson, that in the presence of ultra-violet light (230-400 $m\mu$) the thiouracils appear as dark spots. Quantitative analysis is accomplished by streaking an aqueous solution of the mixture on a line of application rather than spot preparatory to development. The paper is cut into strips containing the individual thiouracils and eluted with water for 48 hours and the eluate analyzed spectrophotometrically via the 'Grote' method. Satisfactory results on the quantitative determinations of the above compounds, $100 \pm 5\%$ have been obtained.

Pancreatic secretions and response to secretin after vagotomy and sympathectomy WILLIAM W. SHINGLETON, BLAKE FAWCETT AND J. STANLEY VETTER (introduced by K. S. GRIMSON)
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Experiments were carried out to measure pancreatic function in fasting normal patients and in patients after vagotomy or transthoracic sympathectomy with splanchnicectomy. A specially designed triple lumen tube, with one balloon at the tip and another 18 cm proximally, was passed through the pylorus of the stomach. Proximal and distal balloons were inflated, the former resting in the pylorus, and the latter in the lower duodenum. Specimens were collected from the portion of duodenum between the balloons. A 30 minute sample of secretion was obtained before and after intravenous injection of secretin (1 unit/kg secretin, Eli Lilly and Co). Measurements of total volume, amylase, and NaHCO_3 , were made on the samples collected. In 4 normal patients the average volume of secretion before secretin was 93 cc, and after secretin, 118 cc. The average total output of amylase units (1 unit = 1 gm starch digested 1 hr) before secretin was 88, and

after secretin, 1500. The average bicarbonate value was 4 cc 1 N NaOH per cc of secretion before secretin. One of the normal patients whose amylase value was 96 units before and 3424 after secretin underwent a transthoracic sympathectomy with splanchnicectomy for hypertension. One week later studies showed 570 amylase units before and 1120 units after secretin. Bicarbonate value before operation was 0.9 cc 1 N NaOH /1 cc secretion, and this value was zero following operation. By contrast, five patients tested three to four years after transthoracic vagotomy for ulcer showed an average of 300 amylase units prior to and only 360 units after secretin.

Studies on the 17-ketosteroid excretion, gonadotrophic inhibition and response of rat seminal vesicle following oral and parenteral administration of testosterone and testosterone propionate in different vehicles BETTY M. SILVER, HERBERT S. KUPPERMAN AND RALPH A. POSTIGLIONE (introduced by ARTHUR C. DEGRAFF)
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The comparative efficacy of testosterone and testosterone propionate in various vehicles was evaluated by a study of 17-ketosteroid excretion in man, and by gonadotrophic inhibition and stimulation of the male accessory glands in the rat. The 17-ketosteroid determinations utilizing the Zimmerman reaction were made on urines obtained from a series of hospitalized patients after parenteral administration of the hormones in oil and as aqueous suspensions, and after oral administration as colloid mixtures. These excretion studies did not indicate the superiority of either vehicle or route of administration, inasmuch as comparable increases in 17-ketosteroid excretion were observed following both oral and parenteral administration. Studies on the ketosteroid excretion following the administration of testosterone propionate in the form of rectal suppositories failed to show an increased excretion over initial control values. The gonadotrophic inhibiting effect of the androgens was measured in parabiotic rats by parenteral administration of testosterone and testosterone propionate in oil or as an aqueous suspension to the castrate partner of littermate parabionts. Testosterone in sesame oil or an aqueous suspension was not as effective in inhibiting the gonadotrophic complex as similar preparations of testosterone propionate. The effect of equivalent doses of the 6 parenteral preparations on the seminal vesicles of the castrate male rat showed that there was a decrease in potency and effectiveness of the compounds in the following order: testosterone propionate aqueous suspension, testosterone aqueous suspension,

testosterone propionate in oil and testosterone in oil. The duration of effect was sustained for a longer period of time with testosterone propionate in an aqueous suspension. Testosterone in oil produced only minimal growth effects on the male accessories and these in turn were rapidly dissipated.

Action of Neo-Coronaril, Erba, upon induced ventricular fibrillation DONALD SLAUGHTER AND J BELOGORSKY * *Dept of Physiology and Pharmacology, Univ of South Dakota, Vermillion*

Studies were made with diethyl-amino ethoxy-4 stilbene-Neo Coronaril-(NC) to determine its ability in preventing the onset of ventricular fibrillations, induced by a chloroform-epinephrine method in dogs as described by Shen and Simon (*Archives Internationales de Pharmacodynamie*, 1938 Vol 59, p 68). Eighteen dogs, weighing from 6.5-28 kg, anesthetized with sodium-pentobarbital, were divided into 4 groups. Using the method referred to, fibrillation occurred in the first group of 9 animals which were used as a control. In the second group of 3 dogs (NC) as the protective drug was injected intravenously, 15 mg/kg (standard used in this study), one-half minute before the epinephrine. In this series no fibrillation occurred. In the third group of 3 dogs (NC) and epinephrine were injected simultaneously, in 2 animals the onset of fibrillation was delayed for approximately 3 minutes, and in one the fibrillation was not checked at all. In the fourth group of 3 dogs (NC) was injected immediately at the onset of fibrillation and no protective action was noted. The results of the experiments indicate that (NC) possesses positive anti-fibrillatory action in dogs if used previously to the agent producing fibrillation (NC) when administered simultaneously with the fibrillatory agent is of questionable value. Finally, (NC) is ineffective if used when the fibrillation is already in progress.

Chemical constitution and analgetic action M B SLOMKA* AND F W SCHUELER *Dept of Pharmacology, State Univ of Iowa, College of Medicine, Iowa City*

Using a rat tail modification of Wolf *et al* technique we have tested 5 types of amine derivatives, most of which are new compounds, synthesized in our laboratory. These materials embraced the following compounds given together with the mean % increase in time for the tail twitch over control time, confidence limit and dosage. (A) 1) 2-phenyl-1-methyl-ethyl-N-methyl-N-(2-acetoxyethyl) amine HCl, 34.3%, 1%, 100 mg/kg, 2) 2-phenyl-2-methyl-ethyl-N-methyl-N-(2-acetoxyethyl) amine HCl, inactive, 200 mg/kg, 3) 2-phenyl-2-methyl-ethyl-N-methyl-N-(3-acetoxypentyl) amine HCl, 17.2%, 1%, 100 mg/kg. (B) 1) N-(2-acetoxyethyl)-1,2,3,4-tetrahydroiso-

quinoline HCl, 44.6%, 2%, 75 mg/kg, 2) N-(2-acetoxy-ethyl) isoquinolinium bromide, inactive, 100 mg/kg. (C) 1) 2-phenyl-2-acetoxy-dimethylaminomethyl cyclohexane HCl, inactive, 100 mg/kg, 2) 2-phenyl-2-acetoxy-diethylaminomethyl cyclohexane HCl, 17.6%, 1%, 25 mg/kg and 52.5%, 1%, 50 mg/kg. (D) 1) morphine sulfate, 102.7%, 1%, 2 mg/kg, 2) morphine methiodide, 103%, 0.1%, 2 mg/kg. (This material was supplied to us by Dr L F Small.) (E) 1) N-phenacyl-N,N-dimethyl-N-(2-hydroxy propyl) ammonium bromide, inactive, 25 mg/kg, 2) N-phenacyl-N,N-diethyl-N-(2-hydroxy ethyl) ammonium bromide, inactive, 10 mg/kg, 3) N-phenacyl-N-methyl morpholinium bromide, inactive, 50 mg/kg, 4) N-phenacyl-4-(γ-hydroxy propyl) pyridinium bromide, inactive, 50 mg/kg. Higher doses than those given in this last group were too toxic for further investigation. The results of this preliminary study lend support, in general, to a qualitative working hypothesis developed previously in our laboratory concerning the relationship between chemical constitution and analgetic action (*J Am Pharm A* 38 74, 1949).

Toxicity and irritation studies on some organic mercurial diuretics J K SMITH,* R E BRIDENSTINE,* C R THOMPSON* AND H W WERNER *Pharmacology Dept, Wm S Merrell Co, Cincinnati, Ohio*

TMS (trisodium salt of N-(2-methoxy-3-(1,2-dicarboxyethylthiomercuri) - propyl) - 0 - carboxymethyl salicylamide), a new thiomercureal, has been compared to meralluride sodium and mercaptomerin sodium with respect to local irritation and acute and chronic toxicity. Continuous intravenous infusion to the point of death in dogs demonstrated meralluride sodium to be significantly more toxic than TMS and mercaptomerin sodium. Intramuscularly in rats and subcutaneously in mice the local irritant effects of TMS and mercaptomerin were similar and less than those of meralluride sodium. The acute 14-day LD₅₀ values expressed as mg of Hg/kg for TMS, meralluride sodium and mercaptomerin sodium were respectively 16.6, 8.6, and 6.7 for i.v. administration to rabbits and 15, 10, and 5 for i.m. administration to rats. TMS and mercaptomerin sodium were administered s.c. to rats and i.v. to rabbits every third day for 8 weeks in doses of 0.7 and 3.5 mg of Hg/kg. Signs of toxicity were not observed in the rats or in rabbits at 0.7 mg of Hg/kg, however, in rabbits at 3.5 mg of Hg/kg both compounds caused upper nephron nephrosis and a vacuolization of the parenchymatous cells of the liver. The histologic alterations in the kidneys were more severe in the mercaptomerin sodium group than in the TMS group. Similar chronic toxicity studies on meralluride sodium are in progress.

Morphological and biochemical changes produced by infectious myxoma virus in chorioallantoic membrane of the developing chick embryo
MARGARET H. D. SMITH* AND ERNEST KUN
Depts. of Pediatrics and Medicine, Division of Infectious Diseases, Tulane Univ., New Orleans, La

The chorioallantoic membranes of developing chick embryos have been inoculated with measured amounts of infectious myxoma virus. The pathogenesis of the ensuing lesions has been studied serially at intervals of a few hours and the titer of virus, histopathological changes and some metabolic disturbances were determined. During the early stage of infection, at the time of most rapid virus multiplication, brilliant acidophilic inclusion bodies appear in almost all the cells of the ectodermal layer. With slightly more delay, marked cellular proliferation develops in all 3 layers of the membrane, also endarteritis and thrombosis of the capillary vessels. After the virus has reached its maximum titer, the inclusion bodies diminish in number and become weakly basophilic, there is some necrosis and a gradual return to a more normal histological pattern. The respiration in Krebs-Henseleit solution, containing glucose, the aerobic lactate formation, the fermentation of hexosediphosphate to diphosphoglycerate, the zymohexase activity and sulfhydryl content of infected and normal membranes was determined in intervals from 2 hours after inoculation till the complete development of the embryo. The first measurable effect was on activation of zymohexase, then increased hexosediphosphate fermentation and an increase in aerobic lactate formation. Respiration was slightly inhibited only 14 days after inoculation of the virus. Correlation was found between metabolic effects and sulfhydryl content. Starting at 48 hours, a marked decrease in sulfhydryl groups occurred in the inoculated membranes.

Biochemical changes in the liver produced by chemical intoxication
H. C. SPENCER AND NORABELLE T. WILLIAMS (introduced by D. D. IRISH)
Biochemical Research Lab., Dow Chemical Co., Midland, Mich

In these studies it has been demonstrated that biochemical changes in the liver furnish reliable criteria for the evaluation of the effects produced by chemical intoxication. In laboratory animals that received repeated exposures to graded vapor concentrations of ethylene dichloride, trichloroethylene, tetrachloroethylene and carbon tetrachloride quantitative determinations of the following constituents of the liver have been made: total lipid, phospholipid, neutral fat, cholesterol fractions, water, nitrogen, sulfur, phosphorus, and collagen. Changes in these

constituents of the liver have been correlated with the type and extent of histopathological effects produced. As an example, the typical fatty changes observed histologically in the liver of animals exposed to carbon tetrachloride have been correlated with an increase in total lipid, due in a large measure to an increase in neutral fat and esterified cholesterol.

Neomycin toxicity studies
JOSEPH N. SPENCER,*
HARTELL G. PAYNE* AND FRED H. SCHULTZ,
JR.
Pharmaceutical Research Division, Commercial Solvents Corp., Terre Haute, Ind

Toxicity studies have been conducted with neomycin, a new antibiotic first described by Waksman (*Science*, 109, 305, 1949). The preparation employed was the neomycin sulfate. These preparations were pyrogen free and devoid of any significant pressor or depressor effects in anesthetized dogs. The following LD₅₀ values were obtained in mice: intravenous, 4,450 units/kg; intraperitoneal, 35,000 units/kg; subcutaneous, 36,000 units/kg; oral, greater than 865,000 units/kg. In rats the subcutaneous LD₅₀ was 92,000 units/kg and oral LD₅₀ was greater than 865,000 units/kg. Mice were given daily subcutaneous doses of 18,000, 9,000, 4,500 units/kg for 30 days. All animals survived the lower doses and 90% survived the highest dose. Four dogs were injected intramuscularly with 2000 units/kg every 6 hours for 8 days. Neomycin blood level determinations indicated that continuous blood levels were maintained in 2 animals. No toxic symptoms were observed and there were no alterations in the hematology, and urine and blood chemistry. Histopathological findings will be reported.

Dosage—reversal of effects of acetylcholine and of adrenaline, as observed in submaxillary salivary gland of cat
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It is known that under the influence of amyl nitrate, bleeding and other conditions which tend to lower blood pressure, the vasomotor effect of chorda tympani stimulation may be reversed, the chorda causing a diminution of the blood flow through the submaxillary salivary gland instead of the usual vasodilatation (FRÖHLICH AND LOEWI, 1906; DALE, 1930; STAVRAKY, 1934). Using the intra-arterial route (anterior lingual artery), it was found in the present investigation, that in cats under chloralose and urethane (1:10) anaesthesia, acetylcholine bromide injected in 10⁻⁴—10⁻⁷ quantities, produced pure vasodilatation in the submaxillary gland, while, when introduced in greater quantities (20–200 γ) it caused vasoconstriction. In both cases a secretion of saliva could be evoked. Eserinization of the gland or intra-

venous injection of eserine facilitated the appearance of the vasoconstrictor effect of acetylcholine and when sufficient quantities of eserine or acetylcholine were injected a reversal of the vasomotor effect of chorda tympani stimulation also took place. Atropinization abolished these vasoconstrictor effects. Similarly intra-arterial injections of small quantities of adrenaline hydrochloride (10^{-10} – 10^{-2} γ) had a vasodilator effect, while larger amounts (10^{-1} – 5γ) produced prolonged vasoconstriction in the submaxillary gland. It was possible to demonstrate with the present technique that while low blood pressure has a predisposing effect on these reversals, the latter could be produced at ordinary blood pressure levels and depended upon the quantity of the injected chemical agents. These observations fit in with other instances of reversal of the effect of acetylcholine described in recent years (SPADOLINI AND DOMINI, 1940, McDOWALL, 1946, BULBRING AND BURNS, 1949, BURN AND VANE, 1949 *et al*).

Action of veriloid upon isolated mammalian heart

NORMAN S STEARNS (introduced by J W STUTZMAN) *Dept of Pharmacology, Boston Univ School of Medicine, Boston, Mass*

This report deals with the investigation of the inotropic, chronotropic and dromotropic effects of Veriloid (J W Dart Labs' purified, reproducible extract of *Veratrum viride*). Sixty-four rabbit hearts were perfused with Locke's solution using a modification of Langendorff's technique. Inotropism was interpreted from isotonic contractions of the heart recorded on Teledeltos paper using a lever attached to the left ventricle. Rate and rhythm were recorded with a visio-cardiette under controlled conditions of temperature and pressure. Satisfactory volume and solvent controls were done. Both positive and negative inotropic actions of Veriloid have been demonstrated. Both moderate Stat doses (up to $10\mu\text{g}$) injected rapidly close to the heart and moderate concentrations (up to $0.075\mu\text{g/cc}$) perfused continuously produce positive inotropism. Effective dose ranges appear to be within probable effective ranges for hypotension in intact dogs and in man. Bradycardia has not been demonstrated with Veriloid in the isolated rabbit heart. Tachycardia has been observed with high Stat doses and with relatively high concentrations (over $0.1\mu\text{g/cc}$) of continuously perfused Veriloid. Inotropic and chronotropic effects may occur independently of each other. Arrhythmias are produced by continuous Veriloid perfusion in high concentrations (over $0.3\mu\text{g/cc}$).

Cardiovascular aspects of acute cocaine intoxication JOHN E STEINHAUS (introduced by A L TATUM) *Dept of Pharmacology, Univ of Wisconsin Med School, Madison*

From a study of the prophylactic use of barbiturates in cocaine intoxication it became evident that the cardiovascular system is involved to a much greater degree than has generally been supposed in the 'slower type' of intoxication. These effects were studied on dogs and rabbits by means of electrocardiograms and blood pressure tracings taken during the administration of cocaine. The marked variation seen clinically and experimentally can be explained in part by variations in absorption. For this reason cocaine was administered intravenously by a motor-driven mechanical injector. Rapid rates of injection, 15 mg/kg/min, caused almost simultaneous cardiovascular and respiratory failure. Rates of 3-4 mg/kg/min produced death by cardiovascular failure and rates of 2.5 and lower caused primarily respiratory failure. Failures of both systems were often seen at rates between the 2 lower figures. Barbiturate antagonism of cocaine intoxication was studied by treating 4 groups of 5 dogs each with doses of 0, 5, 15, and 30 mg/kg of pentobarbital *iv* followed by the administration of cocaine, 2.25 mg/kg/min, until respiratory arrest occurred. The groups receiving 15 and 30 mg/kg of pentobarbital developed no signs of central nervous system stimulation, however, a marked cardiovascular deficiency appeared as indicated by very low blood pressures at respiratory arrest. This could be combatted with small doses of epinephrine suggesting the utility of epinephrine and related drugs in the barbiturate treatment of local anesthetic intoxication.

Hypotensive action of Veriloid, an extract of *Veratrum viride* J W STUTZMAN AND GEORGE L MAISON *Dept of Pharmacology, Boston Univ School of Medicine, Boston, Mass*

Veriloid (J W Dart Labs, Los Angeles) is a purified, reproducible extract of *Veratrum viride*. None of the previously described potent alkaloids have been obtained by its fractionation. Normotensive dogs anesthetized with pentobarbital sodium have been used in the studies of its hypotensive effects. Linear dose-response relationships were found with 151 Stat injections of 1 to $12.5\mu\text{g/kg}$ and with 400 10-minute infusions of 0.25 – $3\mu\text{g/kg/min}$. With an infusion of $1\mu\text{g/kg/min}$ for 10 minutes the average decrease in mean arterial blood pressure was $29.9 \pm 12\%$ in 232 administrations. The hypotensive action of Veriloid was not altered quantitatively by any of the following procedures: 1) complete atropinization (11 animals), 2) bilateral cervical vagotomy (25 animals), 3) bilateral carotid sinus and body denervation (10 animals). Veriloid caused a further fall in blood pressure when administered to 9 animals during the period of hypotension resulting from 20 mg/kg of tetraethylammonium chloride.

This latter drug was sufficient to block the bradycardia from peripheral vagal stimulation and the pressor response from bilateral carotid sinus occlusion. Veriloid lowered the blood pressure of 21 dogs made hypertensive by acute section of all the buffer nerves.

Action of tetraethylammonium bromide and acetylcholine on the kitten phrenic nerve-diaphragm preparation WALTER J. SULLIVAN* AND CHARLES J. KENSLE, *Dept. of Pharmacology, Cornell Univ. Med. College, New York City*

Tetraethylammonium bromide (TEA) restored the response of the rat diaphragm to phrenic nerve stimulation at rates of 5 and 30/min when failure was produced by maintenance in calcium free Ringer-bicarbonate solution, provided sufficient calcium ($\text{ca } 4 \times 10^{-4} \text{ M}$) had been added to permit minimal activity (KENSLE, *Federation Proc.* 8: 307, 1949). Although the calcium threshold for minimal activity was essentially the same for the kitten as for the rat diaphragm, TEA was less effective in restoring function to the kitten preparation (to 45-65% of normal) than to the rat (85-105%). For the kitten maximal calcium 'replacement' was achieved by concentrations of TEA $\text{ca } 7 \times 10^{-4} \text{ M}$. Further increase in TEA concentration decreased the response. The addition of TEA ($1 \times 10^{-3} \text{ M}$) to the kitten preparation in normal calcium Ringer markedly increased the muscle response to nerve stimulation as reported earlier by Ing and Wright (*Proc. Roy. Soc. London, B* 109B: 337, 1931) for a frog nerve-muscle preparation. The response of the kitten diaphragm to nerve stimulation was potentiated by low concentrations of diisopropyl fluorophosphate (DFP) and was reversibly blocked by higher concentrations ($\text{ca } 2.5 \times 10^{-3} \text{ M}$) as had been found for the rat (KENSLE AND BERRY, *Federation Proc.* 8: 307, 1949). Acetylcholine ($1-2 \mu\text{g/ml}$) blocked the response to nerve stimulation in the presence of low concentrations of DFP or after exposure to high concentrations ($2.5 \times 10^{-3} \text{ M}$). After recovery from a DFP-acetylcholine block, low doses of acetylcholine ($\text{ca } 0.1 \mu\text{g/ml}$) usually produced a sustained potentiation of the response of the diaphragm to nerve stimulation. Higher doses decreased the response.

Anticonvulsant properties of optical isomers of nirvanol and mesantoin EWART A. SWINYARD, JACK E. ORR,* JOEL M. JOLLEY* AND LOUIS S. GOODMAN, *Depts. of Pharmacy, Pharmaceutical Chemistry, and Pharmacology, Univ. of Utah College of Pharmacy, and College of Medicine, Salt Lake City*

Optical isomers of Nirvanol and Mesantoin (prepared by J. J. Spurlock, North Texas State College) and Nirvanol (prepared by J. E. Orr)

were tested for their ability to modify maximal electroshock seizure pattern and prevent Metrazol convulsions in rats. Drugs were given orally in a 10% acacia suspension and tested at the time of peak drug effect. Anticonvulsant potencies (ED_{50}) and minimal neurological toxicity (TD_{50}) were determined and protective indices calculated ($\text{TD}_{50}/\text{ED}_{50} = \text{P.I.}$). The data indicate that neither toxicity nor anticonvulsant activity resides exclusively in one isomer. The levo forms were the more active except in the case of antielectroshock potency of Mesantoin. For Nirvanol the racemic mixture showed greater activity than that calculated from the isomers.

Studies on cardiovascular actions of adenylic compounds VIRGINIA L. SYDOW* AND RAYMOND P. AHLQUIST, *Dept. of Pharmacology, Univ. of Georgia School of Medicine, Augusta*

The cardiovascular actions of the following compounds were compared to those of adenosine in the anesthetized dog: adenylic acid, adenine, guanine, guanosine, guanylic acid, cytidine, cytidylic acid, thymine, hypoxanthine, uracil, uridine and ribose. Changes in arterial pressure, femoral and carotid arterial blood flow, heart rate and ECG were used as the criteria of activity and the compounds were administered intravenously, intra-arterially or intra-aortically. Adenosine produced a transient fall in arterial pressure due to peripheral vasodilation. No significant changes in heart rate or ECG were found with the doses tested (1×10^{-6} to 1×10^{-3} moles). On a molar basis adenosine was the most active vasodilator. Sodium adenyate produced about the same degree of dilation but its effect was more prolonged. Sodium guanylate was qualitatively similar to sodium adenyate but only one sixth as effective. Guanosine produced either vasodilation or vasoconstriction depending upon the dose employed and the pretreatment of the animal. All of the other compounds were practically inactive.

Reaction mechanism of diamine oxidase (histaminase) HERBERT TABOR, *Natl. Insts. of Health, Bethesda, Md.*

The preparation of a highly purified diamine oxidase from hog kidney (TABOR, *Federation Proc.* 1949) has been modified by the inclusion of an isoelectric precipitation. The oxidation of 1 M of histamine or putrescine by diamine oxidase (in the presence of catalase) requires $\frac{1}{2}$ M oxygen. One M NH_3 appeared, and 1 M bisulfite was bound by the reaction product. During the oxidation of histamine the imidazol ring remained intact. In addition to the bisulfite binding, evidence for an aldehyde intermediate during histamine oxidation was indicated by the intense rose color obtained upon addition of 2,4-dinitrophenylhydrazine to the reaction product and subsequent alkaliniza-

tion Furthermore, additional experiments showed that aldehyde oxidase (prepared according to Racker) oxidized the intermediate as measured by the stoichiometric reduction of diphosphopyridine nucleotide These data indicate that the most likely reaction mechanism, among the different possibilities proposed by various authors, is the conversion of histamine to imidazol acetaldehyde (ZELLER, STEPHENSON, *et al*)

Metabolism of radioactive S³⁵ thiopental (Pentothal) in the rat and monkey J D TAYLOR,* R K RICHARDS AND D L TABERN* *Depts of Pharmacology and Special Research, Abbott Research Labs, North Chicago, Ill*

The urine of rats injected intraperitoneally with a single dose of 40 mg/kg of radioactive Pentothal (6 M conc /gm) was analyzed for chloroform extractable, inorganic and ethereal sulfate, and neutral sulfur over a period of 4 days At the end of 24 hours, 65% of the total injected radioactivity was extracted At the end of 4 days 85% of the total radioactivity could be accounted for with 7% chloroform extractable, 23% as inorganic sulfate, 8% as ethereal sulfate, 40% as neutral sulfur, 5% in the feces, and only 2% remaining in the carcass In the monkey injected intravenously with 35 mg/kg of radioactive Pentothal, 75% of the injected dose was excreted in the urine in the first day A total of 86% of the radioactivity was accounted for in 4 days with 17% occurring as inorganic sulfate and 14% of the total injected dose chloroform extractable Paper chromatography was used to determine the number of radioactive degradation products in the urine The number and relative amounts of these products varied with species and time after injection In one fraction of monkey urine, at least 9 radioactive compounds were found At least 4 of them can be extracted with chloroform One of these latter compounds is unchanged Pentothal and another is probably the Pentothal carboxylic acid described by Brodie *et al* (personal communication)

Some substituted ethylenimines with actions like nitrogen mustards J B THIERSCH* AND F S PHILIPS *Pharmacology Section, Division of Experimental Chemotherapy, Sloan-Kettering Inst for Cancer Research, New York City*

Nitrogen mustards are tertiary amines having 2 or more 2-haloethyl groups/molecule, for example, methyl-*bis* (2-chloroethyl) amine (HN2) and N,N'-*bis* (2-chloroethyl)-piperazine (I) Such compounds act through *in vivo* transformation into ethylenimmonium derivatives Therefore, it was interesting to compare actions of 2,4,6-*tris* (ethylenimino)-s-triazine (II), 1,1'-hexamethylene-*bis* (3,3-ethylenurea) (III), and other substituted ethylenimines with those of HN2 and I

Characteristically nitrogen mustards in near MLD amounts cause a delayed lethal syndrome associated with marked pancytopenia and severe diarrhea Animals become progressively debilitated after poisoning and succumb between 3 and 7 days Atrophy of all hematopoietic tissues, cytological abnormalities in bone marrow, and damage to intestinal epithelium are typically found Substituted ethylenimines like II and III are as toxic as HN2 and I and near MLD amounts cause a similar delayed lethal syndrome with diarrhea and pancytopenia Lesions noted parallel those observed in animals given nitrogen mustards Structural analogs differing from II in lacking ethylenimine groups are much less toxic and do not act like nitrogen mustards Supralethal doses of II like those of HN2 are convulsant and paralytic However, unlike HN2, II is not outstandingly parasympathomimetic This may be related to the tertiary state of the ethylenimine-derivative in contrast to the quaternary state of ethylenimmonium transformation products of HN2

'Benemid' (p-(di-n-propylsulfamyl)-benzoic acid), an anticatabolite its estimation in body fluids ELIZABETH K TILLSON,* GRACE S SCHUCHARDT* ALICE A PITT,* RICHARD W GASS* AND KARL H BEYER *Dept of Pharmacology, Med Research Division, Sharp & Dohme, Inc, Glendolen, Pa*

Beyer *et al* have reported (*Federation Proc* 1950) that 'Benemid' (p-(di-n-propylsulfamyl)-benzoic acid) decreases the destruction and elimination of certain therapeutically useful organic acids by inhibiting their conjugation with glycine In this sense, it functions as an anticatabolite Two analytical methods have been developed for the estimation of the drug in body fluids The first method depends on the fact that the compound can be extracted into chloroform from an acidic, aqueous medium and in turn reextracted from chloroform into 0.1 N sodium hydroxide The alkaline solution is examined spectrophotometrically at a wave length of 242.5 mμ For the second method the drug is also extracted into chloroform from an acidic aqueous medium The chloroform layer is shaken with an aqueous solution of methylene blue The colored salt, formed at the surface, is soluble in the solvent making it possible to determine the amount present by examining the chloroform layer colorimetrically With the aid of these methods, it has been found that the compound is well absorbed following its oral administration, that it is excreted extremely slowly if at all, and that hydrolyzable metabolites of the drug appear slowly in the urine following its oral or parenteral administration

In vivo antihistamine activity of several antihistamines R TISLOW, S MARGOLIN, M T

SPOERLEIN AND E. VOLAGE (introduced by H. B. HAAG) *Biological Research Labs., Schering Corp., Bloomfield, N. J.*

The oral activity and toxicity of several antihistamines were determined in guinea pigs by the method previously described (LaBelle, A. and R. Tislow *Federation Proc.* 7: 236, 1948). To determine the potency, animals were challenged one hour after feeding of the antihistamine by rapid intravenous injection of 11 mg/kg of histamine dihydrochloride. From the data and from clinical experience, it appears that the *in vivo* test in guinea pigs provides a better estimate of the clinical effective dose of antihistamines as compared with the *in vitro* test.

Effects of parpanit, myanesin, and benzimidazole on properties of frog nerve. J. E. P. TOMAN, W. H. EVANS, S. C. HOUSTON, H. V. MILLER, J. M. NANCE AND B. K. WILSON (introduced by L. S. GOODMAN) *Depts. of Physiology and Pharmacology, Univ. of Utah College of Medicine, Salt Lake City.*

The effects of centrally acting muscular relaxants were studied in frog sciatic nerve by means of a battery of tests previously devised for anticonvulsant investigations. By all tests used molar potency was greatest for parpanit and least for benzimidazole, as previously observed for their relaxant and toxic central actions. Concentrations blocking conduction were found to act by raising threshold rather than by depolarizing. Alterations of normal fiber function were noted in the concentration range of central toxic effects *in vivo*. Protection against hyperexcitability induced by excessive stimulation or by phosphate treatment was found in the concentration range for minimal central actions *in vivo*. In spite of the greater potency of parpanit, the margin of safety was least for this drug and greatest for myanesin, in accord with anticonvulsant tests in mammals. Beyond these parallels no distinct patterns of action were noted which might clarify the known clinical differences between these drugs.

Effect of convulsant and anticonvulsant agents on the activity of cytochrome oxidase. CLARA TORDA AND HAROLD G. WOLFF *New York Hospital, Kingsbridge Hospital (V.A.) and Depts. of Medicine, Neurology and Psychiatry, Cornell Univ. Med. College, New York City.*

The effects of convulsant and anticonvulsant agents on the activity of cytochrome oxidase have been investigated. The activity of cytochrome oxidase increased in the presence of the convulsant agents used (acetylcholine, caffeine, dichlorodiphenyltrichloroethane, mechoyl, methylsalicylate, pentamethylene tetrazol, picrotoxin, scilliroside, and strychnine) in concentrations of 1×10^{-3} M and less. The greatest increase ob-

served was over 250%. The activity of cytochrome oxidase was not modified significantly by the anticonvulsant agents used (hydantoin, methylphenyl-ethyl hydantoin, phenylhydantoin sodium, phenobarbital, and tridione) in concentrations of 1×10^{-3} M and less. Since changes in the electrical activity of brain characteristic for seizures precede the increase of cerebral oxygen consumption (Davies, P. W., and A. Remond *Research Publ. A Nerv. & Ment. Dis.* 26: 205, 1947) the increase of the activity of cytochrome oxidase in the presence of the convulsant agents suggests that these agents actively induce not only the seizures and the changes in the electrical activity of the brain but also the increase of oxygen consumption of the brain during the seizures.

Treatment of sodium fluoroacetate (1080) poisoning in dogs. W. W. TOURTELLOTT* AND J. M. COON *Toxicity Lab. and Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.*

Sodium acetate and ethanol antagonize synergistically 1080 poisoning in mice (*Federation Proc.* 8: 339, 1949). The present report is an attempt to evaluate these compounds and barbiturates in treating 1080 poisoning in dogs. The oral LD-50 of the 1080 powder used in these dog experiments was found to be 0.066 mg/kg. Of 24 dogs poisoned with 0.12 mg/kg of 1080, a surely fatal dose, and treated 30-40 minutes later, none of 6 survived with ethanol-sodium acetate treatment, 6 of 6 survived with pentobarbital-phenobarbital treatment and 12 of 12 survived with ethanol-sodium acetate-barbiturate treatment. Of 13 dogs poisoned with 0.24 mg/kg and treated 30-40 minutes later with ethanol-sodium acetate-barbiturate, 3 dogs survived. The dosage schedule of 50% ethanol and 50% sodium acetate beginning 30-40 minutes after poisoning was 2 cc/kg intravenously and repeated orally at 2 and 5 hours. The dosage schedule of the barbiturates was 15 mg/kg of pentobarbital intravenously 30-40 minutes after poisoning, 25-50 mg/kg of phenobarbital subcutaneously at 3 and/or 9 hours, and 5 mg/kg of pentobarbital p.r.n. for convulsions. The dogs receiving ethanol-sodium acetate required 40% less phenobarbital for prophylaxis against severe convulsions, while approximately the same amount of pentobarbital was given to control convulsions.

Distribution of procaine in squid axons (Loligo).

A. P. TRUANT (introduced by PAUL K. SMITH) *Dept. of Pharmacology, George Washington Univ. School of Medicine, Washington, D. C.*

Recently, the distribution of procaine in sciatic nerves (*Rana pipiens*) has been studied in relation to conduction block (A. P. Truant and E. L. Way, unpublished). The distribution of procaine in the nerve fiber of the giant axon of the squid was

studied The giant nerve fiber was prepared free of adjacent tissue and mounted on electrodes in a moist chamber Normal neurograms of the monophasic action potential were recorded and the axon segment was immersed in buffered artificial sea water, containing procaine hydrochloride When conduction block occurred the treated segment was washed and the axoplasm was extruded The procaine concentrations in both the axoplasm and the axon membrane were determined colorimetrically In 22 experiments the average concentration of procaine at time of conduction block in the axoplasm was $96.7 \mu\text{g}/100 \text{ mg}$ of tissue and that in the axon membrane $9.3 \mu\text{g}/100 \text{ mg}$ of tissue The concentration of procaine in the axoplasm at the time of recovery was $68.9/100 \text{ mg}$ axoplasm while the axon membrane concentration did not significantly change The concentration of procaine in the axoplasm is not the only relevant factor responsible for conduction block In axon segments blocked with 0.01 M procaine and subsequently recovered in sea water the action potential was impeded by carefully blotting the treated segment Control nerves were not affected by this procedure By spraying the axon with sea water conduction returns in 1-2 minutes This phenomena could be repeated until the axoplasm procaine concentration became sub-minimal

Theophylline blood levels after oral, rectal and intravenous administration of Aminophylline
EDWARD B TRUITT, JR AND VICTOR A MCKUSICK (introduced by JOHN C KRANTZ, JR) *Dept of Pharmacology, Univ of Maryland and Cardiovascular Clinic, U S Marine Hospital, Baltimore, Md*

Theophylline blood levels were measured by the method of Truitt, *et al* (*J Pharmacol & Exper Therap* 91 185, 1947) Blood levels were determined in six normal subjects at various intervals up to 8 hours Aminophylline (0.5 gm) was given by intravenous injection and rectal suppository Uncoated tablets of Aminophylline (0.3 gm) were given orally From the data on intravenous administration the average rate of disappearance of theophylline was found to be $0.12 \text{ mg \%}/\text{hour}$ One patient with carbon tetrachloride nephrosis was observed to have a prolonged elevation of

blood level with a slow rate of disappearance The data indicate that rectal administration by suppository gives peak blood levels of only about 20% of the level attained after the same period (2 hr) by intravenous administration of the same dose After oral administration blood levels were more comparable with the intravenous route, 60% as large a dose by mouth resulted in a 2-hour blood level of approximately 65% of the blood level at the same time by the intravenous route

New color reaction for the estimation of steroid ketones having the α, β -unsaturated ketone grouping in ring A ERNEST J UMBERGER (introduced by ARNOLD J LEHMAN) *Division of Pharmacology, Food and Drug Admin Federal Security Agency, Washington, D C*

Feigl's spot test for aldehydes and ketones employing azobenzenephnylhydrazine sulfonic acid (APS) has been developed into a method suitable for the quantitative estimation of testosterone and other steroids having the α, β -unsaturated ketone grouping in Ring A Place 0.5 ml of alcoholic solution of testosterone (about $50 \mu\text{g}$) and 2.0 ml of APS reagent ($0.5 \text{ mg}/\text{ml}$ in diluted H_2SO_4 prepared by mixing 1 volume conc H_2SO_4 with 2 volumes H_2O) in a glass-stoppered flask and let stand in the dark for 30 minutes Add 5.0 ml H_2O and 5.0 ml CCl_4 shake and transfer to a separatory funnel Draw off the CCl_4 layer through a cotton pledget and measure the color at $416 \text{ m}\mu$ Include a blank and a standard with each run for comparison The reaction should be carried out in subdued light to prevent fading If 4.0 ml of the yellow CCl_4 solution is diluted with 1.0 ml of 2.5% HCl in EtOH , a less stable bluish-purple color is obtained which can be utilized by making the readings at $580 \text{ m}\mu$ Of the compounds so far tested, positive reactions are given by testosterone, testosterone propionate, methyl testosterone, ethinyl testosterone, progesterone and desoxycorticosterone acetate The intensity of the colors are inversely proportional to their molecular weights Benzaldehyde and formaldehyde give yellow colors which become reddish-purple and red respectively on making acid Essentially negative reactions are given by 7-ketocholesterol acetate, androsterone, dehydro isoandrosterone, estrone, acetone, ethyl acetoacetate, benzyl styryl ketone, and p-dimethylaminobenzaldehyde

Antagonistic effects of pentamethylene-bis(trimethylammonium iodide) (C5) and of d-tubocurarine (dTC) on action of decamethylene-bis(trimethylammonium bromide) (C10) in man
KLAUS R UNNA AND MAX S SADOVE * *Dept*

TIME	THEOPHYLLINE BLOOD LEVELS mg %/70 kg		
	Intravenous	Oral	Rectal
10 min	1.20		
30 min	1.06	0.18	
1 hr	0.86	0.34	0.04
2 hr	0.73	0.46	0.14
4 hr	0.51	0.39	0.10
8 hr	0.12	0.14	0.06

of Pharmacology, Univ of Illinois, College of Medicine, Chicago, Ill

The curariform action of C10 differs from that of dTC in that it is not antagonized by neostigmine. In animals, both dTC and C5 have been found to counteract the effects of C10. It has been claimed (ORGANE, PATON, AND ZAIMIS *Lancet* 1 21, 1949) that C5 antagonizes the effects of C10 also in man. In 4 volunteers in whom the effects of C10 in doses up to 2 mg on grip strength and vital capacity had been determined repeatedly, pretreatment with 50 mg of C5 five minutes before the administration of C10 failed to alter significantly the response to C10 or to shorten its duration of action. These results are contrasted to the effects of prostigmine on the response to dTC under comparable conditions. In contradistinction, subjects given dTC in doses of 8-10 mg were found after recovery from the paralytic effects—to be remarkably well protected against the curarizing effects of a subsequent injection of C10.

Antagonism of d-tubocurarine by neostigmine methylsulfate E F VAN MAANEN (introduced by G H ACHESON) *Dept of Pharmacology, College of Medicine, Univ of Cincinnati, Ohio*

Adequate doses of neostigmine sometimes decurarine but at other times fail to do so. This can be explained by the relative magnitudes of 1) the increased concentration of acetylcholine resulting from the action of neostigmine and 2) the increased threshold of the receptive substance to acetylcholine produced by d-tubocurarine. Since neostigmine eliminates only the destruction of acetylcholine, it can increase the acetylcholine concentration only to a certain limit. Thus, in the rat sciatic nerve-muscle preparation, varying amounts of neostigmine given intravenously before d-tubocurarine produced no more than a threefold increase in the paralyzing dose of the latter (from 30-80 to 100-220 $\mu\text{g/kg}$ rat). In preparations in which the threshold to acetylcholine can be determined, however, this variable may be increased markedly by increasing the dose of d-tubocurarine. Neostigmine injected intravenously during total paralysis by d-tubocurarine in the cat sciatic nerve-muscle preparation produced either (A) a rapid recovery of muscular contraction or (B) no immediate decurarization but instead, a gradual recovery, like that observed without neostigmine, but occurring sooner. (A) is found when the threshold is only moderately elevated beyond the level of complete paralysis, hence the increase in acetylcholine concentration is immediately effective in overcoming the paralysis. In (B), however, the elevation of threshold is so great that the greatest increase of acetylcholine concentration which can result from neostigmine fails to reach the threshold and is evidenced merely by an earlier recovery from paralysis.

Effect of L-hydrazinophthalazine (C-5968) on cardiovascular system of unanesthetized dogs HARRY A WALKER, SUSANNE WILSON* AND ARTHUR P RICHARDSON *Dept of Pharmacology Emory Univ School of Medicine Emory University Ga*

The effect of 1-hydrazinophthalazine (C-5968) on the response of the cardiovascular system of unanesthetized dogs to the intravenous injections of acetylcholine, pure 1-epinephrine, and pure 1-norepinephrine and to tilting from a horizontal to an 85° upright position has been studied after oral and progressively increasing intravenous doses of the drug. Intravenous doses of 0.25 mg/kg produced a slight decrease (5%) in resting systolic pressure accompanied by a marked increase (50%) in pulse rate. As the dose was increased to 8 mg/kg there was a progressive hypotensive effect with the mean systolic pressure gradually falling from the control level of 124 mm Hg to 90 mm although the mean pulse rate had risen from 93-198 beats/min at the same time. With 0.25 mg/kg there was a postural hypotension upon tilting with a 34% decrease in systolic pressure and a 24% decrease in pulse rate. The only change in the acetylcholine response was a 50% decrease in the reflex tachycardia. This latter effect may be explained by the fact that the resting pulse rate with this dose of C-5968 was already increased. At this same dose the pressor response to intravenous injection of 1-epinephrine and 1-norepinephrine was not materially altered. The homeostatic mechanism of maintaining blood pressure during the tilting procedure apparently is altered by 0.25 mg/kg of C-5968 without marked effects on the compensatory reflexes brought into play by the intravenous injection of acetylcholine and epinephrine. Larger doses of C-5968 depress the epinephrine response. A typical response was an abrupt rise in pressure followed by a gradual decrease which frequently progressed below the resting pressure. The initial pressor response was never completely blocked.

Effect of D- and L-methadone on blood glucose and lactate of dog D T WATTS (introduced by C L GEMMILL) *Dept of Pharmacology, Univ of Virginia Med School, Charlottesville*

Previous experiments have shown that both D- and L-methadone inhibit aerobic oxidative enzymes of brain homogenates but do not inhibit the anaerobic glycolysis of glucose to lactate. The present experiments were undertaken in an attempt to obtain indirect evidence for *in vivo* enzymatic blockage in dogs by demonstrating an increase in intermediate metabolic products such as lactate after the administration of these drugs. Glucose and lactate determinations were made on venous blood drawn at one-hour intervals follow-

ing the intravenous administration of the drugs. The following maximum changes from control levels were obtained

DRUG	DOSE mg/kg	% CHANGE	
		Lac tate	Glu cose
none		-16	-12
D-methadone	3 0	-24	+17
L-methadone	3 0	+60	+60
L-methadone	8 0	-23	+41
L-methadone + Nem- butal	3 0 + 30 0	-31	-7

During control experiments and after D-methadone lactate value dropped slightly. The L-methadone at 30 mg/kg produced a marked increase in lactate which is apparently due to increased excitability and muscular activity rather than enzymatic blockage. When deep depression and muscular relaxation was produced by a higher concentration of L-methadone or by methadone plus nembital the lactate level dropped well below the control value. The D-methadone produced a slight hyperglycemia. The L-methadone produced a marked hyperglycemia. This hyperglycemia was eliminated by nembital, indicating it is probably due to a central nervous system stimulation.

Effects of tetra-ethyl thiuramdisulfide (antabuse) on toxicity of methyl alcohol E. LEONG WAY AND RUSSELL HAUSMAN * *Division of Pharmacology and Experimental Therapeutics, Univ of California School of Medicine, and College of Pharmacy, San Francisco*

The effects of antabuse on the oral toxicity of methanol was investigated experimentally on rats and rabbits. The preliminary results indicate that antabuse increases the toxicity of methyl alcohol but not to the extent that it potentiates the effects of ethyl alcohol. In animals which were given a single dose of antabuse in aqueous suspension daily for 3 days and then methanol, the toxic symptoms arising had a more rapid onset and appeared to be more protracted and severe than in the control animals which received methanol, only or an aqueous suspension of calcium carbonate instead of antabuse prior to the methyl alcohol. The median lethal dose (LD_{50}) of methanol was significantly decreased by antabuse. Death usually occurred earlier in the antabuse animals and the recovery of the survivors was considerably slower than in the control group.

Pitfalls in the treatment of frostbite with heparin

DAVID WEINER AND KURT LANGE (introduced

by M. G. MULINOS) *Dept of Medicine, New York Med College, New York City*

In our original communication in 1945 and in later reports we have demonstrated the efficacy of heparinization in preventing gangrene subsequent to experimental frostbite. Several investigators have attempted to produce similar results, some have encountered success, some failure. Careful analysis of the reports of those who have failed indicates that the failure to prevent gangrene in a large percentage of treated animals is due to inadequate heparinization either in degree or in duration. This is demonstrated by the following findings. One hind leg of each of 83 rabbits was immersed in an alcohol dry ice bath at -12°C to -30°C for 30 to 45 minutes under penthotal sodium anesthesia. Thirty-two were not heparinized. Thirty-four were adequately heparinized for at least 5 days with the coagulation time consistently above 30 minutes (Lee-White method). Seventeen were inadequately heparinized. Of the 32 controls, all (100%) resulted in complete gangrene of the exposed limbs. Of the 17 inadequately heparinized animals, 13 (76%) resulted in either partial or complete gangrene. Of the 34 adequately heparinized animals, 5 (15%) resulted in gangrene. These findings emphasize the importance of maintaining the coagulation time above 30 minutes throughout the treatment period which should be at least 5 to 6 days. The dosage necessary to accomplish this varies widely in individual animals and in different strains necessitating frequent determinations of the coagulation by the Lee-White method.

Effect of anoxia on activity of isolated intestine T. C. WEST,* G. HADDEN* AND A. FARAH *Dept of Pharmacology, University of Washington School of Medicine, Seattle*

The effect of anoxia on contractions of the isolated rabbit intestine was studied with respect to 1) substrate utilization, 2) drug influence and 3) action of enzyme inhibitors. Anoxic influence on substrate utilization and enzyme inhibitor action was measured by alteration in the character of spontaneous contractions and in the acetylcholine response. Anoxic anoxia and sodium cyanide were used to test the effect on various drugs influencing smooth muscle contraction. The substrates glucose, mannose, lactate, pyruvate, acetate, oxalacetate, butyrate, caproate, caprylate and acetoacetate furnish energy for aerobic contractions. Anaerobic contractions occurred only in the presence of glucose or mannose. Acetylcholine, pilocarpine, nicotine and barium chloride produced anaerobic contractions. Eserine and hexaethyltetraphosphate (HETP) were incapable of stimulating the anoxic gut. The anoxic atropine effect was characteristic to that seen aerobically.

The anaerobic nicotine contraction was sensitive to block by d-tubocurarine chloride and tetraethylammonium bromide. Epinephrine was inhibitory to anaerobic spontaneous contractions. Dinitrophenol and iodoacetate completely inhibited all anaerobic activity. Sodium fluoroacetate, malonic and propionic acids were ineffective anaerobically. In high concentration, the cyanide effect resembled that of anoxic anoxia. Low concentrations of cyanide inhibited tonus without greatly affecting spontaneous contractions, and abolished the activity of eserine and HETP. Acetylcholine and pilocarpine produced contractions but the characteristic tonus change was abolished.

Effect of certain dialkyl-substituted aminoalkyl-bearing compounds on apomorphine-induced emesis. JOSEPH M. WHITE,* DANIEL FREEDMAN*, E. L. MCCAWLEY AND WILLIAM D. GRAY*
Dept. of Pharmacology, Yale Univ. School of Medicine, New Haven, Conn.

Diphenhydramine 8-chlorotheophyllinate (Dramamine) has been introduced into therapy for the control of motion sickness. Diphenhydramine (Benadryl) is but one of a number of common substances which (1) contain a substituted aminoalkyl side chain (2) exhibit antihistaminic activity. Apomorphine hydrochloride 30 mg/kg when administered subcutaneously was found consistently to produce emesis in the dog. When 2 mg/kg of the 8-chlorotheophyllinate salt of diphenhydramine were administered orally to dogs, the effect of apomorphine upon the vomiting center was antagonized completely. Similarly 1 mg/kg of diphenhydramine (as the hydrochloride) prevented emesis. No protection could be elicited when 10 mg/kg of 8-chlorotheophylline were administered. This pharmacological antagonism suggests that diphenhydramine acts by depression of the vomiting center. Protection against apomorphine-induced emesis in the dog may be used as a criterion for the screening of compounds which have an action similar to that of diphenhydramine. Relative potency may also be determined. A series of compounds related structurally and/or pharmacologically to diphenhydramine has been studied.

Toxicity studies on tomatine. ROBERT H. WILSON, GEORGE W. POLEY* AND FLOYD DEEDS*
Pharmacology Division, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Admin. U. S. Dept. of Agriculture, Albany, Calif.
Tomatine is an antifungal substance discovered in wilt-resistant tomato plants by Fontaine, Irving, *et al.* (*Arch. Biochem.* 18: 467, 1948). Such a material might be useful as an addition to foods to prevent mold-growth and as a treatment of fungus diseases. Studies on chronic, subacute, acute oral

toxicities and intravenous toxicity are presented. Rats were fed diets containing up to 0.04% of tomatine or its aglycone, tomatidine for 200 days. This concentration is considered well above any amount needed in food preservation. Growth, food intake and appearance of the animals were normal. Tissues at autopsy showed no abnormalities in gross appearance or in weight, histological examination is in progress. Subacute toxicity was studied in rats fed 250 mg tomatine/kg by stomach tube daily for 5 days. No symptoms were noted, autopsy 3 days after the last administration showed grossly normal tissues. Three rats received single oral doses of 800-1000 mg tomatine/kg. Death occurred within 2 days with apparent contracture of the pylorus and, in two, gross infarction in the liver. Preliminary studies indicate that intravenous administration of a few mg of tomatine/kg to mice causes death within seconds. Blood pressure tracings from rats receiving 1-2 mg tomatine/kg i.v. showed a marked drop in pressure, frequently precipitous. Recovery might be rapid or the pressure might remain depressed for some time. Central action was shown by the absence of blood pressure change or a slight rise, after vagal section or atropinization.

Studies on the nervous and humoral control of coronary circulation. MARTIN M. WINBURY*, PATRICIA M. MICHIELS* AND D. M. GREEN*
Pharmacology Dept. Division of Biological Research, G. D. Searle & Co., Chicago, Ill.

The effects of various chemical mediators, drugs and nerve stimulation on the coronary blood inflow (CBF) were investigated in open-chest anesthetized dogs. CBF was recorded continuously with a rotameter. All injections were made into the coronary circuit. CBF was increased by the chemical mediators epinephrine (2 γ), 1-arterenol (2 γ) and acetylcholine (10 γ) and by histamine (15 γ). Pitressin (0.025 units) reduced CBF. These effects were not accompanied by significant changes in blood pressure or cardiac output. The effect of acetylcholine on CBF was blocked by atropine, that of epinephrine and arterenol by Compound 883 F. Apparently the chemical mediators do not effect CBF by a common mechanism. Reduction in CBF by cardiac vagus stimulation was always associated with hypotension and bradycardia. Increase in CBF by cardiac sympathetic nerve stimulation, pre- or post-ganglionic was not always associated with tachycardia and hypertension. Gastric dilatation (> 40 mm Hg) reduced CBF but was associated with simultaneous reduction in blood pressure and cardiac output. These effects were duplicated by a reduction in venous return and were not blocked by atropine. Stimulation of typical somatic motor and sensory nerves did not effect CBF.

Inhibition of the pasteur effect in brain by certain steroids ALBERT WOLLENBERGER (introduced by OTTO KRAYER) *Dept of Pharmacology, Harvard Med School, Boston, Mass*

The metabolism of brain tissue is profoundly altered by low concentrations ($4 \times 10^{-7} - 10^{-5}$ M) of certain steroids which possess the property of producing CNS disturbances and of strengthening the contractile power of failing heart muscle, viz the tertiary amine bases among the ester alkaloids of veratrum (protoveratrine and veratridine) and the cardiac glycosides. In slices of guinea pig cerebral cortex incubated in Krebs-Ringer - glucose - phosphate solution these compounds produce a 30-100% stimulation of the respiration, which in the case of the veratrum alkaloids may last for several hours, raise the aerobic glycolysis to the normal anaerobic level, and cause inhibition up to 100% of the anaerobic glycolysis. The R Q remains unity. These changes strongly resemble the effect of high concentrations of extracellular potassium ions described first by Ashford and Dixon. The inhibitory effect of the steroids on the Pasteur reaction is restricted, like that of potassium, to central nervous tissue. It does not seem to be associated, at least in veratrum-treated brain cortex, with leakage of intracellular potassium ions into the medium. Omission of potassium ions from the medium gives rise to strong aerobic glycolysis which is not enhanced further by the steroids. In contrast to the potassium effect, the steroid effect is not reversed by washing. However, the depression of the anaerobic glycolysis is largely prevented, though not reversed, by nicotinamide (0.04 M) and, to a lesser extent, by pyruvate (0.0017 M) while ATP and DPN are ineffective. Observations on phosphate uptake by respiring brain homogenates provide no evidence that the steroids uncouple oxidation and phosphorylation or otherwise make available inorganic phosphate. The protective action of nicotinamide suggests stimulation of DPN-ase and it is conceivable that release of ordinarily restrained enzyme activity in the cell represents a more general effect of the tertiary veratrum amines and the cardiac glycosides.

Fate of mescaline in serum, plasma and blood

L. A. WOODS, J. COCHIN* AND M. H. SEEVERS
Dept. of Pharmacology, Univ of Michigan, Ann Arbor

Using a specific and sensitive colorimetric method, the details of which will be published elsewhere, it was found that mescaline is not altered chemically when exposed to dog or human plasma for 24 hours at temperatures between 5° and 25°C. However, when serum, plasma, or whole blood is incubated with mescaline at 38°C for

3-4 hours, a considerable loss of the drug occurs. This loss of mescaline may be due to a chemical reaction with carbon dioxide and/or enzymatic alteration. The former is a definite possibility since it is known that primary beta-phenylethylamines, such as mescaline, exhibit a marked affinity for carbon dioxide resulting probably in the formation of carbamates of the type, $RCH_2CH_2NHCOOH \cdot NH_2CH_2CH_2R$. The effect of carbon dioxide on this phenomenon was ascertained by determining the comparative rates of disappearance of mescaline from a buffered solution equilibrated in air and in 100% carbon dioxide. In each instance the drug was dissolved in 0.25 M Sorensens phosphate buffer at pH 7.4 and incubated with shaking for 4 hours at 38°C. In the presence of pure carbon dioxide, 50-65% of mescaline was lost whereas none disappeared in the presence of air. The 'lost' mescaline was recovered quantitatively after reconversion by heating with basic solution. Although enzymatic breakdown is a possibility, heating of serum at 58°C for 1 hour did not modify its capacity to degrade mescaline when incubated with this substance at 38°C.

Veratrine-like properties of the alkaloidal fractions from *Zygadenus venenosus* SUMNER YAFFE AND S. MORRIS KUPCHAN (introduced by OTTO KRAYER) *Dept of Pharmacology, Harvard Med School, Boston and Dept of Chemistry, Harvard Univ Cambridge, Mass*

Since Reid Hunt's discovery (*Am J Physiol* 6: vii, 1902) of a veratrine-like activity of the alkaloids obtained from various species of *Zygadenus*, relatively little has been done to identify the active principles, although Heyl *et al* (*J Am Chem Soc* 35: 258, 1913, 71: 1751, 1949) succeeded in isolating a crystalline alkalamine, zygadenine, as early as 1913, which is considered to be a tertiary amine base like cevine. We have undertaken to study the alkaloids of *Zygadenus* chemically and pharmacologically in view of the increased interest in the veratrum alkaloids and substances related to them. The alkaloidal fractions so far isolated caused the characteristic myotonic response when tested on the isolated sartorius muscle of the frog. In addition small amounts of some of the alkaloidal fractions produced a reflex decrease in blood pressure and heart rate and a stoppage of respiration which were mediated by the vagus nerves, as they could be abolished or prevented by interruption of the conduction of the vagus nerves. It is probable, therefore, that *Zygadenus venenosus* not only contains tertiary amines of the type of zygadenine but also ester alkaloids of such tertiary bases.

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Ketoglutaric acid oxidase an assay procedure

W WILBUR ACKERMANN (introduced by THOMAS FRANCIS, JR.) *Dept of Epidemiology and Virus Laboratory, Univ of Michigan, School of Public Health, Ann Arbor*

An assay procedure was designed specifically for comparing the α -ketoglutaric acid oxidase activity of tissues. A high degree of cell disruption was accomplished by homogenization in isotonic potassium chloride. This resulted in a dilution of the cell contents and inactivation of all the enzymes of the Krebs cycle. The α -ketoglutaric acid oxidase was found to be reactivated by supplementing the homogenate with phosphate and magnesium ions, adenosinetriphosphate, cytochrome C, α -ketoglutaric acid, co-carboxylase, and diphosphopyridinenucleotide. The further oxidation of succinate, the product of the reaction, was prevented by the use of malonate. The rate of the reaction was determined by the oxygen uptake. In this manner it is possible to oxidize α -ketoglutaric acid in a preparation of the whole tissue at which is believed to be the maximum rate. The assay was applied to a survey of various tissues of the mouse. The activities of these tissues were found to vary in the following decreasing order: heart, kidney, liver, brain, spleen, and lung.

Comparison of physico-chemical properties of

isolated skeletal and cardiac myofibrils CHARLES A ASHLEY,* GEORGE M HASS* AND ARMIN SCHICK * *Rush Lab of Pathology, Presbyterian Hospital, in affiliation with Univ of Illinois College of Medicine, Chicago*

Small blocks of skeletal and cardiac muscle were resected from rabbits immediately after death. The muscle was frozen at once with carbon dioxide and cut into thin sections with a freezing microtome. Frozen sections were placed in a solution (0.005%) of crystalline trypsin for about one hour at 0° C. Initial separation of myofibrils from other cellular structure occurred after brief mechanical agitation. Final separation of myofibrils from other constituents of muscle was obtained by differential centrifugation. The final product consisted of large numbers of individual myofibrils. Myofibrils of cardiac and skeletal

origin possessed very similar physico-chemical properties. They contracted irreversibly into small spherical masses in the presence of ATP when the concentration of ATP was greater than 0.0001 molar. The rate of contraction was greatly prolonged by reduction of temperature from 37° C to 0° C and by changes in pH. Fibrils were rendered non-contractile at pH 4 but retained the property of contractility within the range of insolubility of fibrils in phosphate buffer solutions (pH 5–pH 8) of appropriate ionic strength. Solubility of fibrils within near physiological ranges of pH was a function principally of ionic strength and ionic composition of the buffer solutions. At pH 7 in either potassium or sodium phosphate buffer solutions, fibrils were insoluble at intermediate ionic strengths but promptly dissolved at low (0.05 μ) and high (0.5 μ) ionic strengths. The spherical masses resulting from the action of ATP were insoluble in phosphate buffers of pH 7 in the range of ionic strength used (0.005 μ to 0.7 μ).

Experimental study of the virus host cell relationship in fowl pox F B BANG AND G O GEY *

Johns Hopkins Hospital, Baltimore, Md

The growth of the virus of fowl pox in roller tube cultures of chick fibroblasts, epithelium and macrophages has been studied in continuous homologous culture and in some heterologous cells. The virus may be recovered from chick fibroblasts as long as 100 days after inoculation. Any multiplication which takes place under these conditions is unaccompanied by the formation of the classical inclusion bodies. Epithelium derived from lung, cornea and skin was destroyed by the virus and yielded higher titers than the fibroblasts. No large inclusions like those seen overwhelming the epithelium of the chick chorio-allantoic membrane were seen even when the epithelium was set up in slide preparations. It is known, however, from previous work on the development of the inclusions of lymphopathia in roller tube cultures that cells may develop and maintain huge inclusions in such roller tube cultures. Electron microscope sections of the classical fowl pox inclusions show a basic fibrous

structure presumably holding the virus elementary bodies together, thus explaining the rigid nature of these inclusions

Protein loss by complete drainage of intestinal lymph J L BOLLMAN AND E V FLOCK *Division of Experimental Medicine, Mayo Clinic, Rochester, Minn*

Rats cannulated with plastic tubing so that the intestinal lymph is drained externally secrete intestinal lymph equivalent to about one eighth of their body weight daily. The protein lost in this lymph has a higher albumin globulin ratio than plasma, but most of the proteins are plasma proteins in the lymph. The daily loss of protein is about twice that of the total circulating protein (plasma volume \times plasma protein concentration). After this loss is continued 3 or 4 days the plasma protein concentration is reduced about 20% and the plasma volume is also reduced so that the total circulating plasma protein is reduced about 25% in this time. These observations indicate the rapidity with which plasma protein is replaced and also that the intestine (via intestinal lymph) is not a major factor in the formation of plasma protein. Previous studies of intestinal lymph, however, indicated that a specific protein alkaline phosphatase is added to the plasma specifically by the intestinal lymph.

Interrelations between adrenal cortex and lymphoid tissues: growth of adrenal transplants in spleen and thymus with local thymic atrophy EDWARD J BONZE* AND NATHAN B FRIEDMAN *Division of Lab, Cedars of Lebanon Hospital, Los Angeles, Calif*

After bilateral adrenalectomy transplanted fragments of adrenal cortex grew well in both the thymus and spleen of rats. Approximately half of 50 animals survived beyond the 10-day postoperative period. Ninety-five per cent of 20 animals in which a 2-stage bilateral adrenalectomy or unilateral adrenalectomy with transplantation was performed survived. A semblance of normal zonation was established by the regrowth and large sinusoidal blood vessels formed at the periphery. Although no obvious lysis of lymphocytes was noted in either spleen or thymus, the thymic lobules in intimate relation to the proliferating adrenocortical tissue underwent depletion of lymphocytes and extreme atrophy. Peculiar epitheloid cells appeared in the collapsed thymic reticulum. The results suggest that the agent produced by adrenocortical tissue which affects the thymus is probably bound or converted in some way during the course of its activity.

Influence of age on uptake of radioactive phosphate by skeleton of the rat JOHN F BONNER

(introduced by ERIC L ALLING) *Dept of Radiation Biology, Univ of Rochester, School of Medicine and Dentistry, Rochester, N Y*

Radioactive phosphate was administered to rats of 5 age groups from 15 to 170 days. The uptake of labeled phosphate by femur, vertebrae, and skull was determined. The uptake of P^{32} was approximately 5 times as great for the youngest animals (15 days) as for the oldest (170 days). No direct correlation could be obtained with age, weight or the rate of growth of the inorganic portion of bone. Excretion of the radiophosphate was found to have an inverse relation to the bone activity. The highest excretion rate was observed with recently weaned rats.

Use of rat-repletion method for evaluation of amino acid solutions and protein hydrolysates

P R CANNON, L E FRAZIER* AND R HUGHES* *Dept of Pathology, Univ of Chicago, Chicago, Ill*

Data are presented which further demonstrate the usefulness of the rat-repletion method for the evaluation of amino acid solutions and protein hydrolysates. Amino acid solutions or protein hydrolysates were fed in drinking fountains in 5% solutions, designed to yield approximately 200 mg of dietary nitrogen/rat/day. The basal diet supplied 40 non-protein calories/day plus vitamins, salts and roughage. Whenever the fluid component contained an inadequate amount of one or more indispensable amino acids the rats stopped drinking the greater part of the solution, and soon their consumption of the basal ration also diminished. A protein hydrolysate which engendered only fair tissue repletion was markedly improved by the addition to it of a mixture of essential amino acids or by concentration to 70% of original volume. In other words, concentration of ill-smelling constituents did not interfere with its acceptance, but on the contrary improved its utilization. Data are also presented concerning the effects of autoclaving of protein hydrolysates or amino acid solutions in the presence of glucose in relation to amino acid utilization and the Maillard reaction.

Preparation of Ac-globulin free plasma assay of Ac-globulin JOHN R CARTER* AND E D WARNER *Dept of Pathology, State Univ of Iowa, Iowa City*

A method has been devised whereby plasma can be rendered free of Ac-globulin, antithrombin, fibrinogen, and thrombin. The product contains 50-100 units of highly reactive prothrombin. The procedure consists of treating fresh oxalated beef plasma, previously frozen and thawed, with 17 gm/100 cc of kaolin. The kaolin-plasma is then frozen 24 hours, thawed, and centrifuged. The supernatant is treated with equal volumes of

1.5% bichloride of mercury, and insoluble, coagulated egg albumin added. The mixture is then centrifuged, and the supernatant defibrinated, filtered, and stored at -40°C . The amount of Ac-globulin in any given plasma is manifested as a function of 3 variables: 1) the time when conversion begins, 2) the rate of conversion, and 3) the thrombin yield. The quantitative measure of Ac-globulin, which includes the variables, can be represented by an area which is circumscribed by the activation curve and by straight lines through a constant, arbitrarily selected point on each axis. This area can be determined by one of the quadrature methods or with a planimeter, and the amount of Ac-globulin of an unknown can be compared with that of the normal control.

Effect of adrenalectomy and glutathione on x-ray-induced mortality in mice E. P. CRONKITE AND W. H. CHAPMAN *Naval Med Research Inst Bethesda, Md*

The 28-day mortality of mice simultaneously exposed to various doses of 20 mev x-ray was studied on 3 groups of the same age and sex. Group 1 adrenalectomized 10-15 days before exposure and maintained on 1% saline, Group 2 normal control mice, Group 3 injected subcutaneously with glutathione 4 mg/gm immediately before exposure. It is of interest that adrenalectomy 10 days before reduces the total body and hepatic reduced glutathione concentration significantly. The present data indicate that adrenalectomy increases the sensitivity of mice to radiation, this may be related to sulfhydryl levels of tissue. In addition this data refute in part the statement of Straube *et al* (*Proc Soc Exper Biol and Med* 71:539, 1949) "the radiosensitivity of intact or adrenalectomized mice, with or without exogenous adrenal cortical steroids, is similar."

Estimated Percentage Lethal Doses in r from Mortality Dose Curve

	ADRENALECTOMIZED	CONTROL	GLUTATHIONE TREATED
LD ₀	<450	550	550
LD ₅₀	<620	730	840
LD ₁₀₀	<790	940	>1050

Hyperplasia of lymphoid tissues following implantation of cancer cells in resistant hosts JOHN T. ELLIS,* HELENE WALLACE TOOLAN* AND JOHN G. KIDD *Dept of Pathology, New York Hospital—Cornell Med Center, New York City*

The cells of C3H mammary carcinomas and C3H lymphosarcomas regularly proliferate briefly and then regress when implanted subcutaneously in racially-resistant A mice (*Federation Proc* 8:360, 373, 1949). A few days after the tumor cells

are implanted the lymph nodes of the resistant hosts become markedly hyperplastic and exhibit an increase in size and number of the germinal centers (which contain numerous cells in mitosis) and a great thickening of the medullary cords owing to the proliferation in them of elements that seem to be young plasma cells, which later mature. The hyperplasia recedes a short time after the growths regress. The same type of hyperplasia develops even more promptly following implantation of the C3H cancer cells in A mice already immune to them. Furthermore it develops not infrequently after implantation of C3H cancer cells in C3H mice, and it often persists in these hosts as the cancer cells proliferate progressively. The relations of the lymphoid hyperplasia and the resistant state are considered.

Ameboid movement of neoplastic cells H. T. ENTERLINE* AND DALE REX COMAN *Dept of Pathology, Univ of Pennsylvania School of Medicine, Philadelphia*

In previous papers we have attributed the invasive propensities of malignant tumors to ameboid movement of neoplastic cells, which have become detached by loss of their mutual adhesiveness. Although ameboid movement of cancer cells in tissue culture has been described by us and by others, no study has been directed toward determining whether neoplastic cells in general, and especially those of epithelial origin, are capable of ameboid activity. In the present study cinematographic observations were made on a variety of cells in tissue culture. The sources of cells included a variety of human tumors (breast cancer, carcinoma of the kidney, and sarcomas), and 3 animal tumors (Mouse fibrosarcoma 241, Walker rat carcinoma 256, and V₂ carcinoma of rabbits). It was found that the neoplastic cells from all of these tumors displayed active ameboid movement. It was further found that even non-neoplastic glandular epithelial cells (from the human breast) are ameboid, once they become detached from other cells. Of further interest was the observation that even small clusters of from 3-5 cells are able at times to move as a unit. The rates of movement of the cells studied varied from fractions of μ/min to $6\mu/\text{min}$, averaging 1-2 μ/min . Movement was intermittent and no significant differences in rate of movement were found between cells from different tumors.

Influence of steroid hormones on fatty livers EMANUEL FARBER,* DIETER KOCH-WESER* AND HANS POPPER *Hektoen Inst for Med Research and Depts of Pathology, Cook County Hospital and Northwestern Univ Med School, Chicago, Ill*

To elucidate the influence of hormones upon the

development of fatty livers, endocrinological factors in rats intoxicated with ethionine were varied. This analogue of methionine produces a marked fat accumulation in the liver without necrosis or other functional impairment. The fat content of the livers was determined chemically and histologically. Excess fat accumulation was observed by either method only in female but not in male rats. Ovariectomy did not prevent the fatty infiltration, while testosterone administration did. Orchidectomy made the male animals susceptible. Administration of testosterone protected the castrated males. Adrenalectomy prevented fatty livers in female. This suggests the role of testosterone in the prevention of some types of fatty livers.

Histochemical studies on the skeleton in experimental scurvy RICHARD H. FOLLIS, JR. *Dept of Pathology, Johns Hopkins Univ., Baltimore, Md.*

A series of histochemical reactions (*Bull. Johns Hopkins Hosp.* 85:281, 1949) have been carried out on the bones of young guinea pigs placed on a scorbutogenic diet. Some animals were allowed to die of the deficiency, others were treated in time to allow survival in order to study healing sequences. In absolute scorbutus, cytochrome oxidase activity, as qualitatively estimated by the *nad*₁ reaction, is reduced or absent in the cells which make up the *gerustmark*. Alkaline phosphatase activity is completely absent in these cells. In 24-48 hours after treatment intense activity is found in both nuclei and fibers which are being laid down. Similar absence and return is found in the periosteum, as well. The argyrophilic fibers which appear after therapy is instituted are strongly metachromatic.

Comparison of protein and electrolyte changes in tissues during shock from burns and tourniquet trauma CHARLES L. FOX, JR. (introduced by A. R. DOCHETZ) *Dept. of Bacteriology, Columbia Univ. College of Physicians and Surgeons, New York City*

A loss of plasma proteins into injured tissues has been postulated on the basis of the finding of nitrogen in amounts comparable to plasma in the supernatant fluid obtained by centrifuging edematous traumatized tissues. To test whether plasma proteins actually do accumulate in tissues injured by burns or tourniquet trauma, the following experiments were performed. Single hind legs of mice were subjected to the above injuries. When swelling of the injured leg was maximal and shock profound, both hind legs were amputated at the groin and the entire leg analyzed for water, nitrogen, potassium and sodium. Injured legs did not contain more nitrogen than contralateral

uninjured legs although they were 25-75% heavier. If the fluid gained by the edematous injured legs were chiefly plasma, approximately 10-20% (3-8 mg) more nitrogen might be expected in the injured leg. The gain in sodium exceeded the proportionate gain in fluid by 40%, furthermore, the potassium content of these injured legs decreased by an approximately equimolar amount. These data are not in accordance with the view that plasma accumulates in traumatized tissues. They indicate that tissue cells were damaged and exchanged some of their potassium for sodium and that protein-free sodium containing fluid accumulated. Nitrogen previously observed in the supernatant fluid may have been derived chiefly from damaged tissue cells and not from the plasma as assumed.

Fate of transplanted embryonic gonadal tissue into hosts of inbred mouse strains ALFRED GOLDEN *Dept. of Pathology, Veterans Administration Hospital, Buffalo, N. Y.*

Gonadal tissue from about 12-15 day old mouse embryos implanted in the testes of hosts of either of 2 highly inbred mouse strains survived in about $\frac{1}{3}$ of the animals for periods up to 8 months. Of the survivals 25% underwent neoplastic change spontaneously producing small, non-invasive, non-metastasizing multilocular tumors. When methylcholanthrene impregnated wool fibers were implanted in the spleen shortly before or after the transplantation of embryonic gonadal tissue, the ensuing tumors were invasive, but non-metastasizing adenocarcinomas. In these mouse strains methylcholanthrene deposition in the spleen alone produced bulky non-metastasizing fibrosarcomas.

Studies on utilization of antihemophilic factor during clotting JOHN B. GRAHAM,* GEORGE D. PENICK* AND K. M. BRINKHOUS *Dept. of Pathology, Univ. of North Carolina, Chapel Hill*

Transfusion experiments in canine hemophilia, reported last year, showed that normal serum had lost its antihemophilic effect (*Federation Proc.* 8:356, 1949, *J. Exper. Med.* 90:97, 1949). In the present paper studies of the rate of disappearance of the antihemophilic factor (AHF) in clotting dog blood are reported. The method of determining antihemophilic activity consisted of adding standard dilutions of normal plasma or serum samples to freshly drawn whole hemophilic blood. After standing for 30 minutes at 28° C, pH 7.3, residual prothrombin content of the mixtures was determined by the 2-stage method. Free thromboplastin, in contrast to AHF, was determined on each sample by testing the effect on platelet-poor hemophilic plasma. The results indicate that normally AHF is rapidly consumed during and after clotting. Assays of normal sera, 1-3 hours

old, showed only traces of AHF. The rates of disappearance of AHF and of prothrombin during clotting tended to be the same. Ionic calcium, wettable surface and platelets appear to be necessary for a normal rate of utilization of AHF. Plasma allowed to clot in siliconed glassware showed no loss of AHF during the observation period. In plasmas with low platelet levels, AHF disappeared slowly. In citrated plasma freed of fibrinogen by heat coagulation, full antihemophilic activity was present, but on recalcification it rapidly disappeared.

A study of choline deficiency in the rhesus monkey LOUIS D. GREENBERG* AND JAMES F. RINEHART
Div of Pathology, Univ of California School of Medicine, San Francisco

Two rhesus monkeys weighing 2.5 kg, were kept on a modified M-3 diet without choline for 5 months. Because of the presence of sufficient choline precursors (methionine) in the casein of the basal diet, the animals showed no manifestations of choline deficiency at the end of this period and thrived as well as a control animal which was receiving the same diet supplemented with 100 mg of choline citrate daily. For this reason, the casein of the diet was replaced by an equivalent quantity of alpha-protein of soy beans. Within approximately one week after this change the monkeys began to lose weight and have continued to do so. This was followed in 6-8 weeks by the excretion of watery stools, a condition which has persisted to this day. A control animal receiving the same diet supplemented with choline and methionine has gained weight steadily and has remained strong and vigorous. The free and total plasma choline of these monkeys has been followed by microbiological assay during the course of this experiment with the following results. There was no significant alteration in free and total choline of the monkeys on the diet lacking choline while they were receiving casein in the diet. However, the replacement of casein by alpha-protein resulted in a marked decline in the free choline and a significant decrease in the total choline of the plasma.

Effect of penicillin and chloromycetin on oxygen uptake of typhus-infected embryonate eggs DONALD GREIFF* AND HENRY PINKERTON
Depts of Biology and Pathology, St. Louis Univ, St. Louis, Mo

The effect of the injection of 5 mg chloromycetin and 2000 units penicillin G on the oxygen uptake of murine typhus infected embryonate eggs was measured by the technique reported by Greiff and Pinkerton (*J Exper Med* 87:175, 1948). The results obtained are given in the following table.

AGE OF EMBRYOS (DAYS)	UNINJ CONTROLS CC O ₂ /EGG/HR	CHLOROMYCETIN + RICKETTSIAE CC. O ₂ /EGG/HR	PENICILLIN + RICKETTSIAE CC O ₂ /EGG/HR.
5	39	36	45
6		Rickettsiae inoculated	
7	1 33	1 29	1 20
8		Chloromycetin and penicillin-inj	
9	1 68	1 42	2 75 ¹
11	3 08	4 12	5 68 ¹
13	6 32	6 95	7 84 ¹
14	8 50	8 77	10 48 ¹
15	9 93	10 55	11 86 ¹
16	12 30	11 12	10 37
18	17 40	12 90	11 82

¹ Significant increase

The increase in oxygen consumption of the penicillin-injected eggs, as compared with the controls and the lack of such increase in eggs receiving chloromycetin suggest that the rickettsiostatic action of these 2 antibiotics is brought about by different mechanisms. The results are compared with those previously obtained with para-aminobenzoic acid and other reagents.

Immunologic approach to study of treatment of transmitted leukemia in Ak mice P. F. HAHN, E. L. CAROTHERS,* LOUIS BERNARD* AND MARVIN JACKSON *
Meharry Med College, Nashville, Tenn

The Ak strain of mice developed by Furth when inoculated with a spleen brei obtained from mice suffering from leukemia will quite uniformly result in the death of the inoculated animals in a period of from 9-13 days. The Ak strain of mice have been used in this laboratory to study the effect of gold¹⁹³ on the course of such leukemia as well as for the study of various chemotherapeutic agents. Usually, the increased survival time (from 3-10 or more additional days in comparison to the survival time of the average control group) is used to determine the efficacy of the agent employed. In preliminary experiments in which guinea pigs were sensitized by repeated injections of leukemia mouse spleen brei with subsequent treatment of inoculated mice with a serum from such sensitized guinea pigs, it was found that there were very prolonged increases in survival in a few of the mice so treated. In those survivals killed for histological study no evidence of leukemic infiltration was found. Of 70 mice treated with such serum, 5 lived for protracted periods. Only 1 control animal of 289 inoculated survived more than 20 days. That these results can be attributed to chance is felt to have a probability of about 1%. The mice used in these experi-

ments were obtained either from the Sloan-Kettering Institute or Carworth Farms

Experimental cardiovascular disease in monkeys and rabbits TOM R. HAMILTON,* HARRY E. DASCOMB* AND JEROME T. SYVERTON *Dept of Bacteriology and Immunology, Univ of Minnesota, Minneapolis, and Dept of Microbiology, Louisiana State Univ School of Medicine, New Orleans*

In an attempt to learn the reactivity of the cardiovascular system to the constituents of the streptococcus, normal animals and animals with an altered tissue reactivity brought about by hypersensitization with a foreign protein were injected repeatedly with hemolytic streptococci of Group A which had been inactivated *in vitro* by penicillin. Rabbits and monkeys were employed as the host animals. It was learned that streptococci alone or in combination with foreign proteins in normal and hypersensitized hosts were effective in bringing about inflammatory changes in the cardiovascular system. The animals were kept under observation for from 2-11 months, during which time they had repeated injections. The histopathological changes observed ranged from acute necrotizing process, changes suggestive of rheumatic disease with Aschoff-like nodules, to low-grade chronic changes. The most severe reactions in the rabbit were found in the valvular endocardium and the myocardium. Cardiovascular lesions were present in 14 of the 16 monkeys employed. Periarteritis, myocarditis, and valvular endocarditis were more common and the vascular lesions more severe than in rabbits. The group of monkeys that was given streptococci had more pronounced lesions than any other experimental group. Since the results of studies designed to rule out spontaneous cardiovascular disease in this host species were not available, hearts from 16 monkeys dead from accidental or other non-infectious agents were obtained from Cincinnati, Pittsburgh, and Columbia, S. C. The presence of minimal changes limited to a single one of these animals made our findings in the experimental group of more interest.

Effect of esters of linoleic and arachidonic acids in the diet in relation to histologic features of the skin of dogs ARILD E. HANSEN, S. GRANT HOLMES* AND HILDA F. WIESE* *Depts of Pediatrics and Dermatology, Univ of Texas Med Branch, Galveston*

Young dogs showing gross and microscopic changes characteristic for fat deficient animals, at the age of 4 months, were given dietary supplements of methyl linoleate and ethyl arachidonate at the level of 1% of their caloric intake. At the end of 5 weeks, the epidermis had decreased from 6-12 cell layers to about 3 layers in thickness.

There was evidence of a tendency toward orientation of the cells back parallel to the surface. However, a remnant of palisading in the basilar portion was still present. Some prickling remained but few epidermal pegs were present. There was a marked decrease in the stratum granulosum and little evidence of parakeratosis. In the dermis the collagen fibres showed reduced edema and marked regeneration still leaving some cellular infiltration. The intimal cells of the blood vessels were free from edema. The epidermal cells of the hair follicles decreased from 6-8 layers to 2-3 cell layers, hyperkeratinization disappeared. The alveoli of the sebaceous glands returned towards normal. Essentially the same histologic changes were observed at the end of 5 weeks in the skin of the animals given 1% of their calories either as methyl linoleate or ethyl arachidonate. Grossly the skin and hair of both animals were greatly improved, although there was still some evidence of desquamation in the animal receiving arachidonate.

Histologic features of the skin of dogs on a low fat diet ARILD E. HANSEN, S. GRANT HOLMES* AND HILDA F. WIESE* *Depts of Pediatrics and Dermatology, Univ of Texas Med Branch, Galveston*

The skin and hair of dogs receiving 29% of their calories as lard or baconfat appear normal on gross examination. Histologic sections taken from the dorsal surface of the thigh, when stained with hematoxylin-eosin show the following structure: 1) The epidermis is 2-4 cell layers in thickness, the nuclei of the cells are oriented parallel to the surface and no epidermal pegs are present. 2) The cytoplasm appears syncytial, as the intercellular cytoplasmic bridges do not develop upon fixation. 3) The stratum corneum is thin, no nuclei are present in it and no stratum granulosum or stratum lucidum are evident. The dermis consists of sharply demarcated and loosely woven collagen bundles and the blood vessels have normal intimal cells. The hair follicles show an epidermal layer of 2-3 cells with the shafts intact. The sebaceous glands have average alveoli with compact cells. Sections taken from comparable sites of dogs which have been maintained on a low fat diet show distinctly different structures. The epidermis is thickened to 6-12 cell layers, the nuclei of the cells in the first 2-3 layers lie parallel to the surface, however, in the basilar portion the nuclei assume a vertical formation with palisading of cytoplasm and evidence of marked proliferation, driving epidermal pegs into the dermis. Prickling is present. The corneum is tremendously thickened into hyperkeratosis showing parakeratosis with marked disruption of the stratum disjunctum by scaling associated with the development of the stratum granulosum and luci-

dum The dermis is many times thicker than in the normal dog and the collagen although compact and crowded is nevertheless edematous and shows round cell infiltration The intimal cells of the blood vessels are also edematous The hair follicles have layers of 8-12 epidermal cells with papillary formation and hyperkeratinization is evident The alveoli of the sebaceous glands are shrunken with fibroblastic replacement These microscopic alterations are reflected in the gross appearance of the animals as shown by loss of hair, thickened skin and extensive desquamation

Association of mitochondria with the cyclophorase system JOHN W. HARMAN (introduced by D. MURRAY ANGEVINE) *Dept of Pathology, Univ of Wisconsin Med School, Madison*

The cyclophorase system of integrated enzymes obtained from liver, kidney and heart implements the complete oxidation of fatty acids, pyruvic acid and several amino acids by way of the citric acid cycle The suspensions, as thrice washed residue, contain chromosomes, mitochondria and very few ultra-microscopic particles Repeated washing removes completely the ultramicroscopic particles without alteration of oxidative activity Separated chromosomes are inactive, whereas the mitochondria retain the capacity for complete oxidation By use of differential homogenization, in correlation with mitochondrial counts and selective staining of the mitochondria, it is shown that the extent of oxidation is dependent upon both the integrity and number of mitochondria in suspension Both selective stains and phase microscopy reveal a structural complexity which is lost, concomitantly with loss of oxidation when the homogenates are treated with various transforming agents such as deionized water, sodium cholate, dinitrophenol, gramicidin, capryl alcohol and 30% urea Both oxidative phosphorylation and cyclophorase activity are phenomena found only in fractions containing mitochondria It is significant that all transforming agents tested block both processes simultaneously Under the conditions of differential homogenization the locus of various oxidative enzymes is shifted from the mitochondria into the 'microsome' fraction There is evidence that many fine 'microsomal' particles are derived from mitochondria in proportion to the extent of homogenization and that definition of these units is at present arbitrary and operational

Grafting of rabbit lymphatic tissue on chorioallantoic membrane of chick embryo SUSANNA HARRIS,* AND T. N. HARRIS *Children's Hospital of Philadelphia, Dept of Pediatrics, Univ of Pennsylvania School of Medicine, Philadelphia*

In the course of work on the role of lymphatic tissue of the rabbit in the formation of antibodies it was felt desirable to maintain lymph node tissue

outside the rabbit Slices of popliteal lymph node, freshly excised from the rabbit, were placed on the exposed surface of the chorioallantoic membrane (CAM) of developing chick embryos The lymphatic tissue plus the attached chorioallantoic membrane was removed after varying intervals of incubation from 2-10 days and histologic sections were prepared After 4 days it was seen that 1) Vascularization had taken place, extending away from the CAM in the direction of the rabbit tissue with nucleated erythrocytes filling many of the blood vessels 2) The rabbit lymphatic tissue was undergoing degeneration 3) Between the layer of CAM and the lymph node tissue a new cell type appeared which was identified as reticulum cells These cells usually occurred in groups or in a band extending between the CAM and the lymphatic tissue 4) On occasion nests of fresh, mature lymphocytes appeared, always in proximity to the reticulum cells in the direction of the CAM These cells occurred either in round or crescentic nests either at the periphery of the band of reticulum cells or in the midst of them The fresh lymphocytes were never seen in direct proximity to the old, degenerating lymphocytes

Influence of fractions of chick embryonic extracts upon proliferation of chick fibroblasts GEORGE M. HASS, ALBERT SCHWEITZER,* AND HELEN BOSCIA * *Rush Lab of Pathology, Presbyterian Hospital, in affiliation with Univ of Illinois College of Medicine, Chicago*

Small pieces of chick lung were explanted in micro-dishes They were cultivated continuously for 2 weeks *in vitro* in various media The radial growth of fibroblasts was measured periodically The control medium was composed of fresh clotted plasma from young guinea pigs The control medium was supplemented by crude embryonic extract or fractions thereof Crude extract was prepared by extracting crushed 5-7 day chick embryos with Tyrode's solution (1 cc/embryo) Plasma contained growth-promoting factors These accounted for 25% of growth obtained when equal parts of plasma and crude embryonic extract were used The activity of the crude embryonic extract was quantitatively retained on standing at 10° C for at least 7 days Dilution of crude embryonic extract (1:10) reduced its activity to little more than that of plasma Dialysis of crude extract at 10° C for 48 hours against distilled water showed that the non-diffusible, water-soluble fraction contained growth-inhibitory factors The non-diffusible, water-insoluble fraction contained all recovered growth-promoting factors About 50% of this fraction consisted of proteins which could not be redissolved in Tyrode's solution The remainder, which was readily dissolved, possessed 80% of the growth-promoting activity of the initial crude extract

Metabolic alterations in radioactive isotope concentration by malignant tissues induced by hormone pretreatment SAUL HERTZ AND J STEWART ROONEY * *Massachusetts Women's Hospital and Radioactive Isotope Research Inst, Boston*

In the course of study of patients with hopeless metastatic malignant disease of breast and cervix and (bone marrow) myeloma, it was noted that patients who had been treated by current dosage of testosterone had uptakes of P^{32} out of proportion to any such patients who had previously been observed without preliminary testosterone therapy. This led to a controlled study by tracer techniques and radioautographic study of malignant tissue obtained at biopsy and at post-mortem. The data to be presented indicate that such preliminary dosage of patients with these types of malignant disease promotes an increased uptake and retention of P^{32} . This approach to radiotherapeutic application of P^{32} affords a more likely possibility than has the administration of P^{32} to previously untreated subjects. A few results which have been obtained to date by this combination of therapeutic agents will be presented with tracer data on urinary excretion, tissue analysis and radioautographic demonstration of P^{32} in the malignant sites as compared with normal tissue distribution. It is in the direction of the above exemplified effects that the greatest hope of therapeutic application of the radioactive isotopes in cancer lie in our opinion, namely in the induction of increased specific radioactivities in the cancer cells. It is likely that other more potent means of obtaining these effects exist.

Ultra-thin sectioning of tissue for the electron microscope JAMES HILLIER AND MARK GETTNER (introduced by S MUDD) *RCA Labs, Princeton, N J and Sloan-Kettering Inst for Cancer Research, New York City*

Serial sections 0.2 μ thick and showing structure resolution and absence of new artifacts at the 20 m μ level have been cut successfully with a modified Spencer rotary microtome. Osmium-perfused normal mouse liver doubly embedded in collodion and paraffin was used in the development work. The microtome modifications included: 1) A change in the unit of advance from 1 μ to 0.02 μ by a modification of the inclined plane, 2) the addition of a spring to support 90% of the weight of the horizontal member, in order to reduce static friction in the horizontal slides, 3) the addition of a vibration-free motor drive to eliminate distortions of the machine introduced by manual operation, 4) the addition of a liquid trough to the knife edge in order to keep the sections stretched by surface tension, to keep the sections in order and to avoid destruction of the sections by sliding on the facet of the knife. With these modifications, 0.2 μ sections can be cut with a uniformity and accuracy of

better than 5% in thickness. The cut sections floating as a ribbon on the liquid surface are mounted for electron microscopic examination by simply lifting them out on a 200-mesh screen. It is shown that removing the embedding material introduces fine scale artifacts. A number of new but unidentified structures have been revealed in the resulting micrographs.

Further studies on the pathogenesis of experimental necrotizing arteritis RUSSELL L HOLMAN, CARROLL H LIPPARD AND BLAND GIDDINGS * *Dept of Pathology, Louisiana State Univ School of Medicine, New Orleans, and Dept of Biochemistry, Distillation Products, Inc, Rochester, N Y*

Previous studies have shown that, whereas either alone is ineffective, the sequence of 'standard high fat diet' for 8 weeks or longer then 'standard renal insufficiency' is regularly followed by 'typical arterial lesions' in mongrel dogs regardless of age and sex. The pathogenesis of these arterial lesions, which closely resemble those of rheumatic arteritis and periarteritis nodosa, is obscure but is being studied from the standpoints of a 'dietary factor' and a 'renal factor'. Various fatty substances have been bioassayed for the dietary factor by keeping the 'standard diet'—composed of liver, sugar, starch, tomato juice, salt mixture and fatty substance—isocaloric (75 calories/kg/day, 43% fat, 7% protein, and 50% carbohydrate) and varying only the fatty substance. The following fatty substances have yielded negative results: corn oil, lard, olive oil, coconut oil, mutton tallow and oleomargarine. Positive results have been obtained with certain samples of cod liver oil (in 36 of 40 dogs) and with creamery butter (in 8 of 8 dogs). Studies to date indicate that the renal factor (standard renal insufficiency has been produced by uranium nitrate, mercuric chloride, or bilateral nephrectomy but not by bilateral ureteral ligation) is an, as yet, undetermined metabolic product of renal tubular epithelium. The typical arterial lesions can be retarded or prevented by the addition of vitamin E or cholesterol, by omitting the noxious fatty substance for 4 weeks or longer, or by substituting casein for liver in the standard high fat diet. Conjectures on pathogenesis will be hazarded.

Effect of aureomycin and antibiotics on whole body irradiation JOE W HOWLAND, FRANK FURTH AND M COULTER (introduced by FRIEDA S ROBBINS) *Dept of Radiation Biology and Division of Med Services, Atomic Energy Project, Univ of Rochester School of Medicine and Dentistry, Rochester, N Y*

Results are presented on the exposure of more than 1200 rats and 18 dogs to lethal doses of filtered 250 KVP γ -radiation. Clinical doses of aureomycin or other combined antibiotics given to rats

after lethal x-radiation result in almost complete alleviation of the diarrheal state. Survival of life is prolonged an additional 5-7 days. Animals die of a sudden hemorrhagic death. Pre-radiation weight may be regained as early as 4 days after exposure. Bacteriological studies show suppression of normally occurring gram negative organisms in the intestinal tract. Dogs treated with only clinical doses of aureomycin (equivalent to 50-100 mg/kg) or combined aureomycin-streptomycin have shown no evidence of radiation sickness. Occasional bleeding tendencies are associated only with local trauma. Survival of life is prolonged from 10-14 days. Deaths in control animals occur between the 10th and 14th days, in treated animals, after the 22nd day. Mortality in the dog is considerably improved. To date all dying treated animals have shown sepsis with aureomycin or streptomycin resistant organisms 48 hours before death, with the same organism being cultured post mortem from all tissues examined. Pathological findings in the treated animals have not been in general typical of radiation disease. Lesions of the gastro-intestinal canal have not been observed in the treated animals. Bacteriological observations indicate a general alteration in the bacterial flora of the intestinal canal with a suppression of gram-negative organisms.

A possible new factor distilled from lard? HANS KAUNITZ AND CHARLES A. SLANETZ* (with the technical assistance of RUTH ELLEN JOHNSON*)
Depts. of Pathology and Animal Care, College of Physicians and Surgeons, Columbia Univ., New York City

Weanling rats were placed on a simplified diet containing all essential factors. The residue fraction of lard distilled at 215°C and 10⁻³ mm pressure (Distillation Products Inc.) served as source of fat. Gradually a disease developed characterized by weight deficit, roughened fur, eye symptoms and dehydration resulting in death after 3-8 months. Pregnancies terminated in death at parturition, live young frequently being found in the uterus. Epithelial lesions like those in vitamin A deficiency were observed. They were frequently accompanied by severe infections, especially of the urinary tract (pathology by Herbert Stoerk). Rats on the diet with undistilled lard were normal. Aversion to the diet and improper fat absorption were ruled out by determination of the food intake and fecal fat excretion. Toxicity of the residue seems improbable because addition of the distillate prevented the disease. Dried yeast or large doses of beta-carotene (Barnett Laboratories) or alpha-tocopherol (Hoffmann-La Roche) gave protection. Linoleic acid and water-soluble vitamins had no effect. It is improbable that the condition is caused only by oxidative destruction

of known factors by the lard residue inasmuch as addition of the distillate even 1-2 weeks after preparation of the diet also seemed to afford protection. It is therefore possible that the absence of an unknown factor is at least partly responsible for the condition.

Experimental cholesterol atherosclerosis in bilaterally adrenalectomized rabbits AARON KELLNER AND JAMES W. CORRELL* *Dept. of Pathology, New York Hospital-Cornell Med Center, New York City*

Cholesterol-fed rabbits with atherosclerosis were found to have adrenal glands which had a greatly increased lipid content and which were more than twice as heavy as those of normal animals. To determine whether this increased lipid reflected an alteration in the production of adrenal hormones that might play a role in the pathogenesis of atherosclerosis, a group of 8 rabbits was subjected to total bilateral adrenalectomy and then fed a high-cholesterol diet for periods of 2-5 months. The animals were maintained in good health by daily injection of desoxycorticosterone acetate (DCA) in peanut oil, 0.2 mg/kg, and sodium chloride was added to their drinking water. The completeness of the adrenalectomy was verified at the end of the experiment by the fact that every animal died within 26 days after the cessation of DCA injections, and by the failure to find any adrenal tissue at post-mortem examination. The adrenalectomized rabbits fed cholesterol developed significant hypercholesterolemia, although their blood cholesterol levels were somewhat lower than those of normal controls fed comparable amounts of cholesterol. All the adrenalectomized rabbits developed atherosclerosis of the aorta which in extent and severity was equal to that of the controls. It would, therefore, appear that the adrenal hormones, with the exception of the mineral regulating function of DCA, are not essential for the development of experimental cholesterol atherosclerosis in the rabbit.

Properties of nitrogen mustard protein reaction products A. R. KELLY,* GEORGE H. MANGUN,* BENJAMIN E. SANDERS* AND FRANK W. HARTMAN
Dept. of Labs., Henry Ford Hospital, Detroit, Mich.

Experiments on the nitrogen mustard, CH₃N-(CH₂CH₂Cl)₂·HCl, (HN2) sterilization of blood plasma indicate that, under certain conditions, HN2 couples with plasma proteins to form transformation products with heretofore unrecognized leukopenic activity. These substances, collectively called 'protein-imine' have been investigated chemically and pharmacologically and a colorimetric method developed for their detection. As a result of these studies, the HN2 plasma sterilization process, introduced by Hartman and Mangun,

has been modified to insure that inadvertent stabilization of toxic amounts of 'protein-immune' cannot occur. 'Protein-immune' is non-dialyzable, non-ultrafiltrable, and may accumulate in plasma under conditions which permit the complete degradation of unbound HN2. Protein fractionation following treatment of plasma with 500 mg/l of HN2 indicates that albumin contains approximately 56%, Fraction IV, 29% and fibrinogen, 6% of the total 'protein-immunes' found in all fractions. Intravenous and intraperitoneal injections of plasma, prepared to contain high concentrations of 'protein-immune,' produce neutropenia in mice with typical hypoplasia of bone marrow and lymphatic tissue. Young, protein-depleted animals are especially susceptible to this leukotoxic effect. However, in mice, significant toxicity and leukopenic activity of HN2 treated plasma is demonstrable only following repeated injections of 'protein-immune' equivalent to 5 times the usual transfusion volume of plasma treated with 4 times the usual sterilizing dosage of HN2, with stabilization of the active material at low temperatures and pH.

^{I¹³¹} as antigen label in circulating serum of non-immune rabbits WILLIAM C KNOX (introduced by KENNETH M ENDICOTT) *Natl Insts of Health, Bethesda, Md*

Bovine serum albumin (Armour) was labelled by direct iodination with ^{I¹³¹} to the extent of approximately 2 atoms of iodine/molecule of protein. Each of a series of 6 rabbits was given the labelled protein intravenously. Serum samples were obtained at time intervals beginning at 6 minutes and extending to 12 days following the time of injection. The antigen contents of the sera were estimated by both the quantitative precipitin tests and by measurement of the ^{I¹³¹}. On most of the samples, the 2 values were in good agreement. Each of a second series of 5 rabbits was given native bovine serum albumin intravenously. Serum samples were obtained as in the previous series and the antigen contents determined by the quantitative precipitin test. The labelled protein and native protein cleared in essentially the same manner.

Histological and biochemical changes in fatty livers with and without necrosis DIETER KOCH-WESER,* EMMANUEL FARBER,* PAUL B SZANTO* AND HANS POPPER *Hektoen Inst for Med Research and Depts of Pathology, Cook County Hospital and Northwestern Univ Med School, Chicago, Ill*

An attempt was made to investigate the pathogenesis of fatty liver with and without necrosis. The metabolic and morphologic alterations caused by CCl₄ and ethionine were followed in sequence and correlated with each other. Female rats fasted

for 12 hours were injected intraperitoneally with one dose of CCl₄ or ethionine and sacrificed at intervals from 2 to 240 hours thereafter. Histological preparations were stained with hematoxylin-eosin, Sudan IV and methyl-green-pyronine, the latter for the study of ribose nucleic acid distribution. Total lipids, phospholipids, cholesterol, total nitrogen, nucleic acids, acid soluble phosphorus, esterase, alkaline phosphatase and water were determined chemically in the liver and esterase, alkaline phosphatase, phospholipids and bilirubin in the blood. Ethionine produced a very marked fat accumulation in the liver without other structural or biochemical changes. The fatty infiltration appeared later and persisted longer than that produced by CCl₄. A few hours after administration of CCl₄, excess fat appeared prior to enzyme changes or morphologic necrobiosis. Later centrilobular necrosis and chemical changes were found. At that time the most constant biochemical changes were a sharp drop in liver esterase, coincident with the onset of necrosis, increase in total water content and neutral fat with the total phospholipids remaining constant. Biphasic blood changes accompanied the chemical and morphological alterations in the liver. The centrilobular disappearance of pyroninophilia is not accompanied by chemical decrease of ribose nucleic acid. It appears that the processes leading to necrobiosis and to fatty changes are not necessarily interdependent.

Role of aciduria in the development of hemoglobinuric nephrosis in dehydrated rabbits JOSEPH J LALICH AND SEYMOUR SCHWARTZ* *Dept of Pathology, Univ of Wisconsin Med School, Madison*

The influence of acid diets when combined with 5 days of food and water deprivation on the development of hemoglobinuric nephrosis was determined in rabbits. Three of 11 control animals died when ground oats, or oats and alfalfa, were followed by 5 days without food and water. Two of 3 rabbits which died had either necrosis of renal tubules or the liver. In tissues from 8 surviving rabbits we observed hyaline casts in the collecting tubules, moderate to severe swelling and vacuolization of epithelial cells in the proximal convoluted tubules and interstitial medullary edema. Eighteen test rabbits were given 18 gm/kg of homologous hemoglobin intravenously in divided doses. Eleven of 18 animals died of hemoglobinuric nephrosis. All rabbits with fatal hemoglobinuric nephrosis had variable increases in kidney weight with numerous pigment casts, while 9 had elevations of non-protein nitrogen (NPN) in excess of 190 mg %. The pigment casts were principally in the distal convoluted tubules. Associated with pigment casts accumulation there was focal necrosis of tubules in 5 and moderate to

severe degeneration of tubular epithelium in 6. All of these animals also had interstitial medullary edema. In 4 rabbits which survived and 3 which died of other causes, elevations of NPN, increases of kidney weight and pigment cast precipitation were minimal or absent. Experimental evidence, therefore, suggests that pre-injection acid diets and dehydration produce an antecedent tubular injury which predisposes to the accumulation of pigment casts, uremia and death.

Transmissible papilloma in monkeys BALDUIN LUCKÉ, HERBERT RATCLIFFE* AND CHARLES BREEDIS *Dept of Pathology, Univ of Pennsylvania School of Medicine, and Penrose Research Lab, Philadelphia Zoological Garden, Philadelphia, Pa*

Tumors in monkeys are relatively rare, and there is no record of such growths having been transmitted. Hence the occurrence of a spontaneous papilloma in a brown Cebus monkey led to attempts to transmit the growth, first to the same animal, and later to monkeys of the same and of other species. In 11 out of 13 cases the experiments were successful. When freshly prepared cell suspensions were inoculated into areas of scarified skin, new papillomas developed. Suspensions of frozen or of glycerinated material gave similar results. These growths were in turn transmissible to other monkeys. The causal agent of the papillomas appears not to be species specific, since the growths can be transmitted from New World monkeys to Old World monkeys. In all animals the lesions first appear as distinctly hyperemic patches within 2 weeks after inoculation, the epiderm and its supporting tissue in these areas gradually become raised, corrugated, and eventually form isolated or confluent projections up to 15 mm in height. To date (8 months after the beginning of the experiments) there has been no evidence of invasiveness, and most of the growths have regressed within from 4-6 months. Attempts are now being made to transmit the papilloma to animals other than monkeys.

Deficiency of alpha cells of pancreas as possible etiological factor in familial hypoglycemia IRVINE MCQUARRIE, E T BELL, BERNARD ZIMMERMANN* AND W S WRIGHT* *Univ of Minnesota, Minneapolis*

It has been demonstrated experimentally by previous workers that the alpha cells of the islets of Langerhans exert an anti-insulin or hyperglycemic (glycogenolytic) effect. The present report deals with metabolic and histologic studies on a small series of patients suffering from spontaneous hypoglycemia in whom absence of pancreatic alpha cells appears to be the most likely etiological factor. Special tests for dysfunction of the adrenal cortex, the adenohypophysis and the liver were

negative. No evidence of adenoma or hyperplasia of the beta cells of the pancreatic islets could be found in those patients in whom partial pancreatectomy was performed. However, examination of sections of the pancreas stained with Gomori's special stain unexpectedly revealed the total or almost total absence of alpha cells. Cytoplasmic granules in the beta cells were greatly diminished in number. That the patients showing these unusual characteristics represented a heretofore unrecognized endocrinological entity is suggested by the familial occurrence of the syndrome and the initial appearance of symptoms during the first months of life. Adrenocorticotrophic hormone was found to be highly effective in control of the hypoglycemia.

Nitrogen, phosphorus and calcium metabolism in the rachitic rat given excessive doses of vitamin A CHARLOTTE L MADDOCK* AND S B WOLBACH *Division of Nutritional Research, The Children's Hospital, Boston, Mass*

Because of the problems presented by the fact that excessive administration of vitamin A results in rapid repair of the rachitic metaphysis, with resumption of calcification of cartilage matrix and osteoid (WOLBACH AND MADDOCK *Federation Proc* 8:376, 1949), a study of the metabolism of the rachitic rat before and after excessive vitamin A administration was instituted, in the hope of throwing some light on the manner of action of this vitamin. Doses were 1000 IU per gram of body weight. Diets used were the one previously reported on, suggested by Dr Otto A Bessey, and and this diet modified by substitution of fibrin for casein with consequent reduced phosphorus content. Nitrogen, phosphorus and calcium metabolisms were primarily studied. The results indicate that the animal is thrown into negative nitrogen balance at a time when it is still consuming food, though in reduced amounts, that it excretes larger amounts of calcium than be accounted for by food intake, and that there is a shift in calcium excretion from urinary to fecal channels. Initial urinary phosphorus excretion, usually under 1 mgm per 24 hours, may increase terminally 20- to 50-fold. Combined fecal and urinary phosphorus is much in excess of food intake, so that a marked negative phosphorus balance occurs very shortly after giving the vitamin. Companion studies on starving rachitic animals do not give the same picture. The results suggest that vitamin A acts by speeding up metabolic sequences.

Coagulation defect of vitamin K deficiency compared with that caused by Dicumarol F D MANN,* J D MANN* AND J L BOLLMAN *Inst of Experimental Medicine and Division of Clinical Labs, Mayo Clinic, Rochester Minn*

Vitamin K deficiency was produced in rats by total external drainage of the intestinal lymph. By means of two-stage prothrombin determinations and co-thromboplastin assays, the resulting coagulation defect was compared to that produced by Dicumarol in comparable animals and at about the same rate. Dicumarol decreased co-thromboplastin activity much more than prothrombin while vitamin K deficiency produced greater decreases in prothrombin than in co-thromboplastin. Recognizing that the exact interpretation of these (or any other) coagulation tests may be subject to future modification, they do appear to demonstrate a difference between vitamin K deficiency and the effects of Dicumarol under essentially comparable experimental conditions.

Bone and tooth lesions in the rat resulting from ingestion and intraperitoneal administration of beryllium compounds E. A. MAYNARD,* W. L. DOWNS* AND J. K. SCOTT *Dept. of Radiation Biology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

Weanling rats fed a diet containing 5% beryllium sulphate or beryllium carbonate developed rachitic lesions of the bone within 3 weeks from the beginning of treatment. After removal of the beryllium compounds from the diet the epiphyseal line and metaphysis slowly returned to normal. However, the original metaphysis (the 'primary spongiosa') was not resorbed, as would be expected with Steenbock diet rickets, but remained at its original position in the shaft for approximately a year. A new zone of provisional ossification ('secondary spongiosa') appeared and the long bones increased in length as expected. Beryllium (approximately 11 $\mu\text{g}/\text{gm}$) was present in the bone 280 days after removal of beryllium compounds from the diet. Overgrowth of incisors occurred within 2 months after removal of beryllium sulfate from the diet. Within a period of 3 weeks following the 1 p administration of a single dose of beryllium carbonate, or oxide (3000 mg/kg body weight) there appeared zones of hyperossification in the areas of active bone growth.

Further studies on thermostable leucocytosis factor of exudates VALY MENKIN *Agnes Barr Chase Fndn. for Cancer Research, Temple Univ. School of Medicine, Philadelphia, Pa.*

Earlier studies have demonstrated in exudates a thermostable leucocytosis-promoting factor (LPF). Subsequent and recent studies have shown that in exudates, particularly acid in character, in association with the pyrogenic factor, pyrexin, there is also a thermostable leucocytosis factor. It is these 2 factors which offer a reasonable explanation for the mechanism of leucocytosis with inflammation. When pyrexin, the pyrogenic factor obtained from the euglobulin fraction, par-

ticularly from acid exudates, is treated with $(\text{NH}_2)_2\text{SO}_4$ at half saturation and then dialyzed, a crude material results containing some amorphous pyrexin, the leukopenic factor, and the thermostable leucocytosis factor. This crude material is thoroughly centrifuged, the resulting supernatant fluid is diluted with distilled water (1:1) and poured into a tube which is sealed. This sealed tube is then placed in water boiling for 17-18 hours. The sealed tube is withdrawn. It is slightly cooled and needle-like crystals form. These crystals are collected. They induce only leucocytosis. They produce neither fever nor leukopenia. These crystals seem to represent the thermostable leucocytosis factor by itself. The crystalline material is not a polypeptide, but it is either a carbohydrate or at least it contains a carbohydrate group as indicated by the Molisch test. The material induces a leucocytosis by causing also a discharge into the circulation of a large number of immature granulocytes. The fact that the thermolabile leucocytosis factor (LPF) seems to be a polypeptide whereas the thermostable factor shows no evidence of being such a structure favors the view that the 2 leucocytosis factors are distinct entities.

Agglutination of bacteria by living white blood cells ERIC REISS,* ELIZABETH MERTENS* AND WILLIAM E. EHRLICH *Philadelphia General Hospital and Graduate School of Medicine, Univ. of Pennsylvania, Philadelphia*

Rabbits were immunized by injecting various bacteria into the pad of their hind feet. When antibody formation was in progress, the popliteal lymph nodes were removed and their cells studied in supravital preparations. It was found that certain cells of the nodes agglutinated the bacteria with which the animals were immunized. Photos of this phenomenon will be shown, and its significance in connection with the cellular sources of antibody will be discussed.

Neoplasms in rats treated with pituitary growth hormone I. Pulmonary and lymphatic tissues HENRY D. MOON,* MIRIAM E. SIMPSON, CHOH HAO LI AND HERBERT M. EVANS *Division of Pathology, Univ. of California School of Medicine, San Francisco, and Inst. Experimental Biology, Univ. of California, Berkeley, Veterans Admin. Hospital, San Francisco*

A group of 15 plateaued female rats was injected with pituitary growth hormone for periods up to 485 days. The controls received daily injections of albumin. The experimental rats responded to the injections of growth hormone by continuous growth. Many neoplasms of various types occurred in the rats injected with growth hormone. This paper is restricted to the neoplastic and hyperplastic changes involving pulmonary and lymphatic organs. Lymphosarcomas of the lung occurred in

6 of 15 rats injected with pituitary growth hormone whereas no similar tumors occurred in the control rats. In all rats treated with growth hormone there was hyperplasia of peribronchial lymphoid tissue. The tumors appeared to originate in such hyperplastic tissue.

Influence of crystalline vitamin B₁₂ on carbon tetrachloride poisoning CHARLES W. MUSHETT
Merck Inst for Therapeutic Research, Rahway, N. J.

In confirmation of results obtained by Popper and associates (*Proc Soc Exper Biol & Med* 71:688, 1949) with vitamin B₁₂ concentrate, it has been found that crystalline vitamin B₁₂ exerts a protective effect against the fatty metamorphosis, hydropic change and depletion of cytoplasmic ribonucleic acid in the livers of rats given a single oral dose of carbon tetrachloride. However, when both carbon tetrachloride and vitamin B₁₂ were given daily for a period of 3 weeks, vitamin B₁₂ failed to prevent the hepatic changes even at a dose level of 200 µg. In fact, the livers of the B₁₂-treated rats were relatively larger and contained more lipid than those of rats given carbon tetrachloride alone. Despite its inability to protect the livers of the poisoned rats, vitamin B₁₂, even at low doses, enabled the treated animals to grow at a considerably greater rate than the rats not treated with the vitamin. An explanation for the growth effect was found not to be due to differences in renal damage since this was negligible in both groups. In dogs given repeated doses of carbon tetrachloride, vitamin B₁₂ failed to show a protective effect during life as judged by morphologic blood picture, prothrombin time, icteric index and blood biochemical determinations. The continuation of vitamin B₁₂ treatment, however, after cessation of carbon tetrachloride dosing apparently brought about a more rapid reversibility of hepatic changes as indicated by gross and microscopic examination of the livers of the dogs.

Comparison of several chlorinated hydrocarbon insecticides with respect to pathological changes produced in rats by feeding at low levels, 1 to 100 ppm A. A. NELSON, O. G. FITZHUGH AND G. WOODARD *
Division of Pharmacology, Food and Drug Administration, FSA, Washington, D. C.

For this presentation only the chronic (1-2 years) feeding to rats of 100 ppm or less of certain commercially important chlorinated insecticides in the diet is considered. DDT, TDE, chlordane, toxaphene, methoxychlor, technical benzene hexachloride (BHC), and its alpha, beta and gamma isomers were fed, using the same stock diet and, except for BHC, the same rat strain. Preliminary observations show beta BHC and chlordane causing the greatest total damage, liver weight was increased at 10 or more ppm, while chlordane also

caused some kidney damage, and beta BHC at 100 ppm depressed growth. Alpha, gamma and technical BHC, and DDT, all required 50 or 100 ppm to increase liver weight, with only gamma BHC possibly affecting the kidneys. Data on TDE and toxaphene are less complete, but indications are that their effects are somewhat less than in the previous group. Methoxychlor showed no effect whatever at 100 ppm. In rodents only, these compounds (except methoxychlor) cause varying degrees of hepatic cell enlargement, with peripheral migration of granules. Minimal degrees of this effect, which is characteristic but not necessarily the most damaging and reaches a plateau after several weeks, were evident from as little as 5 ppm of DDT and chlordane. The significance of the results presented becomes apparent when it is realized that our human diet does contain small amounts of these chemicals, and that if man reacts as does the rat, the factor of safety is not inordinately high.

Generalized metastatic calcification combined with vascular changes produced by diet DOROTHY NELSON,* ANDREW C. IVY, PAUL B. SZANTO* AND HANS POPPER
Dept of Clinical Science of Univ of Illinois College of Medicine and Heltoen Inst for Med Research, Chicago, Ill.

Guinea pigs were placed on 4 diets for prolonged periods. Group I received milk with rabbit pellets and lettuce, group II received the same diet for 16 months, egg yolk being added for the first four, group III received milk and a semisynthetic diet designed to promote optimal growth. Its constituents apparently pertinent to the results obtained were 30% casein, 2% fat, 144 U.S.P. units of vitamin D and daily 20 mg of ascorbic acid. Group IV received the same diet as III plus cholesterol or egg yolk. The maximum amount of cholesterol ingested by groups II and IV was 100 mg daily. In group I only one animal, maintained for 3 years on the diet, showed vascular changes and metastatic calcification in kidney and adrenal. Group II showed vascular changes and minimal or no calcification. Group III presented extensive calcification in kidney, muscle, skin, stomach and adrenal. In the aorta the vasa vasorum contained lipophages, the intima was thickened, the internal elastic membrane was calcified and in the media the elastica was replaced by fibroblasts. The coronary arteries were partially or completely occluded by lipophages resulting in myocardial necrosis. The liver revealed fatty changes and cirrhosis. Group IV showed similar changes with more severe involvement of cardio-vascular system and liver. It is assumed that the metastatic calcification is explained by the mineral imbalance of these diets while the hepatic and vascular changes are secondary to the excess of cholesterol.

Determination of opsonins for *Br suis* ABOUT D POLLACK* AND JOSEPH VICTOR *Biological Dept, Chemical Corps, Camp Detrick, Md*

Phagocytosis of *Br suis* by blood leucocytes was investigated. Neutrophils were more active than mononuclear or eosinophilic leucocytes. Citrate, unlike heparin, inhibited phagocytosis. Most uniform phagocytosis occurred with agitation at 37°C for 30 minutes. Formalin killed organisms were phagocytosed to the same degree as living ones. Repeated washing of blood cells as frequently as 20 times in a modified Krebs solution containing 3% Knox gelatin P 20 did not impair phagocytic activity. On the basis of these findings a method was developed for determination of opsonin titre in heparinized blood. Cells and plasma were separated. Cells were washed 6 times in the Krebs gelatin solution, each time with 5-10 fold volumes of fluid. Plasma was diluted with Krebs gelatin solution. Cells were mixed with equal volumes of diluted plasma, 0.1 ml of the mixture was added to 0.1 ml of Krebs gelatin solution containing 1×10^9 formalin killed organisms. The mixture of blood cells, diluted plasma and bacteria were rotated 24-36 times each minute at 37°C for 30 minutes. Smears were made on glass slides warmed to facilitate spreading of the mixture. On the basis of statistical analysis of data, opsonin titre was defined as the lowest concentration of plasma in which 94-100% of neutrophils phagocytosed bacteria. The titre of normal human blood was slightly higher than that of dog. In clinical brucellosis, the titre may increase more than 100,000,-000 fold.

Pathogenesis of hypertension induced by renal constriction L J RATHER (introduced by A J Cox) *Dept of Pathology, Stanford Univ School of Medicine, San Francisco, Calif*

Blood pressures of 21 male albino rats were determined with the tail microphonic sphygmomanometer. All rats were then operated on within a 2-hour period, 11 being subjected to one stage unilateral nephrectomy plus contralateral figure-of-eight silk ligature renal constriction, 10 to unilateral nephrectomy and handling of the contralateral kidney. Blood pressures were determined on 20 occasions during the 50 days after the operation. The rats were then exsanguinated from the aorta under ether anesthesia and determinations made of blood creatinine, urea and hematocrit level. Heart and kidney tissue was weighed according to a uniform procedure. A third group of 8 rats was studied in a similar manner before and after removal of 75% of total renal tissue. Mean lines and frequency distribution curves of blood pressure were subjected to statistical analysis. A definitely significant increase in the mean of the measured pressures was observed in the constricted kidney

group within 4 days post-operatively and a probably significant increase within 2 days. In the rats with loss of 50% and 75% of renal tissue there was no post-operative hypertension. These experiments show, 1) the early onset of hypertension after renal constriction, 2) the absence of such hypertension in rats with loss of 50% and 75% of renal tissue, 3) the independence of these effects from the total mass of functioning renal tissue, 4) the independence of the development of hypertension from the development of fibrous constrictive perinephritis.

Sickling processes in anemia and trait erythrocytes with the electron microscopy of their incipient crystallization J W REBUCK, R M STURROCK AND E A MONAGHAN (introduced by F W HARTMAN) *Dept of Laboratories, Henry Ford Hospital, Detroit, Mich*

Sickling was studied dynamically in timed stages through progressive anoxia of erythrocytes from 3 cases each of sickle cell anemia and sickle cell trait. Formalin fixation arrested the processes at many stages in the transformation of promenisocytes to menisocytes. The early stages of sickling presented marked structural modifications of the hemoglobin-containing corpuscular interior leading to major structural patterns. Focal, eccentric thinning of the disc eventually included the entire corpuscular center as the promenisocyte diameter increased. In the thinned center, patchy hemoglobin aggregation and incipient intracellular crystallization were present. When aggregation of hemoglobin about the entire corpuscular rim was accompanied by peripheral angulation, the 'hollywreath' form appeared, if aggregation about the corpuscular rim was incomplete, an appearance of rupture of the rim was obtained. Anisotropoid angulation of central aggregates progressed to eccentric massing of the hemoglobin at one or more areas within the cell. Such hemoglobin-containing, intracellular masses presented intense peripheral spiculation further suggestive of incipient crystallization. As expected, trait cells with only a partial content of Pauling's defective hemoglobin presented far less spiculation than anemia cells containing defective hemoglobin alone, although both cell types eventuated in complete sickling. When Ponder's expansive forces were not operative early in a planoparallel direction, aggregation was tridimensional, central thinning and anisotropoid changes were less apparent, and incipient crystallization was evidenced only in peripheral spiculation or in geometric configuration of the early menisocyte. Unipolar filamentation and unipolar sickling which sometimes occurred substantiated the concept of incipient crystallization.

Effect of thallium, lead and other metals on development of chick embryo I. P. RIDGWAY, P. A. PATTERSON AND D. A. KARNOFSKY (introduced by C. P. RHODES) *Division of Experimental Chemotherapy, Sloan-Kettering Inst. for Cancer Research, New York City*

The effects of various metals on embryonic development were tested by injecting them into the yolk sac of the 4 day old chick embryo. *Thallium sulfate* produced a severe degree of achondroplasia at a dose of 1 mg/egg, and the embryos usually survived to hatching, although they failed to hatch. These embryos, at term, had parrot beaks, small heads, abnormal eyes and brains, presumably due to compression of these organs by the failure of skull growth, thick trunks and shortened extremities. The achondroplastic embryos were similar in appearance to those reported in hereditary chondrodystrophy, and in certain nutritional deficiencies. *Lead nitrate* was toxic to the 4 day embryo at 0.2 mg/egg, and it produced a remarkable hydrocephalic condition, with atrophic changes in the brain. A number of the embryos survived to hatching, although they failed to hatch. The simultaneous injection of effective doses of thallium and lead produced achondroplasia and hydrocephalus in the same embryo. Further studies on the localization of these elements and their mechanism of action in the chick embryo are under study. Twenty-six other metals have been injected into the chick embryo, but specific morphological changes have not occurred consistently. Their toxicity and effects on the development of the chick embryo will be presented.

Radioactive phosphorus studies in neoplastic disease J. STEWART ROONEY* AND SAUL HERTZ *Massachusetts Women's Hospital and Radioactive Isotope Research Inst., Boston*

P^{32} balances, external Geiger-Mueller distribution, and radioautographic demonstration of correlated studies on a variety of cancer patients following P^{32} administration will be discussed. The data to date in these experiments indicate that P^{32} uptake of certain tumor types can be modified by pretreatment of the patients by means of large dosage of steroid hormones and by the administration of ACTH. The clinical significance of these observations and the related therapeutic trials being carried out will be discussed in the exhibit planned in association with this presentation. A 'windfall' finding in the course of these studies is the demonstration that the thyroid gland is a site of P^{32} concentration. External Geiger-Mueller studies and radioautographs of P^{32} distribution in the normal thyroid will be demonstrated.

In vitro change of streptococcus from pneumotropic to neurotropic type and in vivo genesis of

filtrable pneumotropic and neurotropic phases of streptococcus EDWARD C. ROSENOW *Bacteriologic Research, Longview Hospital, Cincinnati, Ohio*

The strain of streptococcus studied was isolated from outdoor air in winter during a severe epidemic of influenza currently considered as due to Virus X. A pure culture in dextrose-brain broth from a single colony on blood agar after 3 consecutive serial dilution cultures representing a dilution of original inoculum of at least 10^{-22} was inoculated intracerebrally and/or nasally January 10 into mice and into a tube of chick-embryo medium. The chick-embryo culture was incubated at 35°C for 24 hours, then stored at room temperature in the dark until July 2. Pneumonitis developed in high incidence in the mice receiving the streptococcus. Emulsions and filtrates of emulsions of pneumonitic lungs or of the brain were then passed successively through 9 series of mice. Pneumonitis developed in increasing incidence both in those receiving emulsions and filtrates. The streptococcus isolated from the stored chick-embryo culture had lost affinity for the lungs and produced encephalomyelitis in high incidence. Emulsions and filtrates of emulsions of the encephalomyelitic brains were then passed through 12 series of mice. The incidence of encephalomyelitis increased greatly on successive passages irrespective of whether emulsions or filtrates of emulsions were inoculated. The filtrates which produced pneumonitis and encephalomyelitis respectively on successive mouse passages usually proved sterile but corresponding cultures of emulsions of pneumonitic and encephalomyelitic brains respectively yielded the streptococcus true to type in pure culture in high incidence. Control experiments proved negative. The 2 types of streptococci were agglutinated differentially by respective antisera and thermal antibody and convalescent sera.

Biology of granulomatous inflammation experimental spermatic granulomas MURRAY RUSSELL* AND NATHAN B. FRIEDMAN *Division of Labs, Cedars of Lebanon Hospital, Los Angeles, Calif.*

Previous work (*J. Urol.* 62:363, 1949) has shown that a peculiar granulomatous response results in response to extravasation of sperm from various genital ducts. Similar granulomas have been produced experimentally in rats by severing the vas deferens and permitting leakage of sperm into the surrounding tissues. Granulomas are not produced if previous atrophy of the testes is induced by intra-abdominal transfer. Some granulomas form in and about dilated tubules just as do those which occur spontaneously in spermatocele in man. Histologic studies suggest that the reaction is provoked by the sperm themselves rather than by the

accompanying fluid Lipoid materials as judged by stains for fat do not appear to be involved The histiocytic and macrophage response to presumably living cells rich in specialized nucleoprotein is a unique tissue reaction and deserves further study

Effect of diacetylene on viruses, bacteria and blood BENJAMIN E SANDERS, JOAN C WILSON, A R KELLY AND GEORGE H MANGUN (introduced by FRANK W HARTMAN) *Dept of Labs, Henry Ford Hospital, Detroit, Mich*

Diacetylene, $\text{CH}_3\text{COCH} = \text{CHCOCH}_3$, (DAE) has been found to be one of the most active virucides and bactericides investigated in connection with the problem of sterilizing blood plasma and whole blood In 95% ACD citrated human blood plasma, the following concentrations of DAE inactivated the test viruses (1% infected brain suspensions) after 5 days at 5°C (inoculations were made using 0.03 ml I C)

New Jersey Vesicular Stomatitis	10^{-4}	30 mg/l
Lymphocytic Choriomeningitis	10^{-4}	<20 mg/l
St Louis Encephalitis	10^{-5}	275 mg/l
Equine Encephalomyelitis	10^{-6}	<250 mg/l

In similar experiments with plasma containing 5×10^7 organisms/ml, the following organisms were inactivated after 48 hours, with the concentrations shown *E coli*, 200 mg/l, *A aerogenes*, 100 mg/l, *Staph aureus*, 300 mg/l On longer standing, the concentrations required decreased even further When added to plasma, DAE (500 mg/l) had no effect on the clotting mechanism Complement was completely inactivated and *B abortus* antibodies and syphilis antibodies were approximately inactivated by 500 mg/l No hemolysis was observed in whole blood treated up to 1000 mg/l Some retardation of the sedimentation rate was observed The intravenous LD₅₀ to adult mice is approximately 24 mg/kg Plasma treated with 300 mg/l of DAE, a dose found to be lethal to all test organisms, was incubated 48 hours at 5°C and injected into mice Five daily i.p. or i.v. injections of maximum tolerated volumes exerted no effect on survival or body weight Liquid oxygen was used for interim storage of the plasma

Kinetic pathology (motion picture) MACHTELD E SANO (introduced by VALY MENKIN) *Dept of Tissue Culture, Temple Med School, Philadelphia, Pa*

Kinetic pathology is the study, *in vitro*, of cells which have been under the influence of disease or are the direct result thereof Cells under the influence of disease may have had certain potentials activated so that these can be exteriorised These slides, of a classical Boeck's sarcoid, show the transition from epithelioid cells and lymphocytes

to almost a pure culture of cells 10 times as large In kinetic pathology one must differentiate between characteristic growth and fibroblastic growth 75% of diseased tissues give more characteristic growth in autogenous or human plasma as demonstrated by the study of 50 pleural effusions and 150 lymphnodes A cell present in all brain tumors, malignant or benign which resembles in many of its characteristics the 'gemistocyte', once called degenerate, is demonstrated in this movie of a glioblastoma multiforme It may have an altered metabolism but it is certainly not degenerate In its movement and behavior, it resembles to a certain degree the lymphocyte It needs further identification Cellular mitosis, separation from, and formation of the brain tumor are seen Routine tissue culture and microcinematography of tumors reveal to us unsuspected properties of cells which we cannot see by histopathologic methods Cells which have been under the influence of disease and which are histologically similar behave differently in tissue culture revealing activated potentialities which are of prognostic and diagnostic value

Polycystic mesonephros (goldfish kidney) HANS G SCHLUMBERGER *Dept of Pathology, Ohio State Univ College of Medicine, Columbus*

Twenty-three goldfish with polycystic kidneys were obtained from two ponds 150 miles apart In each, the fish were descendants of an original stock placed there 20 years before The fish in one of the ponds were also subject to neurofibromatosis The hereditary background of polycystic kidneys and neurofibromatosis in man may have a counterpart in these lesions of goldfish The cysts were multiple, measured from less than 1 mm to 6 cm in diameter, and occasionally greatly distended the abdomen Most of the cysts arose in the glomerular capsule, few involved the tubules Smith has shown (*J Biol Chem* 81 727, 1929) that in fishes the breakdown products of proteins are excreted by the gills, and that the kidneys of fresh water fish serve chiefly to get rid of excess water Renal function studies were carried out on 4 fish with polycystic kidneys and 5 normal controls The tail and body were encased in a thin rubber bag to collect all urine, and the fish placed in 200 cc of aerated water kept at about 18°C during the 24 hours of the test At the end of that time the urine, water, and blood were tested for the presence of nonprotein nitrogen, urea, ammonia nitrogen, and creatinine The results indicate that even when the kidneys are almost replaced by thin walled cysts, excretion of waste products by the gills and of water by the kidneys is not significantly affected

Malignant lymphoma, reticulum cell type (reticulum cell sarcoma), in albino rats F E SHAFFER, F P CLEVELAND, AND F R DUTRA (introduced

by E A GALL) *From the Kettering Laboratory of Applied Physiology, University of Cincinnati College of Medicine, Cincinnati, Ohio*

White rats procured in equal numbers from Albino Farms and Carworth Farms were used during a two year feeding experiment with a compound which was found to be innocuous. Neoplasms appeared in both treated and control rats with equal frequency, with an overall incidence of 20%. The compound used, therefore, had no significance in this respect. Of the 26 neoplasms observed in 130 animals, 8 were mostly fibro-adenomata of the breast. Eighteen (69%) were classified as malignant lymphoma, reticulum cell type (histiocytic). Primary sites of origin were as follows: lung (10), ileo cecal lymph nodes (4), thymus or lung (1), and undetermined (3). It is of interest that, while the primary tumors of the lung showed cytologic characteristics of malignancy, metastases did not occur. Primary sites could not be determined in 3 animals because of extensive metastases, however, it appeared probable that ileo-cecal nodes were primary sites in 2 of these, in the third, the largest tumor mass was one completely replacing the right kidney. Histologic pattern varied, in the number of giant cells, bizarre mitotic figures, and size and shape of cells, but all were consistent with malignant lymphoma of the reticulum cell type.

Effects of adrenal steroids in adrenalectomized phloridzinized rats ALBERT SEGALOFF AND ANNE S MANY * *Dept of Medicine, Tulane Univ of Louisiana and Alton Ochsner Med Foundation, New Orleans*

Young adult male rats of the inbred Fisher strain were adrenalectomized and were started on a 48 hour fast 2 days after adrenalectomy. At the beginning of the second 24 hours, the animals were injected subcutaneously with the necessary amount of steroid in 1 cc of sesame oil, and 50 mg of phloridzin suspended in 1 cc of olive oil and then placed in metabolic cages and urine collected for 24 hours with sodium fluoride and mineral oil as preservatives. Glucose, total ketones and total nitrogen were determined on the 24-hour collection on each animal. Five adrenal steroids were employed in this study. Compound S acetate in a dosage of 1 mg/animal gave essentially the control values found for adrenalectomized untreated animals. For all of the other steroids, Compounds A acetate E, F, and desoxycorticosterone acetate, there was increasing glucose, nitrogen and ketone excretion in the urine with increase of dosage of steroid. Our results would seem to indicate that at dosages which produce equivalent levels of glycosuria, Compound A acetate and DCA produce greater ketonuria than the other compounds. On the basis of the glycosuria attained, the steroids could be arranged in the following descending order of activity: Compound F, Compound E, Com-

pound A acetate, DCA and Compound S acetate. In none of the doses of steroids employed in this study was it possible to attain the excretion of glucose, ketones or nitrogen during the 24-hour period that was attained by normal intact animals handled in the same manner.

Fate of gold colloids introduced into circulation of the mouse C W SHEPPARD,* J FURTH AND LEON WISH * *Biology Division, Oak Ridge National Lab, Oak Ridge, Tenn*

The disappearance from the circulation of gold colloids labeled with the isotope¹⁹⁸ Au has been investigated in mice. Particle size was determined by electron microscopy. Animals of an inbred strain of uniform age and sex were injected in groups and sacrificed serially. At autopsy the activities of blood and tissues including whole organs were obtained, with an automatically recording gamma-ray ionization chamber. By including determinations of the initial activity of the total animal and of the carcass, excellent material balances were obtained. Highly consistent behavior was observed due to the uniformity of the animals. During the first few minutes following injection most of the activity was found in the circulation. After 3 hours, 88-92% was found in the liver and spleen. No further change was observed up to 3 days following injection. The rate of disappearance of activity from the circulation was slower than in other species hitherto studied. Autoradiographs of tissues obtained shortly after injection show a diffuse localization primarily on the endothelial linings of the capillaries of different organs and of the sinusoids of the liver and spleen. This may be due to the adherence of circulating activity at the moment of death. With increasing time between injection and death a progressive relative increase of activity in the macrophages of the liver and spleen is observed with a corresponding decrease in the activity adhering to the linings.

Sex differences in skeletal development of mice fed a high fat diet MARTIN SILBERBERG AND RUTH SILBERBERG *Snodgrass Lab, City Hospital, and Dept of Pathology, Washington Univ School of Medicine, St Louis, Mo*

In growing mice, the course of the skeletal time curve could be accelerated and articular age changes produced prematurely by feeding a ration of Purina Laboratory Chow with 25% lard added. This diet contains adequate amounts of protein, carbohydrates, minerals and vitamins. Since the female skeleton develops more rapidly and responds to hormonal stimulation less conspicuously than the male skeleton, it seemed interesting to compare the skeletal effects of the high fat diet in both sexes. Twenty-six virgin females of strain C57 black were fed the high fat diet from the time of weaning on and killed together with 26 controls

fed the stock ration after $\frac{1}{2}$, 1, 2, 3, or 5 months' observation. The females fed the high fat diet showed better gain in weight and, histologically, faster epiphyseal development and earlier onset of articular age changes than controls fed the stock diet. However, the differences between the 2 groups of females were less pronounced than those occurring in corresponding groups of males. The weaker reaction of the female may be due to sex differences in food intake or to the comparatively more advanced physiological age of the female skeleton at the beginning of the experiment. Whatever the mechanism involved, because of their weaker response females are less suited than males for studies concerning effects of nutritional factors in skeletal growth and ageing.

Strain differences in skeletal growth and ageing of mice fed a high fat diet. RUTH SILBERBERG AND MARTIN SILBERBERG *Snodgrass Lab, City Hospital, and Dept of Pathology, Washington Univ, St Louis, Mo*

Under the influence of a high fat diet, female C57 mice showed less acceleration of epiphyseal development and articular ageing than males. The faster physiological ageing of the female skeleton was considered to be a possible cause for this sex difference. If this assumption was correct, animals belonging to a strain with a rapid rate of skeletal ageing (dba) should likewise be less susceptible to the influence of such a diet than animals belonging to a slowly ageing strain (C57 black). At weaning, 60 male and female dba mice were put on a diet of Purina Laboratory Chow with 25% lard added. Together with 48 male and female controls fed the stock ration, the test animals were killed after $\frac{1}{2}$, 1, 2, 3, or 5 months' observation. The dba mice fed the high fat diet did not gain more weight than their controls kept on the stock ration, the corresponding C57 males gained 25% and the females 10% more than their controls. Strain differences manifested themselves also histologically. In dba males, the high fat diet caused only slight acceleration of epiphyseal development and articular ageing, dba females were hardly affected. As compared with the response of C57 males and females, there was thus in both sexes of the dba strain a distinctly decreased effect of the high fat diet. Possibly, an as yet undetermined age change in the growing cartilage may be the common denominator for strain and sex differences in the response to various nutritional and hormonal stimuli.

Metabolite antagonists in experimental cancer guanazolo. BORIS SOKOLOFF, JAMES B REDD AND RAYMOND DUTCHER (introduced by LEO LOEB) *Southern Bio-Research Lab, Florida Southern College, Lakeland*

Assuming that cancerous cells, contrary to nor-

mal cells, are unable to synthesize guanine from adenine, G Kidder *et al* submitted mice, bearers of transplanted adenocarcinoma Eo 771 and of spontaneous mammary cancer, to treatment with guanazolo. In the case of adenocarcinoma, treatment started 6 days after transplantation, with a daily dose of 0.5 mg given twice a day. They noted a complete inhibition of growth, which regained its force as soon as the treatment was discontinued. In our experiments we used August Rat Carcinoma, a fast-growing tumor which never regresses, at least in our strain. Altogether 200 rats, with an average weight of 180 gm, were used for experimentation. Fifty rats were given daily injections (subcutaneous) of guanazolo, twice a day, with a total daily dose of 40 mg/kg of weight. The treatment was started on the 6th day after inoculation when the tumor was of pinhead size. We obtained a complete inhibition in 75% of tumors (36 rats) with a slow growth in the rest of the tumors. At the end of 3 weeks of the treatment, the average size of the tumor was 2.2 cu cm against 9.1 cu cm in the control group (25 rats). At the end of the 3rd week the treated rats showed some sluggishness and loss in weight (12% of original weight), with one rat dead. In the 2nd series of experiments, the treatment began when the tumors had reached the average size of 3.2 cu cm (50 rats). The dose of guanazolo was increased to a daily total dose of 100 mg/kg of weight. After 3 weeks of treatment the average size of the tumor had increased to 5.6 cu cm as against 9.9 cu cm in the control group. This dose, however, produced a much stronger toxic effect on the animals with 8 rats dead at the end of 3 weeks' treatment while in the control group no mortality was observed. Histological examination of the adrenal gland revealed some pathological changes in the reticular and fasciculata zones of the gland.

Autoantibodies to in vivo damaged kidney. Relationship to nephrotoxin. DOUGLAS H SPRUNT, WAYNE R ROGERS* AND ANNA DEAN DULANEY* *Inst of Pathology, Univ of Tennessee, Memphis*

The usual method for production of autoantibodies (brain, kidney, heart, placenta, liver) has consisted of immunization of laboratory animals with normal tissues in combination with bacterial toxins or other injurious agents. Complement fixation, precipitin tests, and collodion agglutination have been used for the *in vitro* demonstration of such antibodies. We have employed a new procedure, namely *in vivo* injury of kidneys with Staphylococcus toxin and the subsequent use of these kidneys for the production of autoantibodies. The toxin was given to rabbits in 4 daily doses of 0.1 cc by the intraperitoneal route, or in single doses of 1 cc, incorporated in a water-in-oil emulsion with *M. tuberculosis* (Freund), and introduced subcutaneously. The kidneys were per-

fused with physiological saline solution, portions removed for histological examination, and a finely divided saline suspension prepared from the remaining tissues. When evidence of the pathologic state of the kidneys was obtained these preparations were used for immunization of rabbits by i.v., i.p., and s.c. routes over a period of 3 or 6 weeks. The sera of rabbits were used in complement fixation tests with kidney (normal and damaged) and other rabbit tissues. Sera of rabbits which had received normal kidney plus toxin, kidney or toxin served as controls. Antibodies were demonstrated for all rabbit tissues in sera of rabbits who had received damaged kidney or normal kidney plus toxin. The highest titers were obtained with the anti-damaged-kidney sera and the corresponding antigen. These sera were used *in vivo* to test their nephrotoxic property.

Cytochemical changes in the adrenal cortex of the rat following the administration of cortisone

ROBERT B. STEBBINS (introduced by H. C. STOERK) *Merck Inst for Therapeutic Research, Rahway, N. J.*

Daily subcutaneous administration of cortisone acetate (3 mg/day) produced marked atrophy of the inner zones of the adrenal cortex, while the glomerulosa remained intact. After from 10-42 days of treatment, the lipid and ketosteroid-like material of the subcapsular zone were, in general, unchanged. In contrast, the inner cells of the cortex showed a marked depletion of these substances. Following 42 days treatment, histochemical examinations demonstrated the almost complete loss of these components from the inner zones of the cortex. The glandular parenchyma resumed essentially a normal appearance within 17 days after discontinuance of the drug.

Marrow extracts affecting blood and marrow leukocytes

BERNHARD STEINBERG, ALBERT A. DIETZ* AND RUTH A. MARTIN* *Toledo Hospital Inst of Med Research, Toledo, Ohio*

We extracted from cattle marrow several fractions which decreased and raised leukocytes and platelets in the blood. This presentation deals with the extract which lowered leukocytes. The marrow was aged at 5° C for 5 days and at 26° for 2 days. Each 1000 gm were extracted with 500 ml of water at 75° C for 30 minutes. The fat was discarded. The water extract was precipitated with acetone at pH 4.5. Residue of 5.2 gm was suspended in water at pH 7 and ammonium sulfate added to $\frac{1}{2}$ saturation. The supernatant was dialyzed, filtered and precipitated with acetone at pH 4.5. The residue, 1.2 gm, was suspended in water and heated 3 minutes at 95° C. Acetone was added to supernatant at pH 4.5. The residue, 0.66 gm, was suspended in water at pH 8.1 and digested with trypsin for 24 hours. The trypsin was inactivated

at 80° C for 30 minutes. Acetone was added to supernatant at pH 4.5 and a water soluble residue of 0.4 gm obtained. Each of 50 rabbits were injected subcutaneously with 0.1 gm of extract. A decrease in blood leukocytes occurred in 4 hours and continued for 3-5 days. Greatest decrease was 88.8%, remaining at 75% of normal for 3 days. The bone marrow showed granulocytic hypoplasia due to inhibition of maturation. With an increase in peripheral leukocytes, immature granulocytes appeared. It is postulated that marrow contains substances which maintain a physiological equilibrium of blood cells.

Inhibition of the tuberculin reaction by cortisone in vaccinated guinea pigs

H. C. STOERK *Merck Inst for Therapeutic Research, Rahway, N. J.*

The group of diseases which Hench *et al* observed to be benefited by cortisone are generally assumed to be due to a hypersensitivity of the necrotizing (tuberculin) type. Cortisone is known to produce a marked loss of lymphoid tissue. The latter for a long time has been suspected to be related to antibody formation and has been shown to carry the antibody specific to the tuberculo-proteins. In sensitized animals a failure of the tuberculin reaction (or of anaphylaxis) to appear has been observed following a great variety of injurious stimuli. It seemed plausible to assume that this phenomenon of non specific desensitization may be related to increased adrenal cortical activity. Accordingly, the effect of cortisone has been investigated in the various types of hypersensitivity in guinea pigs. It was found that hypersensitivity of the anaphylactic type was not prevented with cortisone. In the Arthus reaction, cortisone administration reduced the amount of fluid exudation, but not the cellular infiltration in the tissues. The amount of necrosis and of hemorrhage was not appreciably diminished. In guinea pigs vaccinated with heat killed human tubercle bacilli, the tuberculin reaction could not be elicited when cortisone was administered prior to the injection of old tuberculin. Previously, a reduction of antibody concentrations has been observed following the administration of cortisone. It is therefore likely that the suppression of the tuberculin type of hypersensitivity, is at least in part related to an increased degradation of antibody protein by cortisone.

Alterations of cytoplasmic basophilia in liver injury and its relation to serum proteins

PAUL B. SZANTO*, HANS POPPER, DIETER KOCH-WESER* AND J. DE LA HUERGA* *Hektoen Inst for Med Research and Depts of Pathology, Cook County Hospital and Northwestern Univ Med School, Chicago, Ill.*

The alterations of cytoplasmic basophilic material in various examples of human and experi-

mental liver injury were studied and in the humans correlated with serum proteins. This material was identified as ribonucleic acid by pyronine, thionine, galloxyanin, azure II and by specific digestion with ribonuclease. The RNA of the liver cells was reduced in various forms of liver damage such as viral hepatitis, toxic hepatitis and active cirrhosis and less so in even long-standing biliary obstruction. It appeared increased in tumors, regenerating and compressed liver cells. It was similarly decreased in various forms of experimental liver cell damage. In chronic liver injury the decrease ran parallel with nucleolar changes. In individual human cases it ran parallel to reduction of serum albumin. In liver diseases, especially so in viral hepatitis and active cirrhosis, the cytoplasm of the Kupffer cells and the mesenchymal cells in portal triads revealed marked RNA reaction. Markedly bile-laden Kupffer cells, even if proliferated, were free of RNA as chiefly seen in obstructive jaundice. In the human the RNA in Kupffer cells and other mesenchymal cells occurred mainly in conditions with elevated serum gamma globulin. RNA reaction in hepatic epithelial and mesenchymal cells seems to indicate cellular activity. The results support the opinion that albumin is formed by the hepatic epithelial cells and indicate that at least some gamma globulins are formed by the Kupffer and portal mesenchymal cells. The latter process appears stimulated by most forms of liver damage.

Study of fibro-elastic intimal arteriosclerosis following hypothermal medial degeneration of young and old rabbits. C. BRUCE TAYLOR,* DAVID BALDWIN* AND GEORGE M. HASS. *Rush Lab of Pathology, Presbyterian Hospital, in affiliation with Univ of Illinois College of Medicine, Chicago*

Local lesions were produced in aortas of juvenile and senile rabbits with a hypothermal instrument. Animals were killed at intervals up to 24 weeks. Lesions were studied microscopically. Aneurysmal dilatation occurred immediately. Inflammatory reaction was insignificant at any stage. Degeneration of medial smooth muscle was advanced one week after injury and complete at 2 weeks. Elastic lamellae were fragmented at 2 weeks. Medial calcification appeared at 3 weeks and was complete at 5 weeks. In juvenile rabbits calcium slowly resorbed. In senile rabbits cartilage and bone appeared. Lesions of juvenile rabbits gradually contracted, those of senile rabbits showed persistent aneurysmal dilatation. Intimal proliferation with collagen deposition begun at 2 weeks and was complete at 5 weeks. Intimal proliferation was much greater in juvenile rabbits. In all animals new formation of elastic tissue was apparent in the thickened intima in 3 weeks. At

24 weeks, elastic fibrils in the proliferated intima resembled normal medial elastic lamellae. Smooth muscle cells appeared in the thickened intima at 2 weeks. At 6 weeks they were occasionally as abundant as those in normal media. At 4 weeks, a new internal elastic membrane was detectable. It was almost completely formed at 20 weeks. Since proliferated intima provided a matrix for new elastic tissue lamellae and smooth muscle cells, quantities of all elements were much greater in juvenile rabbits. Essentially, a new vessel wall was partly reproduced within the framework of the proliferated intima.

Action of 4-amino-pteroylglutamic acid on the early pregnancy of rats and mice. J. B. THIERSCH AND F. S. PHILIPS (introduced by C. P. RHODES). *Pharmacology Section, Division of Exper Chemotherapy, Sloan-Kettering Inst for Cancer Research, New York City*

4-Amino-pteroylglutamic acid was given to pregnant mice and rats in small doses which caused transient depletion of their marrow to approximately half its cellular contents. The mothers were not otherwise affected significantly. The compound when given in the first week of pregnancy produced death of the foeti but when given during the second week the litters survived. A single observation in a bitch suggested a similar action in this species. The results of these experiments and their bearing on fertility, seasonal breeding, spontaneous abortion and chlorosis are shortly discussed.

Sarcomatoid growths resulting from mammary carcinoma cells that had sojourned in immune mice. HELENE WALLACE TOOLAN* AND JOHN G. KIDD. *Dept of Pathology, New York Hospital-Cornell Med Center, New York City*

Tumors often result when the cells of a transplantable C3H mammary carcinoma are transferred back to susceptible C3H hosts after several days' sojourn in the subcutaneous tissues of A mice immune to them, as previous studies have shown (*Federation Proc* 8:360, 373, 1949). The growths, however, are not mammary carcinomas but fibrosarcomas, as judged histologically. This has proved true in 13 of 15 experiments, in no instance have ordinary mammary carcinomas been obtained from cells that had lain as long as 6 days in the subcutaneous tissues of immune hosts. The sarcomatoid growths are comprised of spindle-shaped cells that often lie whorled in parallel bundles and regularly produce large amounts of collagen. One of them has been transplanted in series in C3H hosts, during many months its cells have retained their elongated form and continued to lay down collagen. They differ notably from the polyhedral elements that comprise the transplantable mammary carcinoma, and they con-

tinue to arrange themselves in bundles that differ distinctively from the solid cords and pseudo-papillary masses of the original growth, a carcinoma simplex alveolare. It is noteworthy, however, that some time ago, after repeated transfers, a small proportion of the cells of the mammary carcinoma, especially those at the margins of the syncytial masses, assumed a more elongated form and were not infrequently surrounded by small amounts of collagen.

Lipo-protein of Gaucher's Disease L. L. UZMAN
(introduced by SIDNEY FARBER) *Children's Medical Center, Boston, Mass*

The spleens of two cases of Gaucher's Disease were removed surgically, frozen in dry ice and processed as follows: 100 gm of tissue were first extracted with 500 ml cold distilled water in a Waring blender. Following centrifugation, the residue was next extracted with cold 10% NaCl. This was centrifuged at 9,000 *g* and the residue again extracted at +5° C with 10% NaCl for 16 hours. This second saline extract was centrifuged at 9,000 *g* and supernatant diluted with 7 vols distilled water. After 24 hours standing in the cold, the ppt was collected, dissolved in 4% NaCl, alkali added to pH 8.0 then brought to pH 4.2 with acid. The precipitate was collected, re-dissolved and re-precipitated in the same manner 4 times, dialyzed and lyophilized. The fluffy white material thus obtained is a lipo-protein containing 62% lipid. The lipid moiety is kerosene and accounts for more than 70% of the total tissue cerebroside. The lipid is very strongly bound, being split from the protein only after 6½ hours boiling with CHCl₃, CH₃OH, or on treating the lipo-protein with 8M-guanidine hydrochloride. Osmotic pressure data reveal a number-average *M* wt of above 300,000 for this lipo-protein which also exhibits a high organic anion affinity comparable to serum albumin. No free -SH groups are detectable before denaturation, and the intact lipo-protein is resistant to tryptic and catheptic digestion. It appears that the lipid in Gaucher's Disease is 'bound' within the cell as a lipo-protein.

Quantitative relations between acute hepatic necrosis and survival in rabbits GORDON VAWTER,* GEORGE M. HASS AND C. BRUCE TAYLOR*
Rush Lab of Pathology, Presbyterian Hospital, in affiliation with Univ of Illinois College of Medicine, Chicago

Albino rabbits, weighting 3-9 pounds were used. During ether anesthesia, the liver was exposed surgically. Using partial aseptic precautions, multiple local areas of acute necrosis were produced in the liver by direct application of an instrument cooled to -60° C. Experience indicated that if animals survived for 3 days they would show no future symptoms attributable to the hepatic

injury. Hence, experiments were terminated either by death following hepatic damage or by killing the animal at the end of 3 days. Bacteriologic studies were made. The liver was removed and its weight and volume were determined. Volume of acute hepatic necrosis was calculated from direct measurements of multiple sharply defined lesions. No anaerobic or significant aerobic bacteria were isolated from the liver. Results indicated that the most important factor responsible for death was the volume percentage of liver, rendered non-viable by freezing. A second factor, the area of contact between normal and non-viable hepatic tissue, was also important. By combining these 2 factors an index for defining with considerable accuracy, the relations between acute hepatic necrosis and survival of the animal was established. This affords a quantitative basis for further study of causes of death and of associated degree of renal damage observed in these animals. The principal advantages of the method are that the hepatic damage is quantitatively and qualitatively reproducible and that no other organ is directly damaged by the method of producing acute hepatic necrosis.

Demyelination induced experimentally by means of lipase F. STEPHEN VOGEL (introduced by JOHN G. KIDD) *Dept of Pathology, New York Hospital-Cornell Med Center, New York City*

Purified lipase made from hog pancreas has regularly produced focal areas of demyelination when injected intracerebrally in rabbits. The lesions became manifest within 48 hours and persisted for at least ten days. They were characterized by complete loss of myelin and moderate gliosis with little or no destruction of other neuronal structures and no noteworthy inflammation, in these respects resembling closely the plaques of multiple sclerosis. The demyelination was often accompanied by paresis. Neither resulted when trypsin and chymotrypsin were injected intracerebrally in control animals. An area of demyelination involving the basal ganglia, together with atrophy of the regional ganglion cells without inflammation, developed following repeated injections of lipase intravenously in a single rabbit. When segments of normal spinal cord from various species were incubated with solutions containing the purified lipase, demyelination regularly resulted, the change being similar to that produced by the plasma of patients with multiple sclerosis (*Arch Neurol & Psychiat* 23:713, 1930, 27:367, 27:375, 1932). Trypsin and chymotrypsin likewise again failed to produce this effect in control experiments.

Adenosinase, adenase and xanthine oxidase of lymphoid tissues BERNARD M. WAGNER* AND WILLIAM E. EHRLICH *Philadelphia General Hos-*

pital and Graduate School of Medicine, Univ of Pennsylvania, Philadelphia

The enzyme content of lymphoid tissues associated with nucleoprotein chemistry has been studied by Barnes. Using a bio-assay method, he found that these tissues excelled in adenosinase. As the enzyme is important in nucleoprotein synthesis, and this appears to be closely related to antibody formation, it was hoped that a systematic study of the various enzymes present in the lymphoid tissues associated with nucleic acid metabolism would throw light on the protein-synthesizing function of their cells. The method of differential ultra-violet spectrophotometry of Kalckar was employed. The results obtained will be presented and discussed.

Obesity and food requirements of albino mice following administration of goldthioglucose S H WAXLER* AND G BRECHER *Army Med Dept Research and Graduate School and National Insts of Health, Bethesda, Md*

Brecher and Waxler recently reported the experimental production of obesity in mice by single injections of goldthioglucose. About 30% of the survivors of a toxic dose of the compound reached weights of 50-80 gm 6-12 months after injection. The excess weight was due to a marked increase of depot fat, but no morphologic changes were found in any organs except for a centrilobular fatty infiltration of the liver. A study of the food requirements of the injected mice showed that the food consumption of injected animals which become obese was increased both during the first 3 months of rapid weight gains and during a subsequent 3-month period of relatively slight additional increments in body weight. The obese animals tolerated prolonged starvation (up to 19 days) utilizing practically all of their excess fat during that period. On refeeding, the animals regained their pre-starvation weights, but weight gains could be delayed by restriction of food intake.

Cellular dynamics in intestinal mucosa, quantitative measurements of effects of antimetabolic agents, effect of nitrogen mustard on cell division and differentiation BANICE WEBBER,* BENJAMIN R CRAIG* AND NATHAN B FRIEDMAN *Division of Labs and Dept of Radiotherapy, Cedars of Lebanon Hospital, Los Angeles, Calif*

Mitotic counts are easily performed on sections of rat duodenum. By doing counts at intervals after treatment with various agents, clear-cut curves of antimetabolic activity, for example, may be obtained. There is usually a period of excessive mitotic activity which follows the phase of depression by irradiation or nitrogen mustard. It is simple by this method to appraise the effects of combinations of these agents with various time

and dosage factors. Study of rats treated with nitrogen mustard reveals that the inhibition of mitotic activity without interference in the differentiation of goblet cells which characterizes radiation reaction in the rat duodenum (*J Exper Med* 81:553, 1945) can be duplicated by this agent.

Histologic and enzymologic changes in lungs of rabbits with airborne tuberculosis CHARLES WEISS, MARIAL L BOYAR* AND H L RATCLIFFE* *Jewish Hospital and Penrose Research Labs, Zoological Society, Philadelphia, Pa*

Female albino rabbits were infected with either a human virulent strain H37-Rv or a bovine, Ravenel culture of tubercle bacilli, employing the Wells apparatus for the quantitative study of droplet nuclei infection (*Am J Hyg* 47:1948). In animals killed 52 days after infection when the tuberculin reaction was positive, the lungs showed from 10 to 16 discrete tubercles, about 10 mm in diameter and protruding about 5 mm above the pleural surface. In the interior of the tubercles there were distinct areas of caseation surrounded by inflammatory zones. Numerous small, scattered secondary tubercles were also observed. Employing chemical methods previously described (*Proc Soc Exper Biol & Med* 72:236, 1949) it was shown that caseous material, which had been freed as far as possible from surrounding inflammatory tissue, is not enzymatically inert but hydrolyzes BAA and LA. However, its rate of hydrolysis is very much slower than that of the surrounding inflammatory 'wall'. Other rabbits were infected with either human or bovine tubercle bacilli by means of the Wells apparatus and, 6 to 7 weeks later, exposed in the same manner to aerosol solutions of PPD in doses of approximately 8×10^{-3} mg. These animals were killed 48 hours later. The tubercles now presented increased areas of caseation which showed decreased density and an increased tendency toward liquefaction.

Cytoplasmic reactions of hepatic parenchymal cells to experimental injury in rodents and dogs W LANE WILLIAMS AND ALBERT J GREENBERG* *Depts of Anatomy and Medicine, Univ of Minnesota, Minneapolis*

Liver injury was produced in mice by CCl_4 or by starvation for 1-8 days, dogs by CCl_4 for 5 days-1 year, by starvation for 35 days or low protein diets for 30-300 days (3% to 10%, with and without a large intake of alcohol), and young rats by a low protein-high fat diet for 4-8 weeks. Initial lesions were centrilobular and mutual cytoplasmic responses were a decrease in ribonucleic acid, a positive alkaline phosphatase reaction (absent in normal liver cells) and beginning liposis. In dietary deficient animals fat deposition was progressive. In CCl_4 -treated animals necrosis of central cells ensued. Continued treatment (dogs) with

CCl_4 produced massive cirrhosis with parenchyma consisting of nodules of hyperplastic cells. Such cells were large and hyperbasophilic (cytoplasm). Considerable basophilia remained after digestion with ribonuclease but was removed by the trichloroacetic acid (Schneider) method for extraction of nucleic acids. Significant cirrhosis did not occur in dogs on low protein diets (with or without alcohol). Such livers were fatty. Most parenchymal cells contained fat and were hypobasophilic, but other cells were non-fatty and hyperbasophilic like those of chronic CCl_4 -treated dogs. It seems significant that a decrease in cytoplasmic basophilia of centrilobular cells was an obvious reaction in 3 species to several methods of liver injury. A similar hypobasophilia was produced in extracentral cells by treating sections with ribonuclease. Trichloroacetic acid removed all cytoplasmic basophilia and Feulgen-positive nuclear material.

Protective action of sulfa drugs against CCl_4 poisoning in mice. J. WALTER WILSON, ELIZABETH H. LEDUC AND LOIS E. ARNOLD (introduced by H. S. N. GREENE) *Dept. of Biology, Brown Univ., Providence, R. I.*

That the more soluble sulfa drugs, particularly sulfanilamide, protect rats against liver damage by CCl_4 was reported by Leach and Forbes (*Proc. Soc. Exper. Biol. & Med.*, 51: 47-48). We have investigated in mice the effect of sulfanilamide and also of sulfaguanidine and formosulphathiazole, which are poorly absorbed. Young adult mice were placed on a low fat (8%) or high fat (26%) semi-synthetic diet. Experimental animals received in the diet one of the following: 0.1% sulfanilamide, 0.5% sulfaguanidine, 2.0% sulfaguanidine, or 0.5% formosulphathiazole. After three days on the diet they were injected subcutaneously with 0.1 cc. of a 40% solution of CCl_4 in sesame oil. Animals were sacrificed on the second and third days afterward, and the livers studied histologically. Animals with CCl_4 but without sulfa drugs showed somewhat less liver injury on the low fat than on the high fat diet. From $\frac{1}{4}$ to $\frac{1}{2}$ of the lobule was necrotic. There was about the same injury with the formosulphathiazole. The 0.5% sulfaguanidine group showed very much less injury, and there was practically none in the 2.0% sulfaguanidine or the 0.1% sulfanilamide groups. In the sulfaguanidine groups, the only indication of CCl_4 poisoning was a reversible hydropic change in the liver cells, e.g. in the two day, 2% sulfaguanidine livers there were many "balloon cells", which had recovered by the third day. The protective action of these sulfa drugs seems to be related to the degree of absorption from the intestine.

Rate of clearance from blood of ^{131}I tagged plasma, determinations of cell and plasma volumes with radioisotopes and Evans blue. L. WISH,* R. H. STOREY* AND J. FURTH. *Oak Ridge Natl. Lab., Biology Division, Oak Ridge, Tenn.*

Rabbit and dog plasma were tagged with ^{131}I . The technique of Fine and Seligman was modified by removing the excess of unbound inorganic iodide by passage through an anion-exchange column. Seventeen rabbits were given a total body exposure of 1000 r of x-rays. Pairs of x-rayed and normal rabbits were injected intravenously with tagged homologous or heterologous plasma from 1-13 days after irradiation. Samples of blood were withdrawn at various intervals after injection and the relative activity of the ^{131}I in the plasma measured. Both homologous and heterologous plasma disappeared from the blood faster in the irradiated than in the normal rabbits. This difference was greater with homologous plasma. The highest concentration of radioactivity in the plasma, 3-5 minutes after injection, was taken to indicate the plasma volume. This volume, calculated in percentage of body weight, was less in animals that had been injected with homologous plasma, 4-13 days after irradiation, than in the normal rabbits. Evans blue plasma values determined on several of these animals were consistently higher than those obtained with iodinated plasma. ^{32}P tagged erythrocytes of the rabbits reintroduced in the animal likewise disappeared faster in irradiated than in normal animals. Experiments are being performed to obtain information on the meaning of these differences observed between x-rayed and normal rabbits.

Influence of estrogenic hormone on calcium and phosphorus balances in osteogenesis imperfecta congenita. HARRY H. WOMACK, JR.,* HILDA F. WIESE* AND ARILD E. HANSEN. *Dept. of Pediatrics, Univ. of Texas Med. Branch, Galveston.*

A 4-month old negro male infant with marked osteoporosis and bodily deformity due to numerous fractures because of osteogenesis imperfecta of the congenita type was studied. He was placed on a metabolic frame and given a weighed milk diet with vitamin supplements considered adequate to meet his fundamental nutritional requirements. The control period comprised two 3-day and two 6-day intervals for a total of 18 days, the separate urine and stool collections beginning after the patient had been on the experimental diet for over 2 weeks. The total calcium intake was 11.483 gm. and the output, urine 0.766 gm. and stool 8.439 gm.; the phosphorus intake was 8.542 gm. and the output, urine 2.603 gm. and stools 3.458 gm. Estrogenic substance, Progyon B (Schering) 1, ml. intramuscularly was given for

30 days, the diet and other conditions remaining unaltered. The calcium intake was 20.963 gm and the output, urine 0.499 and stool 15.865; the phosphorus intake was 15.779 gm and the output, urine 5.033 and stools 6.471. The average daily urinary calcium decreased following the administration of the estrogenic substance. However, average daily calcium retention was 0.154 gm for the control period and 0.153 for the estrogen period. Somewhat less phosphorus was retained during the administration of the estrogen, the daily average retention being 0.234 and 0.142 respectively for the control and estrogen periods. Roentgenograms revealed no change in the degree of osteoporosis. Estrogenic substance was continued for a short time thereafter without stool and urine collections. Fractures continued to occur with slight trauma.

Excretion and tissue distribution of C^{14} following intravenous injection of labeled plasma protein. C. L. YUILE, B. G. LAMSON,* L. B. ANDERSON, JR.* AND G. H. WHIPPLE. *Dept. of Pathology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

Plasma with a high C^{14} content, from a donor

dog which had been fed DL-lysine labeled with C^{14} in the ϵ position, was collected at a time when at least 98% of the total plasma activity was present in the plasma proteins. Following intravenous injection of this labeled plasma into a recipient dog, the C^{14} content of aliquots of expired CO_2 was measured at intervals during a 48-hour period. A low and diminishing amount of the isotope was observed in these samples which indicated that not more than 6-7% of the dose was eliminated by this route during the period of the experiment. Urinary excretion accounted for loss of less than 1% of the injected C^{14} . The rate of decline of the plasma protein activity in the recipient dog confirmed the previously reported conclusion that plasma proteins are utilized and replaced at a minimum rate of 10%/24 hours. Following viviperfusion at 48 hours, significant amounts of activity, predominantly in protein, were detected in all tissues examined. The liver alone contained as much as 6.8% of the dose. The findings in this experiment are consistent with the theory of a rapid and labile exchange between plasma and tissue proteins, evolved from past metabolism studies.

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(An asterisk * following an author's name indicates "by invitation")

Riboflavin deficiency in dogs produced by 2-acetylaminofluorene JAMES B ALLISON AND ARTHUR W WASE * *Bureau of Biological Research, Rutgers Univ, New Brunswick, N J*

Feeding 0.03% 2 acetylaminofluorene in a synthetic diet to adult dogs produced a riboflavin deficiency which was characterized clinically by a seborrheic erythema with alopecia, a condition which was cured rapidly by increasing the riboflavin intake. Riboflavin retention tests demonstrated that the excretion of vitamin in the animals with lesions was greatly reduced below the controls. The riboflavin content of the livers of dogs with lesions was approximately 15 gamma per gram of tissue which is about half the amount found in the controls. The drug also reduced the riboflavin associated with the bacterial flora of the intestinal tract. A deficiency was not produced on a riboflavin-free diet in the absence of the drug. It was demonstrated that dogs fed the riboflavin-free diet had a normal riboflavin content of the feces and liver and that the retention of the vitamin was the same as in the controls. It was shown that the absorption of vitamin could be adequate from the region of the intestine where the bacteria were most abundant. These experiments indicate that a riboflavin deficiency can be prevented by the synthesis of vitamin in the bacterial flora of the intestinal tract and that 2-acetylaminofluorene will reduce the vitamin content of the bacterial culture in the feces as well as the content of the animal tissues. Continued feeding of the drug induced hepatomas in some and depletion of protein stores in all dogs.

A folic acid-derived growth factor for *Leuconostoc citrovorum* in human urine R M ANKER,* J W BOEHNE* AND ARNOLD D WELCH *Dept of Pharmacology, Western Reserve Univ School of Medicine, Cleveland, Ohio*

Urine contains a factor which stimulates the growth of *Leuconostoc citrovorum* 8081 under conditions where pteroylglutamic acid (PGA) does not itself cause growth of the organism. As noted by Sauberlich (*J Biol Chem* 181:467, 1949), administration of PGA greatly increases the urinary excretion of the citrovorum factor. In man, the effect of PGA on urinary excretion is subject to individual and daily variations which, in part, appear to be due to constituents of the diet, however,

the amount excreted remains roughly proportional to the amount of PGA ingested. Concentration of the factor from the urine of individuals ingesting 50-100 mg of PGA daily has been attained. Unmodified PGA is removed on Amberlite IR-4B without significant loss of activity for *L citrovorum*, while activity for *L casei* is greatly reduced. The factor behaves as an anion in alkaline media, is unionized at neutrality, is very unstable below pH 3, and is adsorbed strongly on Darco G-60 charcoal (pH 5-6) from which it is eluted by ammoniacal ethanol. Counter-current distribution, utilizing pyridine and aqueous sodium carbonate, yields a product with ultraviolet absorption suggesting the presence of a pteridine derivative. On paper strips, the factor in untreated urine moved but little with 2,4,6-collidine, with 2,4-lutidine either 2 or 3 zones of activity were obtained, while with butanol-acetic acid only one band resulted (with much loss of activity, as expected). The partially purified factor from urine gave but one band with 2,4-lutidine. Further studies on the concentration and properties of the factor are in progress.

Inactivation of biotin by hydrochloric acid A E AXELROD AND KLAUS HOFMANN *Inst of Pathology, Western Pennsylvania Hospital and Dept of Chemistry, Univ of Pittsburgh, Pittsburgh, Pa*

In the course of studies involving the utilization of different microorganisms for the assay of biotin in natural products, it was observed that hydrochloric acid extracts of grass juice powder possessed growth-promoting activity for *L arabinosus* and *S cerevisiae* but not for *L casei* and *S fecalis*. In contrast, sulfuric acid extracts of grass juice powder, as well as hydrochloric acid extracts of a large number of other natural products, were active for all 4 organisms. In attempts to elucidate the mechanism of the effect of hydrochloric acid upon grass juice powder the following observations were made: 1) Autoclaving of extremely dilute solutions of biotin with hydrochloric acid resulted in almost complete loss of their growth-promoting potency for *L casei* and *S fecalis* and approximately 50% loss for *L arabinosus* and *S cerevisiae*. Autoclaving with sulfuric acid was without deleterious effect. 2) Solutions of oxybiotin were not affected by autoclaving with either hydrochloric or sulfuric acid and 3) biotin sulfoxide was as ac-

tive as biotin for *L. arabinosus* and *S. cerevisiae* on a molar basis, and inactive for *L. casei* or *S. fecalis*. In view of the fact that biotin sulfone is inactive under these conditions for the 4 organisms, it is postulated that hydrochloric acid converts dilute solutions of biotin into a mixture of biotin sulfoxide and biotin sulfone. Experiments are presented which are in agreement with the concept that many natural materials are capable of preventing the conversion of biotin to its sulfoxide and sulfone through the action of hydrochloric acid. Such a 'protective' action is absent in grass juice powder.

Deposition of thiamine, riboflavin, and pantothenic acid in placental and fetal tissues as a basis for estimating vitamin requirements for normal reproduction in rats MARGARET BARRITT,* HARVEY LEWIS* AND GLADYS EVERSON *Nutrition Lab, Home Economics Section, Iowa Agricultural Exper. Station, Iowa State College, Ames*

Rats fed a modification of the Steenbock stock ration have been killed at intervals during pregnancy so that data could be secured on the quantity of vitamins present in fetal and placental tissues at any stage of pregnancy. Analyses for thiamine, riboflavin and pantothenic acid concentrations have been completed for such tissues. Since reproductive performance was known to be slightly more uniform among stock animals of our colony during second pregnancies, the second pregnancy was chosen for study. Females included in the present experiment, therefore, were known to have borne one satisfactory litter and were producing at least 9 young at the time they were killed. The concentrations of the vitamins of the uterus and its contents were determined for non-pregnant females and for rats in the 6th, 10th, 11th and 12th days of pregnancy. From the 13th day to parturition, animals were killed at one day intervals and both fetal and placental tissues were assayed. The results of this study bring out some interesting differences in the vitamin needs of the rat at various stages of pregnancy.

Effect of pyridoxine deprivation on amino acid metabolism in rats J. R. BEATON,* R. M. BALANTYNE,* R. E. LAU,* A. STECKLEY* AND E. W. MCHENRY *Dept. of Public Health Nutrition, Univ. of Toronto, Toronto, Canada*

To determine the relation of pyridoxine to amino acid metabolism, alanine was given intraperitoneally to fasting rats. At suitable intervals after administration of alanine, animals were killed to obtain blood for the estimation of urea, uric acid, glutamic acid, α amino nitrogen and sugar. Comparison was made between rats which had received pyridoxine and ones which had been deprived of that vitamin. The degree of deprivation was de-

termined by microbiological assay of liver stores of vitamin B₆. Pyridoxine insufficiency had no apparent effect on the production of urea, sugar, and glutamic acid after alanine administration, but there seemed to be delayed utilization of all of the metabolites studied. Blood concentration, as measured by packed cell volume, accounted for only a small proportion of the observed effects. The evidence indicated that pyridoxine deprivation, to the extent attained, did not interfere with the transamination of alanine to glutamic acid but did, possibly, delay deamination.

Influence of low levels of protein on heat production ALEX. BLACK, K. H. MADDY* AND R. W. SWIFT *Dept. of Animal Nutrition, Pennsylvania State College, State College, Pa.*

This study represents a continuation of previous on the same general subject (FORBES, et al. J. NUTRITION, 28: 189, 1944) in which it was found that the heat production of rats decreased with increased protein content of isocaloric diets. The protein content of the diets ranged from 10 to 45%. The experiment reported here was designed to include diets of lower protein content than were previously employed, namely, 6, 8, and 10%. All rations were compounded to be equicaloric and to contain approximately the same amount of vitamins and minerals. The experimental subjects were male weanling albino rats fed by the paired-method modified to include three littermate animals in a group, with one rat fed each level of protein studied. The heat production was measured by the body balance procedure involving analysis of control animals at the start of the experiment and the experimental subjects at the end. The average live weight of the three groups increased during 10 weeks from 48 grams at the start of the experiment to 123, 150, and 169 grams at the end for the 6, 8 and 10% protein groups respectively. Other data to be determined include metabolizable energy, nitrogen in the urine and feces, body gain of energy as protein and as fat and the heat production.

Toxicological studies of a new mercurial diuretic 8-(beta-acetoxymethyl-gamma-methoxypropyl)-3-carboxycoumarin-theophylline (EN-564) HAROLD BLUMBERG, ALBERT SCHLESINGER* AND S. M. GORDON* *Research Labs., Endo Products Inc., Richmond Hill, N. Y.*

A new mercurial diuretic, 8-(beta-acetoxymethyl-gamma-methoxypropyl)-3-carboxy-coumarin-theophylline, designated as EN-564, was synthesized in our laboratories. Clinical trials by Shapiro and Weiner have demonstrated that the intramuscular administration of EN-564 produces an effective and satisfactory diuresis in humans. In this compound the propyl group is attached to a ring carbon atom instead of to the carbamido

nitrogen of the currently available mercurial diuretics. Dissolved as the sodium salt at pH 7.3, EN-564 forms a stable solution containing 136 mg/cc of the compound, or 39 mg/cc Hg, which is the customary strength of mercurial diuretics for clinical use. The acute toxicity of EN-564 was determined in several species. The following values were found for the $LD_{50} \pm SE$, in mg Hg/kg intravenous—mouse 40.5 ± 1.7 , rat 9.8 ± 0.82 , rabbit 7.4 ± 1.4 , intramuscular—rat 12.3 ± 0.48 , rabbit 10.5 ± 1.0 , subcutaneous—mouse 83 ± 2.6 , oral—rat 238 ± 11 . These values, as well as those obtained for the estimated LD_{50} , indicate that EN-564 has approximately the same acute toxicity as the currently used mercurial diuretics. In a side-by-side comparison, the following values for the LD_{50} in rats were obtained: mersalyl-theophylline 10.8 ± 0.20 , EN-564 11.8 ± 0.17 . Similarly, a subcutaneous comparison in mice gave mersalyl-theophylline 74 ± 4.4 , meralluride 84 ± 2.1 , and EN-564 83 ± 2.6 . In a chronic toxicity study, young rabbits received weekly i.m. injections of EN-564 for 6 months. The rabbits tolerated the drug and gained weight at dosages as high as 8 mg Hg/kg/wk. This level corresponds to 0.20 cc/kg of the EN-564 solution, or approximately 5 times the clinical dosage.

Effect of dietary level of tocopherols on their metabolism in swine J. W. BRATZLER,* J. K. LOOSLI, V. N. KRAKOVSKY* AND L. A. MAYNARD
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Five male weanling pigs were fed a tocopherol-low purified ration consisting of 25% casein, 40.3% starch, 25% corn sugar, 4% fiber, 0.2% methionine, 5% minerals, and liberal quantities of 9 vitamins. Two animals served as controls and the other 3 received supplements of mixed tocopherols in the form of a concentrate at the following levels in mg/100 lbs of live weight daily: 133.5, 2,500, and 5,000. All animals were slaughtered after 75 days on the experimental diet. Samples of whole blood, liver, heart, kidney, spleen, muscle and fat from 5 different storage sites were analyzed for their content of total and γ -plus δ -tocopherols using molecular distillation. Blood plasma was analyzed by the usual extraction procedure. Iodine and thiocyanogen values were determined on all samples of fat. With each increase in the level of tocopherols ingested, the tocopherol content of the tissues increased. The increases were most marked in the liver and body fats. Analyses of blood plasma and whole blood showed that the cells contained a much larger percentage of total tocopherols than the plasma. From the control animals zero values were obtained for blood plasma as contrasted with 197 μ g/100 ml of whole blood. Blood plasma to-

copherols reflected the levels ingested, but the increases were greater in the whole blood than in the plasma. Tocopherol supplementation affected markedly the fatty acid composition of the body fats by increasing the percentage of oleic acid at the expense of saturated fatty acids.

Lysine requirement for growing swine M. J. BRINEGAR,* H. H. WILLIAMS, J. K. LOOSLI AND L. A. MAYNARD
Depts. of Animal Husbandry and Biochemistry and Nutrition, Cornell Univ., Ithaca, N. Y.

L-lysine monohydrochloride was added to a diet containing sesame meal supplemented with methionine and histidine and fed to weanling pigs for 4 weeks. The basal diet consisting of 21.1% protein contained 0.57% lysine as determined microbiologically using *Leuconostoc mesenteroides*. Experimental diets were made to contain 0.57%, 0.75%, 0.97%, 1.07%, 1.17%, 1.32% and 1.63% pure L-lysine. These diets were distributed among 34 pigs. Daily feed intakes of the pigs were equalized weekly at approximately 5% of body weight. Each increased lysine level up to 1.17% improved the growth rate and feed efficiency. In another trial lysine was added to a 21.3% protein diet containing meat scraps, zein and wheat supplemented with methionine, histidine and tryptophan. Lysine levels of 0.96%, 1.00%, 1.20% and 1.40% were each fed to 5 pigs. Increases in growth rate and feed efficiency were noted up to the 1.20% lysine level. The data of these two experiments show that with diets containing approximately 22% protein (after lysine supplementation) weanling pigs require approximately 1.2% L-lysine in the ration. This is double a lysine requirement previously found by the same authors (*Federation Proc.* 8:379, 1949) when the protein level was only 10.6% furnished by linseed meal supplemented with methionine and histidine. In related amino acid studies it was shown that isoleucine is an essential component of swine rations. The addition of DL-allo-isoleucine to a 20.9% protein diet containing blood flour supplemented with methionine increased the rate and efficiency of gains of weanling pigs.

Vitamin C nutrition of the aged M. H. BROOKES, K. HEITSU* AND M. J. ALLEN*
Dept. of Home Economics, University of Chicago, Chicago, Ill.

The power of old people to use ascorbic acid efficiently has been questioned, but the data were incomplete because the usual food intakes of vitamin C, which preceded the tests, have been largely undetermined. In this study the ascorbic acid values of the food eaten for a 3-week test period, as well as the blood plasma levels of reduced ascorbic acid, were determined for 9 subjects, healthy old people, living in a home for the aged. The 3 men and 6 women studied ranged in age from 62-85

years, with an average age of 76 years. For 14 days the subjects ate their regular diet, which was self-selected, and for 5 days this was supplemented with 60 mg ascorbic acid from orange juice. The regular vitamin C intake, ranging from 29-76 mg between the subjects, had averaged 48 mg/day. The corresponding blood plasmas ranged from 0.27-0.51 mg/100 cc, with an average of 0.41 mg. The supplemented diets averaged 94 mg ascorbic acid and the subjects responded normally to this supplementation with a resulting plasma average of 0.54 mg reduced ascorbic acid/100 cc in 5 days.

Effect of some human-type diets upon growth and health of the laboratory rat ELIZABETH CROFTS CALLISON, ELSA ORENT-KEILES AND RACHEL UHVITS MAKOWER * *Bureau of Human Nutrition and Home Economics, U S D A, Beltsville, Md*

Five experimental diets, based on different food consumption patterns prevalent in the United States and composed of foods prepared as for human consumption, were assessed for their nutritive adequacy by feeding them to groups of laboratory rats from weaning to 500 days of age. Similar groups of rats were fed a stock ration for comparison. The diets were analyzed for their nutrient content. Growth, sexual maturity, ageing, and mortality were some of the physiological responses studied. The general physical condition of the experimental animals was also observed. The biological observations, as related to dietary composition, and the suitability of this type of experiment for the appraisal of human diets will be discussed.

Effect of rich, bulky, and poor diets on fertility in rats ANTON J. CARLSON AND FREDERICK HOELZEL *Dept of Physiology, Univ of Chicago, Chicago, Ill*

A comparative study was made of the effect of a rich diet, a bulky diet and a poor diet on the fertility of successive generations of early bred rats (littermates raised together) and later bred rats (segregated or isolated littermates mated when 130-230 days old). The rich and bulky diets were similar to diets used in our previous study (*J Nutrition* 36:27, 1948). The poor diet was a marginally adequate Sherman-type diet. In 30 months, 6-8 generations of inbred early bred rats were raised on each diet but the fertility of later bred rats was greatly impaired and apparently a cumulative impairment in successive generations prevented the raising of even 2 generations of inbred later bred rats on any diet. Ninety-eight male and 127 female early bred rats and 106 male and 150 female later bred rats were used. Fertility was least impaired in early and later bred rats on the bulky diet. This diet served to reduce the caloric intake of an otherwise rich diet. The results suggest that the low fertility of high grade live stock and the human upper classes is due to the use of

too much rich food and delayed procreation in successive generations.

Occurrence of dehydro-L-ascorbic and diketo-L-gulonic acid in the blood SHIH DZUNG CHEN* AND CECELIA SCHUCK *Nutrition Lab, Purdue University, Lafayette, Ind*

In an earlier study on the excretion of ascorbic acid with intake derived chiefly from crystalline ascorbic acid or from food sources the form found in the urine was largely L-ascorbic acid. However appreciable amounts of dehydro-L-ascorbic and diketo-L-gulonic acid were also detected. The quantities of the latter compounds excreted were rather constant for a given subject and appeared to be irrespective of intake. The question arose as to whether these compounds were excreted as such or whether as the result of incomplete protection of the reduced ascorbic from oxidation they might be formed during the 24-hour period of collection. Analysis of fresh samples of urine showed the presence of both dehydro and diketogulonic acid, with little change in amounts after the samples were preserved for 24 hours. The occurrence of these substances in freshly excreted urine suggested that they should be present at some time in the blood. Both were found in fasting blood samples in some instances and at intervals after the ingestion of crystalline ascorbic acid.

Role of B₁₂ on nitrogen retention of rats fed on soy bean protein diets at different caloric levels BACON F. CHOW AND LOIS BARROWS * *Dept of Biochemistry, School of Hygiene and Public Health, Johns Hopkins Univ, Baltimore, Md*

Supplementation of vitamin B₁₂ to soy bean protein diet accelerates the rate of growth of young rats, deficient of this vitamin. It is therefore of interest to ascertain whether B₁₂ enhances the efficiency of utilization of soy proteins. To this end, nitrogen balance experiments were performed to determine the amount of nitrogen retained by 2 groups of B₁₂ deficient rats, with and without supplementation of B₁₂. Their basal diet consisted of 40% sucrose and 60% Sobee, a commercial product containing 32% soy protein. Adequate vitamin supplement was also given. Urine and stool samples were collected weekly and analyzed for nitrogen, for a period of 6-8 weeks. It was found that when both groups of animals were given a restricted intake (6-8 gm of diet/rat/day), supplementation of vitamin B₁₂ did not increase the growth rate nor the retention of nitrogen. When the dietary allowance was increased by 50%, vitamin B₁₂ brought about a greater rate of growth but no better protein utilization. This is interesting since the added growth points to the effect of B₁₂ on carbohydrate or fat metabolism rather than protein metabolism. In another experiment the daily ration was further increased to *ad lib* feed-

ing The growth rate but not the efficiency of nitrogen utilization was increased by the addition of this vitamin These results therefore indicate that B₁₂ does not enhance the biological value of soy bean proteins under our experimental conditions but may play an important role in carbohydrate or fat metabolism

Effect of dietary restriction and rehabilitation on sodium, potassium and chloride of skin and total carcass of male rats RUTH CLAYTON AND ESTHER DACOSTA (introduced by CARL J KOEHN) *U S Army Med Nutrition Lab, Chicago, Ill*

The average sodium, potassium and chloride in mEq/kg of fresh weight for 14 control rats was 54.7, 30.2, and 24.0 respectively for the skin and 50.0, 52.1, and 19.0 respectively for the carcass. Of 80 rats restricted approximately 95 days, 14 on a low calorie diet had 54.7 mEq of sodium, 30.2 of potassium, and 23.4 of chloride in the skin and 52.0, 52.1, and 19.1 mEq respectively in the carcass. Eleven rats fed a modified carrot diet (2% protein) showed a 50% increase in the skin and carcass potassium. Rats fed a high salt, low protein diet had a 60% increase in both the potassium of the carcass and the chloride of the skin. Animals rehabilitated on a high protein or high carbohydrate diet showed a rapid rise in the potassium of the carcass and the chloride of the skin during the first 4 days of rehabilitation. Sodium in the skin rose to 144.9 mEq in rats on the high protein after the low calorie diet, and 143.9 on the high protein after the carrot diet. After one week of rehabilitation the skin sodiums were still definitely above the normal. The rats rehabilitated on high fat showed little deviation from the control during early rehabilitation.

Significance of vitamin B₁₂ in milk diets R. A. COLLINS,* L. S. DIETRICH* AND C. A. ELVEHJEM *Dept of Biochemistry, College of Agriculture, Univ of Wisconsin, Madison*

The inferior growth observed in rats fed goat's milk mineralized with iron, copper and manganese as compared to the good growth of rats fed mineralized cow's milk was studied. This inferior growth was counteracted by a daily addition to the mineralized goat's milk diet of 50 µg of folic acid plus 0.1 µg of vitamin B₁₂, 0.5 µg vitamin B₁₂ alone, or 1.0 gm of fresh beef liver. An inconsistent half maximal growth stimulation resulted when 50 µg of folic acid or 0.1 µg of vitamin B₁₂ was added separately to this diet. Goat's milk was found to contain only traces of vitamin B₁₂ in contrast to the occurrence of 2-3 µg of vitamin B₁₂ /l in cow's milk. The folic acid content of goat's milk was found to be very low and comparable to cow's milk. Folic acid or vitamin B₁₂ additions to mineralized cow's milk diets did not affect growth in

the young rat. The addition of vitamin C to the cow's milk diets produced a large increase in the vitamin B₁₂ content of the livers, while on the goat's milk diets added vitamin C displayed no activity. Folic acid and vitamin B₁₂ additions to mineralized cow's milk or goat's milk did not affect the rate of hemoglobin formation in the weanling rat or in rats made anemic on milk diets.

Vitamin B₁₂ content of chick tissues as influenced by diet J. R. COUCH, ORLANDO OLCESE AND H. L. GERMAN (introduced by L. R. RICHARDSON) *Depts of Biochemistry and Nutrition and Poultry Husbandry, Agricultural and Mechanical College of Texas, College Station*

New Hampshire chicks from hens fed an adequate ration were used in these tests and were maintained in batteries with raised screen floors. A vitamin B₁₂ low basal diet which contained 35% soybean oil meal was fed to the first group. A 2nd group received a supplement of approximately 20 µg of B₁₂/kg of diet. Supplementing with vitamin B₁₂ increased the B₁₂ content of the liver about 5 times, of the kidney, approximately 2 times, of the pancreas, slightly, and of the spleen, none. The chicks which received B₁₂ averaged 280.4 gm more at 10 weeks than those which did not receive it. In the same series, 10% of dehydrated alfalfa leaf was substituted for an equivalent amount of corn. The ration containing alfalfa was supplemented with 0, 20 and 130 µg of vitamin B₁₂/kg of diet, respectively. The alfalfa appeared to inhibit the growth of the chicks even when the diet was supplemented with the large amount of B₁₂. The alfalfa tended to decrease the B₁₂ content of the tissues below that of comparable groups not fed alfalfa. However, the tissues contained larger amounts of vitamin B₁₂ when the alfalfa diet was supplemented with 130 µg of B₁₂ than when it was supplemented with the lower level or with no vitamin B₁₂. Vitamin B₁₂ content of the tissues was determined with *Lactobacillus leichmanni* 4797 (ATCC). Thiomalic acid was used as a reducing agent in the media.

Quantitative estimation of effect of rutin on biological potency of vitamin C EARLE W. CRAMPTON AND LEWIS E. LLOYD* *Dept of Nutrition, Macdonald College, P. O., (McGill University), Quebec, Canada*

Considerable evidence has been advanced in the literature that rutin, and other vitamin P-active materials, carry out their physiological function as antioxidants. The beneficial effect of some of these materials on vitamin C has been shown, but the results have been of a qualitative nature. This study, involving the administration of vitamin C to 128 guinea pigs, was designed to determine the extent to which rutin enhanced the apparent biological value of ascorbic acid. The odontoblast

method of vitamin C bioassay was used. Rutin increased the apparent biological value of ascorbic acid by approximately 50%, when the vitamin was supplied in sub-maximum amounts, either in crystalline form or from a natural source. As the level of ascorbic acid intake approached that which without rutin will permit normal tooth development, the beneficial effect of added rutin was negligible. These results could be explained by the assumption that rutin prevented *in vivo* oxidative destruction of the administered ascorbic acid.

Effect of dietary restriction and rehabilitation on weight of adrenal gland of male albino rat

ESTHER DaCOSTA and RUTH CLAYTON (introduced by CARL J KOEHN) *U S Army Med Nutrition Lab, Chicago, Ill*

Weights of the adrenal glands of 340 male albino rats were determined after 3 types of dietary restriction (low calorie, modified carrot, and high salt-low protein) and 3 types of dietary rehabilitation (high protein, high fat, and high carbohydrate) following each type of restriction. The average weight of the adrenals of 54 control rats was 75 mg, of 24 rats on the low calorie diet, 66 mg, of 24 rats on the carrot diet, 49.5 mg, and of 24 rats on the high salt diet, 44.5 mg. On an organ/body weight basis instead of the absolute weight, a significant increase was found during restriction, particularly in the group fed the low calorie diet. During the first 4 days of rehabilitation, absolute adrenal weights and adrenal/body weight ratios increased in all rats to values exceeding those of the controls and even after 97 days of rehabilitation only the adrenal weights of the rats rehabilitated on the high carbohydrate diet had declined below that of the controls. In these rats, the average adrenal/body weight ratio was 15.2 as compared to a ratio of 18.7 for the control. The difference in adrenal weight response to different rehabilitation diets may indicate the beneficial value of high protein or high fat as compared to high carbohydrate in rehabilitation therapy.

Metabolism of cobalt 56 in the hen's ovary

H H DARBY (introduced by E M NELSON) *Carnegie Inst of Washington, Dept of Terrestrial Magnetism, Washington, D C*

With the publication of the fact that cobalt is incorporated in vitamin B₁₂ and that B₁₂ is found in the droppings of chickens, it became of interest to study the fate of cobalt fed to hens. It had already been shown that increased growth could be obtained by injecting small amounts of B₁₂ into developing eggs. This would lead one to believe that there was a shortage of B₁₂ in the average hen's diet. Cobalt 56 was fed in pellet form to 2 hens and the eggs collected for some 3 weeks thereafter. The eggs were tested as a whole first for

radioactivity and then they were separated into shell, albumen, and yolk. The amount of cobalt in each fraction was ascertained. The shell and the albumen followed a typical excretory pattern. There was a rise of radioactive cobalt in the shell and the albumen on the 4th-5th day, followed by a fall to almost zero by the 10th day. The rise of radioactive cobalt in the yolk, however, was somewhat slower and although there was a high level for some 10 days, no fall of cobalt was found similar to that in the albumen and the shell. It leads one to believe that the yolk of the egg follows a different pattern of metabolism than does that of the shell and the albumen. From these experiments it seems that cobalt once laid down in the yolk is retained in these formative eggs.

Excretion studies of biotin in urine and feces of normal and protein-depleted rats

ROBERT L DAVIS* and BACON F CHOW *Dept of Biochemistry, School of Hygiene and Public Health of Johns Hopkins Univ, Baltimore, Md*

Biotin requirements of rats can be met by bacterial synthesis in the intestinal tract. This investigation deals with the effect of dietary proteins on this bacterial synthesis of biotin as reflected by its excretion. Male adult rats, which were protein-depleted as evidenced by blood protein concentration and weight loss, were placed in metabolism cages as were casein diet controls. Urine and feces, collected every 4 days, were assayed for free biotin using *Lactobacillus arabinosus* 17-5. Throughout this experiment, both groups received a biotin-free vitamin supplement. Fecal results were also expressed in mg biotin/gram wet feces. Some differences were noted re-

COLLECTION PERIOD	BIOTIN GIVEN	MODE OF ADMIN
1st (Baseline)	none	Intramuscular
2nd	10 mg every 4 days	
3rd	10 mg every 4 days	Oral
4th (Baseline)	none	

garding urinary and fecal excretion of biotin in the protein-depleted and normal rats when this vitamin was withheld or the mode of administration varied. 1) Urinary and fecal biotin was greater in the casein diet rats during the initial baseline period. 2) The administration of biotin either *im* or orally resulted in no marked difference in the urinary excretion of this vitamin between the two groups. 3) When biotin was given orally, fecal biotin was considerably higher in the protein-depleted rats. 4) During the final baseline, urinary and fecal biotin was about equal in both groups.

Methionine deficiency by force-feeding J N WILLIAMS, JR,* A E DENTON,* FUNG-HAAN FUNG* AND C A ELVEHJEM *Dept of Biochemistry, College of Agriculture, Univ of Wisconsin, Madison*

In the course of investigations concerning the effects of a methionine deficiency upon general metabolism of the young growing rat, a force-feeding procedure was undertaken. One group of rats was force-fed a complete ration in which protein was supplied as an amino acid mixture. Another group was force-fed the same ration in the same amounts except that methionine was omitted. Nitrogen balance of the 2 groups of animals was determined. The animals of all groups receiving the complete ration gained weight and remained in strongly positive nitrogen balance. The rats force-fed the methionine-free ration for up to 3 weeks retained the same weight and remained in strongly positive nitrogen balance throughout the period. Moreover, they showed no tendency to go into negative nitrogen balance. The methionine-deficient group, however, exhibited some striking differences from the control group in other respects: bleeding of the feet and around the mouth, fatty livers, and very low liver xanthine oxidase activity.

Inhibition of chick bone marrow choline oxidase by aminopterin in vivo JAMES S DINNING,* CECILIA K KEITH* AND PAUL L DAY *Dept of Biochemistry, Univ of Arkansas School of Medicine, Little Rock*

A study was made of the effects of aminopterin on adult chick bone marrow cell counts, peripheral blood counts, and on the oxygen consumption of marrow cell suspensions. After 6 daily injections of 1 mg of aminopterin the tibia and femur marrow cell counts were reduced to 11% of control values, blood leucocyte counts to 18% of control values, and red cell counts to 63% of control values. Marrow cell suspensions from aminopterin treated chicks consumed an average of $11.5 \mu\text{l O}_2/10^8$ cells/hr, similar preparations from control chicks consumed an average of $5.4 \mu\text{l O}_2/10^8$ cells/hr. The addition of choline to cell suspensions from control chick bone marrow increased oxygen consumption 28% while the addition of choline to cell suspensions from aminopterin treated chick bone marrow depressed oxygen consumption 18%. The addition of sodium succinate to cell suspensions from control and aminopterin treated chick bone marrow increased oxygen consumption 22% and 21% respectively. These data suggest that normal chick bone marrow exhibits choline oxidase activity and that the choline oxidase activity is completely inhibited by aminopterin. Barron *et al* (*J Exper Med* 87: 489, 1948) have shown that the nitrogen mustards, which produce a

leucopenia, strongly inhibit oxidase, and we have found that urethane, another substance which lowers white cell count, inhibits choline oxidase *in vitro*. In view of these findings it is suggested that the choline oxidase enzyme system plays a significant role in blood cell formation.

Metabolism of ascorbic acid in rats I Influence of age and diet DOROTHY A EHMEKE,* BESSIE L DAVEY* AND E NEIGE TODHUNTER *Research Lab of Human Nutrition, Univ of Alabama, University*

Earlier studies showed plasma and whole blood levels of ascorbic acid in mature rats were higher for males than females (*J Nutrition* 31: 573, 1946). The present study was undertaken in an attempt to determine the cause of the difference. Groups of rats were analyzed at 1) 63 days and 2) 112 days of age. Each age group was subdivided and received for 3 weeks prior to analysis one of the following diets: a) stock diet of laboratory checkers, containing some vitamin C, b) synthetic vitamin-C free diet, c) stock diet plus 50 mg ascorbic acid daily. There were approximately 10 rats of each sex in each diet group within each age group. Analyses for ascorbic acid were made in plasma, whole blood and white cell-platelet fraction, hematocrit and hemoglobin were also determined. The following organs were analyzed: liver, adrenals, kidneys, ovaries and testes. 24-hour urine collections were made for 3 days immediately prior to death of the animals. Maturity does not appear to be a determining factor in ascorbic acid in blood. Plasma, whole blood and white cell-platelet values were lower for these young females than for males. Intakes of 50 mg ascorbic acid daily caused no increase in blood ascorbic acid and apparently no increase in the organ stores. Urinary excretion of ascorbic acid increased when ascorbic acid was administered, but not in proportion to the amount fed. The same factors are being studied in senile rats.

Regression of lymphosarcoma transplants following the administration of cortisone to riboflavin-deficient mice GLADYS A EMERSON, ELIZABETH WURTZ AND MARY E ZANETTI* *Merck Inst for Therapeutic Research, Rahway, N J*

Heilman and Kendall reported that the administration of compound E (cortisone) to mice with lymphosarcoma transplants resulted in a regression of tumor tissue; however, the tumors recurred following withdrawal of the drug and the animals succumbed despite the resumption of therapy. Stoerk and Emerson observed a regression of lymphosarcoma transplants in riboflavin-deficient mice; furthermore animals so treated were refractory to subsequent transplants. Therapy with cortisone superimposed upon riboflavin avitaminosis might be expected to bring about a sup-

pression of tumor growth with minimal injury to the host. Accordingly cortisone and other adrenocorticosteroids were administered to mice with well established (13 to 16-day post-inoculation) lymphosarcoma transplants. The animals were maintained on either riboflavin-deficient or natural food rations. A rapid regression of tumor tissue occurred in mice receiving 500 μ g of cortisone daily in conjunction with a riboflavin-deficient diet. The tumors were non-palpable following 4 days of treatment while the transplants of controls (deficient in riboflavin) were still apparent at 10 days. When the same level of cortisone was given to mice fed a stock diet only a transient retardation of tumor growth occurred and the animals succumbed despite continuous treatment. ACTH (500 μ U) when given in divided dosage (twice daily) to mice maintained on the riboflavin-deficient diet, failed to enhance the effect of the avitaminosis upon tumor growth. A regression of transplants was not observed following daily administration of 500 μ g of Compound S acetate or of 500 μ g of desoxycorticosterone acetate.

Influence of dietary protein on tumor production by 2-acetylaminofluorene R. W. ENGEL *Lab of Animal Nutrition, Alabama Polytechnic Inst, Auburn, Ala*

Weanling female rats of the AES strain were fed a basal diet containing 03% 2-acetylaminofluorene. The diet consisted of corn grits 20, salts 4, lard 15, cod liver oil 1, water-extracted casein 9, l-cystine 0.3, sucrose 50.7 and was supplemented with adequate amounts of thiamine, riboflavin, pyridoxine, pantothenate, choline, inositol, niacin and alpha-tocopherol. This diet consistently produced mammary tumors in an average of 20 weeks if the body weight was 165 gm or more at 16 weeks. When body weight was less than 165 gm as a result of voluntary food restriction the mammary tumor incidence was only 25%, the remainder of the animals dying with face or liver tumors in 24-30 weeks. Increasing the dietary casein to 20% assured the attainment of body weights of 165 gm or more in 16 weeks and resulted in the consistent appearance of mammary tumors. When the casein was increased to 40% body weights averaged 185 gm in 16 weeks and 50% of the animals had not developed external tumors at 32 weeks while 25% had mammary tumors and 25% had face tumors. When the casein in the diet was increased to 60% body weight gains averaged 165 gm in 16 weeks but all animals were still tumor-free after 33 weeks at which time all control animals had died with mammary or face tumors.

Failure of folic acid or xanthopterin to replace thiamine in mice and rats maintained on thiamine-deficient diet ALBERT J. EUSEBI* AND

LEOPOLD R. CERECEDO *Dept of Biochemistry, Fordham Univ, New York City*

The relationship between thiamine and certain pterins, particularly folic acid and xanthopterin, studied by Busnel and co-workers (*Compt rend Soc de biol* 224:237, 1947) in pigeons, was investigated in mice and rats. The basal diet consisted of purified casein, 25%, sucrose, 53, Crisco, 10, lard, 5, salts, 5, and Ruffex, 2. The following supplements were added/kg of diet: pyridoxin, 10 mg; riboflavin, 10 mg; calcium pantothenate, 100 mg; alpha-tocopherol, 40 mg; beta-carotene, 20 mg; choline 1.5 g; and vitamin D 5000 IU. Swiss, Rockland and Fordham mice were used. Folic acid was administered orally by incorporation in the diet at a level of 20 mg/kg, or by subcutaneous injection at a level of 50 γ /day. Young mice were placed on the thiamine deficient diet until depleted. At this time the experimental group received folic acid orally or by injection, and the controls maintained on the deficient diet. Ten control mice survived for periods of 23-30 days. Sixteen mice receiving folic acid survived from 17-31 days. Further, the weights of the animals receiving folic acid ran parallel to those of the controls. Once the mice were deficient in thiamine, folic acid failed to restore the appetite or the body weights. These results were confirmed with young Sherman rats to which both folic acid and xanthopterin were administered. As in the case of the mouse, these pterins failed to exhibit any 'thiamine-like activity' in the rat.

Human digestibility studies suggesting unexpected limitations in use of certain common foods as sources of riboflavin GLADYS EVERSON, ELINOR PEARSON* AND ROBERTA MATTESON* *Nutrition Lab, Home Economics Section, Iowa Agricultural Experiment Station, Iowa State College, Ames, U S Bureau of Human Nutrition and Home Economics*

During earlier studies concerned with the apparent availability of the riboflavin of green peas, ice cream, soybeans and almonds, we observed significant differences in the urinary excretion of riboflavin when one mg quantities of this vitamin were supplied from the above food sources. These findings have led us to investigate the degree to which these foods are digested by young adult women. Nitrogen, crude fiber, fat and heat of combustion values have been determined on the feces of 4 young adult women tested during a series of five 9-day metabolism experiments. Each test food has been incorporated into an adequate mixed diet so that the subjects consumed 2 average serving portions of the food/day. The basal diet was adjusted from one test period to another so that the total intake contributed the same dry food weight daily. The results of these studies have revealed surprisingly large fecal losses of

calories and fat. Nitrogen losses have been increased when certain of the test foods were consumed. We believe these differences in digestibility explain in the main the variations in urinary riboflavin noted during our past experiments and that incomplete digestion of certain of these foods has caused them to be of reduced value as sources of riboflavin.

Studies on fat deposition and nitrogen metabolism in four strains of mice PAUL F. FENTON AND CLAIRE J. CARR * *Dept. of Biology, Brown Univ., Providence, R. I.*

Earlier investigations have suggested the possibility that rations could be devised which might be ingested by the mouse in quantities sufficient to lead to obesity. Body weight gains were determined in 4 strains of mice (C57, I, A and C3H) fed highly purified rations differing in the amount of protein, fat and carbohydrate. Increasing the fat content of the diet above 5% resulted in greater weight gain. Mice fed a diet containing 90% casein grew more slowly than mice fed a ration containing only 30% casein. When lactalbumin was used at the 90% level, growth was better than with 90% casein. In each case the nature of the weight gain was studied by carcass analysis for water, protein and fat. Making use of the diet which so far has given the most rapid weight gain, the four strains of mice were compared with respect to the efficiency of food utilization for growth. The weight gain per gram of food consumed was greater for the C57 than for the other three strains. Nitrogen metabolism has been studied in the 4 strains of mice by two methods: a) by determining the body weight gain per gram of nitrogen ingested (making use of a 10% protein diet), and b) by measuring nitrogen balance. The latter was accomplished by feeding a nearly nitrogen-free diet, administering different amounts of nitrogen by stomach tube after a suitable period of depletion, and determining the fecal and urinary nitrogen.

Use of the mealworm, *Tenebrio molitor*, in nutritional studies G. FRAENKEL *Dept. of Entomology, Univ. of Illinois, Urbana*

The technique of using mealworms as subjects in nutrition research is described in detail. Valid and repeatable results can only be obtained if the temperature and relative humidity of the culture room are rigidly controlled. At 25° C and 70% relative humidity, on an optimal diet, larvae grow from 0.5 mg to 120–150 mg during a period of 2½ to 3 months, but during the period of fastest growth, between 10 and 100 mg, weight is nearly doubled every week. The rate of growth varies greatly with the water content of the food, being fastest when in equilibrium with 70–80% R.H., and virtually at a standstill at 13% R.H. *Tenebrio* requires 80–85% of a carbohydrate in the diet, the

requirements being equally satisfied with glucose or starch, also a suitable sterol, but no fat or fat soluble vitamins. Of proteins casein, lactalbumin, peanut protein or edestin give optimal effects, soy bean protein is of moderate value, and only after extensive heating, zein or gliadin fail to support growth, the deficiencies being only partly rectified by the addition of lysine and tryptophane. On wheat flour, even from whole wheat, growth is very slow, on account of a partial riboflavin deficiency. *Tenebrio* requires the following vitamins of the B complex, minimum optimal amounts, expressed as µg/gm of the dry diet, being given in () —thiamin (1), riboflavin (2–8), nicotinic acid (16), pyridoxin (2), pantothenic acid (8), biotin (0.16), pteroylglutamic acid (0.12) and choline (300).

Importance of time factor upon utilization of amino acids in 'maintenance' of adult rats E. GEIGER AND E. B. HAGERTY (introduced by HARRY J. DEUEL) *Dept. of Pharmacology, Univ. of Southern California Med. School, Los Angeles, and Van Camp Seafood Co., Terminal Island, Calif.*

It has been shown earlier that growth of infantile rats occurs only if all the essential amino acids are supplied simultaneously (*J. Nutrition* 34:97). Cannon (*Federation Proc.* 6:390) has similar results investigating the repletion of protein-depleted adult rats. The repletion, i.e. repair of lost tissue, is however essentially equivalent to protein formation in growth. We have investigated the possibility that there are some basic differences regarding the time factor in the utilization of amino acids for 'maintenance' as compared with growth. Twelve adult rats were kept for 12 hours on a diet containing a tryptophan free amino acid mixture, the missing amino acid was supplied 12 hours later. In one control group the diet did not contain any amino acids, these rats lost weight progressively. In the other control group the acid hydrolysate (casein) and the tryptophan supplement have been fed simultaneously. These rats maintained their weight. To assure a satisfactory intake of the amino acids, the tryptophan free mixture as well as the tryptophan supplement, were force-fed to the paired animals. Adult rats receiving tryptophan as a delayed supplement for 21 days lost weight as fast or faster than those kept on an amino acid free diet. This indicates that 'maintenance', similar to growth, requires the simultaneous presence of all essential amino acids, i.e. the aging protein cannot be mended by a 'piece-by-piece' replacement of the individual amino acids. On force feeding of the tryptophan free amino acid mixture, the animals' appetites deteriorated progressively, indicating the damaging action (Allison, Elvehjem) of 'incomplete' mixtures.

Acetylation of PABA and of sulfadiazine by human subjects HERBERT GERSHBERG,* W JAMES KUHLE* AND ELAINE P RALLI *Labs of the Department of Medicine, New York Univ College of Medicine, New York City*

Acetylation of orally administered PABA and intravenously administered sulfadiazine was measured over a period of 7 months. The free and acetylated form of the compounds excreted in the urine was determined by the method of Bratton and Marshall. Results are expressed as the percentage acetylated of the amount recovered. In normal subjects acetylation remained fairly constant during the period studied. The percentage of PABA acetylated decreased as the dose administered increased. When the dose of PABA was 100 mg, 90% was acetylated. As the dose increased to 500 mg, the percentage acetylated decreased slightly. With doses from 500 to 800 mg sharper decline in acetylation occurred, with doses of 800 to 1700 mg, acetylation decreased further to 55%. Calculated on the basis of body weight, the sharpest decline in percentage acetylated occurred with doses from 6-10 mg/kg, and remained fairly constant up to 20 mg/kg. Administration of large amounts of pantothenic acid (10 gm daily) did not alter acetylation of PABA or sulfadiazine. Acetylation studies were done in 6 patients with cirrhosis of the liver, and 2 patients with adrenal insufficiency. The capacity of these patients to acetylate PABA fell within the normal range. Apparently in human subjects acetylation occurs principally in the tissues, and neither the liver nor the adrenal cortex are significantly involved. In patients with hyperthyroidism, however, acetylation was decreased, and the administration of thyroid to hypothyroid individuals decreased their capacity to acetylate.

Nutritional requirements of syrian hamster for growth HUMBERTO GRANADOS* AND HENRIK DAM *Dept of Biology, Polytechnic Inst, Copenhagen, Denmark*

They contain an unrecognized growth factor (s) for hamsters (GRANADOS, GLAVIND AND DAM *Nordisk Mejeri-Tidsskrift* 12: 237, 1946). The published studies on the need for growth by hamsters of dietary biotin, choline, inositol, niacin, and p-aminobenzoic acid are contradictory (SCHWEIGERT *Vitamins and Hormones* 6: 55, 1948). Two experiments with newly weaned hamsters were carried out using a basal diet containing 4% salts, 25% casein, 63.5% sucrose, and 7% lard. First experiment *group I* (control) received all chemically known vitamins in abundance. *Groups II, III, and IV* were deprived of choline, inositol or biotin, respectively. Average weight gains, in gm, during 49 days: *I*, 44, *II*, 39, *III*, 44, and *IV*, 42. Second experiment *groups V* and *V-a*

(controls) received vitamins as *group I*. *Groups VI, VII, VIII, and IX* and *IX-a* were deprived of p-aminobenzoic acid, niacin, folic acid or vitamin K, respectively. Average weight gains, in gm, during 63 days: *group V*, 56, *VI*, 53, *VII*, 57, *VIII*, 57, and *IX*, 53. The prothrombin times of blood taken at the 4th, 5th, 6th and 7th weeks were essentially the same for *groups V-a* (control) and *IX-a* (fed no vitamin K). At autopsy none of the hamsters from *groups IX* and *IX-a* exhibited hemorrhages. Thus these studies show that hamsters grow somewhat better with dietary choline, but that they do not require dietary inositol, biotin, p-aminobenzoic acid, niacin, folic acid or vitamin K for growth. Since the hamsters fed the vitamin K-free diet neither grew slower nor developed hypoprothrombinemia or hemorrhages, these results are at variance with what Hamilton and Hogan have reported, i.e., that hamsters require dietary vitamin K (*J Nutrition* 27: 213, 1944).

Interrelation of linolenate and linoleate in growth studies on rats SAMUEL M GREENBERG,* CLARENCE M CALBERT,* EVELYN E SAVAGE* AND HARRY J DEUEL, JR *Dept of Biochemistry and Nutrition, Univ of Southern California, Los Angeles*

Weanling rats were placed on fat-free diets until essential fatty deficiency was established by growth failure. The depleted rats were then divided into the following groups each consisting of 5 males and 5 females: (1) Positive controls (30% cottonseed oil diet), fat-free diets with the following daily supplements: (2) negative control (ethyl laurate), methyl linoleate in (3) 5 mg (4) 10 mg, (5) 20 mg, (6) 50 mg, methyl linolenate in (7) 10 mg, (8) 5 mg plus 5 mg linoleate and (9) 10 mg plus 10 mg linoleate. With male rats the curve for weight gain vs log-dose over a 11-week period was a straight line function. By extrapolation to the growth attained in (1), optimum level of linoleate was estimated at 100 mg daily. With female rats, optimum level of linoleate was found to be 20 mg and some growth depression results at 50 mg intake. Superior growth on fat diet in *group 1* could not be obtained with linoleate in females. Linolenate alone (7) gave only slight growth stimulus but when combined with linoleate (9) gave identical growth with equivalent dose of linoleate (5). Results of males on (8) were somewhat less than on (4). Male and female rats on (2) lost weight during the test. It is concluded that the utilization of linolenate for growth depends on the concomitant administration of linoleate and that a sex difference in utilization of essential acids obtains. In female rats fat has growth effect not attributable to linoleate.

Iodine in Nutrition

ISIDOR GREENWALD *Dept of Chemistry, New York Univ College of Medicine, New York City*

A recent review (*J A M A* 139 28, 1949) claimed that goiter in Yunnan, China was due to the cessation of the previous supply of salt from the coastal provinces, due to the Japanese occupation. As is well attested by the literature, goiter has been prevalent in Yunnan since before 1867, salt from the coastal provinces was not rich in iodine and, moreover, never or rarely reached Yunnan. The same review stated, "Baumann and Roos and also Oswald were the first to observe that if iodine is withheld compensatory hypertrophy of the thyroid occurs." There is nothing about withholding iodine in the papers cited. Both state that the concentration of iodine in the thyroids from goitrous regions was less than in those from non-goitrous districts but Oswald emphasized that the iodine content was greater in the thyroids from goitrous regions than in those from places in which goiter was less prevalent and that the total iodine was greater in goiters than in normal glands from the same districts. No one has yet demonstrated enlargement of the thyroid in animals on an iodine-poor diet of purified constituents. Enlargements have been produced with diets that must be considered to have contained goitrogenic agents. Presumably, iodine is a dietary essential but there is no accurate information regarding the amounts needed for maintenance or the effects of withdrawal.

Influence of previous diet on metabolism of rat diaphragm

R G HANSEN,* W J RUTTER* AND L T SAMUELS *Dept of Biological Chemistry, Univ of Utah College of Medicine, Salt Lake City*

Diaphragms from rats under different nutritive conditions were incubated in glucose buffer in the presence of O₂, and the changes in glucose, pyruvate and lactate were determined. If the rats had been fed a high fat diet, the diaphragms used less glucose and accumulated more pyruvate and lactate than diaphragms of carbohydrate-fed rats. If insulin was added glucose uptake and glycogen storage were increased in all diaphragms, but proportionately to the rate without insulin. The effect of fat-feeding, therefore, could not be explained on the basis of a difference in insulin content. Since there was no significant difference in O₂ uptake between the different groups, the evidence here would indicate the inflow of substrate into the oxidative cycle beyond pyruvate in the case of the fat fed rats.

Effect of surface active agents when fed in diets of rats

ROBERT S HARRIS, HENRY SHERMAN* AND WALTER W JETTER *Massachusetts Inst of Technology and Boston Univ School of Medicine, Boston, Mass*

Sorbitan monolaurate (SL), polyoxyethylene sorbitan monolaurate (PSL), polyoxyethylene monolaurate (PL), polyoxyethylene monostearate (PS) and hydrogenated oil (HO) were fed as 25% of 2 experimental diets to groups of 14 and 30 weanling rats for 60 and 70 days, respectively. *Diet I* contained casein 18, sucrose 52.7, salt mixture 4, vitamin mixture (vitamin B₁, B₂, B₆, A and D, niacin, pyridoxine, Ca pantothenate choline) 0.3 and test substance 25. *Diet II* contained casein 27, sucrose 40.5, salt mixture 4, liver extract (1:20) 1, corn oil 1, celluloflour 1, and vitamin mixture (same vitamins as diet I plus E, C, inositol, PGA, biotin, PABA) 0.5, and test substance 25. Results on *diet I* tabulated below. *Diet SL* produced nasal hemorrhage, gangrenous tails and legs, PSL and PL caused severe diarrhea, PL produced extensive bladder stones. *Diet II* produced similar results. Paired feeding of one HO group with SL group indicated SL results not due to starvation. Extra group fed 12.5% HO plus 12.5% Celluloflour indicated results not due to inert material in test compounds. Organ tissues from groups on *diet II* were studied histologically, results will be reported. The results indicate that SL, PL and PSL are toxic to rats when fed as 25% of nutritionally complete diet.

TEST COMPOUND	SL	PSL	PL	PS	HO
Wt increase (gm)	-19	45	74	88	193
Wt incr/gm diet	—	10	11	15	28
Mortality (%)	70	36	0	0	0

Comparison of the 2,6-dichlorophenolindophenol and 2,4-dinitrophenylhydrazine methods with the Crampton bioassay for determining vitamin C values in foods

ELIZABETH M HEWSTON,* MURRAY FISHER* AND ELSA ORENT-KEILES *Bureau of Human Nutrition and Home Economics, U S D A, Beltsville, Md*

The application of chemical methods to the measurement of ascorbic acid in foods, especially those processed or stored, is often complicated by the presence of interfering substances. The most troublesome of these are the reductones and diketogulonic acid which react like ascorbic acid with two commonly used reagents, 2,6-dichlorophenolindophenol and 2,4-dinitrophenylhydrazine. Since these substances are biologically inactive, the vitamin C values obtained are falsely high. To determine the specificity of the 2,6 dichlorophenolindophenol and 2,4-dinitrophenylhydrazine methods when interfering substances are present, comparison was made of the vitamin C values obtained by these two chemical procedures with those obtained by bioassay employing omdotoblast length as the criterion. Canned foods before and after storage at several temperatures were used. Diketogulonic acid does not interfere with the indophenol reaction and reductones could be

differentiated Correction of the phenylhydrazine method for diketogulonic acid and partial correction for reductones was possible Thus, suitable corrections for interfering substances could be made with the indophenol method, but not always with the phenylhydrazine method The extent of interference, methods of correction, and agreement of both methods with the biologically determined values will be reported

Role of fat in protein metabolism CECILE HOOVER* AND PEARL SWANSON *Nutrition Lab , Iowa Agricultural Experiment Station, Iowa State College, Ames*

It has been demonstrated previously in this laboratory that protein metabolism in a rat partially depleted of its reserves of nitrogen is affected not only by the energy intake of the animal but also by the source of calories in the low-nitrogen ration Omission of fat from this diet results in a doubling of the rate of protein catabolism when the caloric intake is restricted to 25% of the normal ingestion Supplementary methionine prevents this effect Extension of the study has provided data that give some insight into the mechanisms underlying the regulatory effect of fat and methionine on protein metabolism Evidence is based on studies showing the respective concentrations of moisture, nitrogen, fat, and riboflavin in the liver, and changes in the distribution of amino nitrogen and urea in the blood of rats under the dietary conditions imposed in the experiment That fat under conditions of stress may provide building blocks for certain enzyme systems is indicated

Relation of vitamin B₁₂ to vitamin E in nutrition of young rats E L HOVE AND J O HARDIN * *Lab of Animal Nutrition, Alabama Polytechnic Inst , Auburn*

Previous work has shown that supplements of alpha tocopherol given to young rats on a low casein, vitamin E-free diet increased growth and protein utilization, gave marked protection against CCl₄ and prevented a fatal lung hemorrhage-liver necrosis syndrome which occurred after about 3 months The diets used in this work contained no added source of vitamin B₁₂, folic acid or biotin (HOVE, E L, D H COPELAND AND W D SALMON *J Nutrition* 39: 397, 1949) When boiled egg white or fibrin replaced casein as the protein source at 10%, supplements of 1 mg alpha tocopherol daily did not increase growth or give protection against CCl₄ However, on diets with defatted whole wheat or soybean meal as the protein source, vitamin E increased growth and protein utilization When vitamin B₁₂ concentrate was added at a level equivalent to 30 µg/kg to diets containing soybean meal, casein or whole wheat, the protein efficiency ratios increased

sharply and vitamin E supplements were without apparent benefit Folic acid or biotin did not influence the results Supplements of vitamin B₁₂ offered some protection against CCl₄, but under the conditions used could not replace vitamin E, the combination was more effective than either alone Against the fatal lung hemorrhage-liver necrosis syndrome vitamin B₁₂ gave little protection although in some experiments the time of onset was delayed

Use of chromium oxide as indicator of digestibility E A KANE,* W C JACOBSON* AND L A MOORE *Division of Nutrition and Physiology, Bureau of Dairy Industry, Agricultural Research Administration, U S Dept of Agriculture, Washington, D C*

It was shown by a direct comparison, that digestibility coefficients obtained with both lignin and chromium oxide ratios by total collection of feces, were in excellent agreement with those determined by the standard total collection method The next problem was to determine how many samples of feces/day are necessary to attain the same accuracy and reliability with the ratio technique as with the standard total collection method In order to obtain fundamental data on this point, the following experiment was undertaken Around 15 gm of chromium oxide had been fed to 3 cows for approximately 90 days in connection with the previous trials Then, during a period of 24 hours, each passage of feces from each cow was collected, mixed, and sampled Each sample was analyzed separately for dry matter and chromium oxide The percentage of chromium oxide in each passage from each cow was determined and averaged for the 24-hour period, the averages for the 3 cows being 0.370, 0.350, and 0.367%, respectively These results compare favorably with the averages of total daily collections for the 9-day period immediately preceding this 24 hour experiment, which were 0.375, 0.354, and 0.362%, respectively The range of variation in percentage of chromium oxide in the feces of the 3 cows, during the 24-hour period, was 0.060, 0.081, and 0.082%, respectively An analysis of variance revealed a highly significant difference between cows and between A M and P M samples Therefore, the time of day of sampling, in addition to the number of samples, is important and should be considered in deciding on a reliable procedure for accurate sampling of feces for digestibility studies where the ratio technique is used

Improvement in the nutritive value of milled rice MARINUS C KIK *Univ of Arkansas, Fayetteville*

The proteins of milled rice rations (percentage composition milled rice, 89, salt mixture, 4, cellulose flour, 2, wheat germ oil, 3, cod liver oil, 4) were

supplemented by those of dry skim milk, dry whole milk, and dry whole egg. The rations were *ad libitum* and paired fed to rats receiving daily an ample supply of members of the vitamin B complex. Six males and 6 females were used in experiments of 90 days duration. The supplements were fed at a 1, 3, and 5% level replacing an equivalent amount of protein in the milled rice ration. The rice ration had a protein content of 5.46%, milled rice, 6.14%, dry skim milk, 33.8%, whole milk, 24.0%, and whole egg, 45.2%. The average difference in gain/1000 gm of food intake in the *ad libitum* feeding experiments at the 1, 3, and 5% level was for dry skim milk 14, 27, and 35 gm respectively. For whole milk 1, 27, and 31, and for whole egg 5.2, 23.3, and 18.7 gm. Lower values were found in the paired feeding experiments. The quality and quantity of the milk solids proteins favorably influence the nutritive value of milled rice, as do also their vitamin A, thiamine, riboflavin, and calcium content.

Utilization of a pantothenic acid conjugate (PAC) for rat growth TSOO E. KING,* FRANK M. STRONG* AND VERNON H. CHELDELIN, *Dept of Biochemistry and the Enzyme Inst., Univ of Wisconsin, Madison*

A pantothenic acid conjugate (PAC) described by the authors has been found more active than the free vitamin in certain microorganisms (KING, FELS AND CHELDELIN, *J Am Chem Soc* 71: 131, 1949). It exists in various animal tissues. The present experiment was designed to test the degree of activity of the conjugate in growing rats. Eighty albino rats of the Sprague-Dawley strain, of body weight 40 ± 2 gm, were separated into 11 groups of 6 to 10 animals each. They were maintained on a purified diet which was supplemented with graded amounts of PAC or calcium pantothenate, administered either orally or intraperitoneally. PAC was prepared according to previously described methods and was freed from coenzyme A and from free pantothenic acid by prolonged dialysis against distilled water. The intraperitoneal administration of a sample of PAC containing 5% bound pantothenic acid showed that the conjugate was at least as active as equivalent amounts of pantothenic acid. PAC by oral administration was slightly less effective than the free vitamin. The feasibility of developing a rat growth method for total pantothenic acid in tissues is discussed.

Lysine deficiency in rats fed zein diets W. A. KREHL AND DAVID KLIGLER,* *Yale Nutrition Lab, Dept of Physiological Chemistry, Yale Univ., New Haven, Conn.*

Attempts were made to produce an uncomplicated lysine deficiency in growing albino male and female rats of the Sprague-Dawley strain. Even when zein was supplemented to theoretically ade-

quate levels with missing amino acids and used in an otherwise adequate diet, it did not promote optimum growth. Experiments were conducted to determine the reasons for this observation. Supplementation of the zein diet with additional essential amino acids and a variety of growth factors, proteolytic hydrolysis of the zein, and supplementation with small amounts of an adequate protein (casein) were all attempted. The results obtained indicate that lack of growth in rats fed supplemented zein diets can be attributed to inadequate digestion of the protein with the result that certain of the essential amino acids are released too slowly to be absorbed and join the metabolic pool of other essential amino acids. Addition of arginine, valine and threonine simultaneously provided the additional essential amino acids necessary for protein synthesis required for growth. Animals on the lysine deficient diet, in addition to their failure to grow, exhibited marked symptoms of deficiency characterized by ruffled hair, unsteady stance and gait and severe emaciation. These symptoms but not the growth failure were corrected by lysine supplementation. Histological studies of the liver, heart, kidneys, adrenals, and thyroid of the 'lysine-deficient' animals revealed no pathology although the liver appeared to contain less than the normal amount of fat.

Acetylation studies in patients with rheumatoid arthritis W. JAMES KUHLM,* HERBERT GERSHBERG* AND ELAINE P. RALLI, *Labs of Dept of Medicine, New York Univ College of Medicine, New York City*

The acetylation of PABA and of sulfadiazine by patients with rheumatoid arthritis was determined and compared to that of normal subjects. Acetylation was measured either by the oral administration of 500 mg of PABA or by the intravenous injection of 1 gm of sulfadiazine. Eight normals, and, up to the present, 8 patients with rheumatoid arthritis have been studied, both before and after the administration of calcium pantothenate. In both the normal subjects and in the patients with rheumatoid arthritis, the ability to acetylate was variable, but remained constant in a given individual. As measured by PABA the degree of acetylation of the amount excreted in the urine varied from 82-96% in both groups. Following the injection of sulfadiazine, from 7-20% of sulfadiazine excreted as acetylated. The oral administration of 10 gm of calcium pantothenate daily for periods of from 3 weeks-4 months to patients with rheumatoid arthritis did not appreciably influence the acetylation of either PABA or sulfadiazine. The results suggest that acetylation is not disturbed in patients with rheumatoid arthritis.

Urinary loss of amino acids in normal and undernourished males following single injection of amino acid mixture STANLEY LEVEY, ELLEN D HOGANSON AND JOHN E HARROUN (introduced by ARTHUR H SMITH) *Dept of Physiological Chemistry, Wayne Univ College of Medicine, Detroit and Wayne County General Hospital, Eloise, Mich*

The excretion of 9 amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine) was determined in the urine of 4 normal and 4 undernourished male subjects following the intravenous infusion of an assayed amino acid mixture. The amino acids were estimated using microbiological methods. The degree of undernutrition in the subjects studied ranged from moderate to severe as shown by clinical and laboratory studies. No difference was found in either the excretion of the individual amino acids or the relative excretion pattern which could be correlated with the nutritional state of the subjects. The amount of any amino acid retained was considered to be the difference between that injected and that amount excreted in the urine. The retention of the individual amino acids studied ranged from 99% for arginine to 81% for threonine. Retention of the individual amino acids could not be correlated with the nutritional status of the subject. The excretion of bound amino acids (those freed by acid hydrolysis) was also studied. In spite of the fact that the infused amino acid mixture was free of bound amino acids, an increased urinary loss of bound leucine, lysine, and valine was found following the injection of this amino acid preparation as compared to the urinary loss following an infusion of physiological saline. The 'saturation or load' test used for the evaluation of the nutritional state in regard to certain vitamins, does not appear applicable for studying protein nutrition.

Studies on vitamin A deficiency in the mouse

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A study of vitamin A deficiency was made in 3 strains of white mice in order to determine what changes occur throughout the body, and whether these changes are the same as those described for the rat. Weanling mice placed upon a 30% casein, 15% fat, vitamin A-free diet grew normally and did not exhibit the typical vitamin A deficiency syndrome, although rats responded to this regime, within 4 to 7 weeks. In order to decrease the depletion period, and to secure uniformity in the time of appearance of symptoms, different dietary regimes were devised. By varying the nutritional background of the weanling it has been possible to set up conditions under which 80% of the young

mice will develop the typical deficiency syndrome within a relatively narrow range. The time of appearance of symptoms is determined by the stringency of pre-natal and lactational treatment. The effect of vitamin E-free and fat-free diets on the depletion period was also investigated. Histological studies were carried out on various tissues. Reproduction studies indicate that the reproductive function in the male has been virtually destroyed by the absence of vitamin A from weaning. In the female, the reproductive function has been greatly impaired, but not destroyed. The young of such females are viable. In a study of the minimum daily requirement, our data indicate that the minimum requirement is in the neighborhood of 1 IU per day.

Effect of various amino acids and vitamin B₁₂ on chick growth L J MACHLIN,* J L MILLIGAN,* C A DENTON* AND H R BIRD *Bureau of Animal Industry, U S Dept of Agriculture, Beltsville, Md*

Amino acids and vitamin B₁₂ were investigated as supplements to corn-soybean meal and cottonseed meal diets for growing chickens. Protein concentrates were added so as to supply 22% protein. In addition the diets contained 3% alfalfa leaf meal, butyl fermentation solubles, tricalcium phosphate, limestone, salt, manganese sulfate, niacin, and a vitamin B₁₂ concentrate on charcoal. Choline was added to the cottonseed meal diet. The diets were assayed microbiologically for all essential amino acids. The soybean meal diet contained 0.22% methionine and the cottonseed meal diet 0.7% lysine. Addition of 0.1% DL-methionine to the soybean meal diet gave maximal growth response of the chicks. A level of 0.8% DL lysine monohydrochloride gave the best growth on the cottonseed meal diet. No additional growth was obtained when amino acids other than methionine or lysine were fed although tryptophane and glycine levels in both diets and methionine and tyrosine levels in the cottonseed meal diet were on the border line. In studying the effect of B₁₂, chickens from animal-protein-depleted dams were used and the B₁₂ concentrate omitted from the basal diets. Average weight in gm at 5 weeks in a typical experiment were: soybean meal basal 253, plus B₁₂ 304, plus methionine 282, plus B₁₂ and methionine 335, cottonseed meal basal 200, plus B₁₂ 217, plus lysine 296, plus B₁₂ and lysine 311. When B₁₂ was replaced by an animal protein factor concentrate the chickens weighed 267 without and 359 with lysine.

Variations in nitrogen-balance data on college girls BEULA V McKEY,* DOLORES MALONEY COREY* AND JANICE M SMITH *Agricultural Experiment Station, Dept of Home Economics, Univ of Illinois, Urbana*

Five girls, 19-23 years of age, cooperated in a study of the reproducibility of nitrogen balance data from September, 1947 to June, 1948. During this time, determinations of nitrogen balances were compared with values obtained on the same subjects 3-5 months later. In 11-day test periods, 3.2, 2.4, 1.9, 1.4 or 0.2 gm nitrogen daily were fed. Except at the lowest intake, nitrogen was supplied by bread, rolled oats, lean beef, light cream and potatoes. The composition of the basic diet and proportions of protein foods are described in the publication by Bricker *et al* (*J Nutrition* 37: 163, 1949). The average nitrogen balance for all subjects at the 2nd time of feeding was significantly lower than at the 1st in 4 of the 5 comparisons (exception at 1.9 gm). These average nitrogen balances (expressed in gm/day) with the standard errors for the 1st and 2nd tests respectively, were as follows for the 5 nitrogen levels: 3.2 gm, -0.403 ± 0.0960 , -0.008 ± 0.0534 ; 2.4 gm, -0.219 ± 0.0389 , -0.028 ± 0.0464 ; 1.9 gm, -0.654 ± 0.0571 , -0.319 ± 0.0395 ; 1.4 gm, -1.1059 ± 0.0498 , -0.815 ± 0.0436 ; 0.2 gm, -1.814 ± 0.0274 , -1.488 ± 0.0418 . Biological values were 61 ± 4.0 , 74 ± 2.8 and $85 \pm 2.9\%$ at the 3.2, 2.4 and 1.4 intakes respectively. These biological values were significantly different in all possible comparisons indicating that the biological value estimated in this study was influenced by the amount of nitrogen fed.

Studies on lactalbumin heated with carbohydrate

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A comparison of *in vitro* and *in vivo* studies was made in an attempt to evaluate the role of heat and carbohydrate concentration in the non-enzymatic browning of proteins as exhibited in the Maillard Reaction. Commercial and 'carbohydrate-free' lactalbumin, heated under various conditions, was used in *in vitro* digestion experiments. The conventional formol titration procedure, showed that the rate and amount of digestion of the protein by pancreatin is inversely proportional to the severity of heat treatment. Removal of reducing substances from lactalbumin before heating permits a greater rate and degree of enzyme hydrolysis of the heated product. Microbiological assays for valine, histidine, leucine, phenylalanine, lysine and methionine in acid hydrolysates of the various proteins showed that only lysine was partially destroyed. These observations suggest that the carbohydrate reacts with the protein to form enzyme-resistant materials. A nitrogen balance study in dogs was used to determine the relative effect of heat and of carbohydrate in the alteration of the nutritive

value of heated protein. 'Carbohydrate-free' lactalbumin and extracted lactalbumin to which had been added 2 and 5% lactose served as the protein nitrogen sources. The time of autoclaving of these proteins was varied from one-half to one hour. The biological value was high in all cases. However, as either the carbohydrate concentration or the time of heating was increased, the nutritive index of the protein decreased. Lowered digestibility, shown by increased fecal nitrogen, accounted for the decrease in nutritive value.

Chronic riboflavin deficiency in Cebus monkeys

GEORGE V. MANN AND PATRICIA L. STEVENS (introduced by FREDRICK J. STARE) *Dept of Nutrition, Harvard School of Public Health, and Dept of Biological Chemistry, Harvard Med School, Boston, Mass*

Young Cebus monkeys were studied upon a purified diet with and without the addition of crystalline riboflavin. Without added riboflavin the diet furnished 25 γ or less of riboflavin per 100 gm of diet. With added riboflavin this diet is adequate for periods of at least 2 years. Without added riboflavin animals on this diet began a steady decline of weight within 2 weeks. Although appetite remained good and food consumption 80% or more of normal, after 6 months weight loss amounted to 30%-40% of the original weight. No mucous membrane or ocular lesions were seen. In one of 6 animals a seborrheic dermatitis with yellow encrustations developed over the dorsum of the hands and on the tail with slight loss of hair. In another instance a pre-existing fungal dermatitis showed extension and exacerbation. Plasma levels of free and combined riboflavin decreased to levels 20% of normal. The sequence was diminution of free riboflavin plus flavin-mononucleotide followed by a fall of flavin-adenine-dinucleotide. Total 'flavin' concentration reached a minimal level of 0.5-1.0 $\gamma\%$ after 80-100 days and remained there until death. Hematological studies reveal no evidence of anemia but rather of a polycythemia which progresses until death. Cell indices, white blood count, and differential remain unchanged. Plasma volume and total protein determinations indicate no contraction of fluid volume to account for this polycythemia. Glucose tolerance tests reveal no abnormality of carbohydrate metabolism. Response to riboflavin suggests that minimal daily requirement for growth is between 30 and 50 γ /kg/day.

Nutrient intake and serum blood levels of children showing physical signs of vitamin deficiencies. SUSAN B. MERROW,* R. F. KRAUSF,* J. H. BROWE,* C. A. NEWHAIL* AND H. B. PIERCE, *Depts of Biochemistry and Anatomy, Univ of Vermont College of Medicine, Burlington*

Twenty-four hour food intake records were secured in the winter, spring and fall of 3 consecutive years as part of a study investigating relationships between physical findings, blood chemistry and food intakes of school children. All subjects exhibited generally accepted signs of vitamin deficiencies attributed to inadequate amounts of vitamin A, riboflavin, niacinamide and/or ascorbic acid. Three groups of subjects were selected that had 'high' or 'low' blood levels of vitamin A, carotene or ascorbic acid during the 3 years. The vitamin A, carotene and ascorbic acid intake of the higher and lower blood level groups for each of the above mentioned 3 blood constituents were compared. Study was also made of the blood values of subjects whose nutrient intake for each of the 3 food constituents was above or below a certain value. Blood levels of these 3 nutrients were compared for each of the 3 higher and lower nutrient intake groups. The higher dietary intakes of carotene, total vitamin A, and ascorbic acid appear to be more indicative of the higher blood levels of these 3 nutrients than the lower dietary intakes of carotene, total vitamin A and ascorbic acid are indicative of the lower blood levels of these same nutrients. Blood content of vitamin A and carotene appear to be a poor indication of dietary intake of these nutrients. The higher blood values of ascorbic acid reflect dietaries of above 40 mg of ascorbic acid and the lower blood values dietaries under 40 mg of this nutrient.

Physiological and histological changes in the folic acid-deficient duckling O NEAL MILLER, J W GODDARD AND ROBERT E OLSON (introduced by FREDRICK J STARE)

In addition to anemia, folic acid deficiency in ducklings causes a depression in the respiration of erythrocytes and a decrease in pyruvate utilization by cardiac muscle (*Federation Proc* 8 390, 1949). The failure of these birds to gain weight despite a high food intake suggested that additional lesions might be found in the gastrointestinal tract or in its associated glands. Young ducklings were fed a purified diet low in folic acid and supplemented with sulfaguanidine. When deficiency signs became manifest, these birds were killed and samples of liver, gut, and pancreas were fixed for histological examination. Determinations of blood sugar, liver glycogen and fat, and the titre of duodenal amylase and trypsin were made at the same time. Deficient ducks showed markedly decreased liver glycogen, slightly increased liver fat, normal blood sugar levels, and markedly decreased duodenal amylase and tryptic activity. Histological and histochemical examination of the liver, pancreas, and intestine showed 1) decrease in alkaline phosphatase and glycogen in the liver,

2) clumping and reduction in number of mitochondria and dislocation and decrease in the number of zymogen granules with evidence of inhibition of their release in the pancreas, and 3) virtually no change in the intestine. It is concluded that experimental folic-acid deficiency in the duck causes biochemical and pathological changes in the liver and pancreas, which may account for some of the signs noted in this disease.

Nitrogen balance index of adult rats fed amino acid mixtures low in valine E S NASSET AND JOSEPH T ANDERSON * *Dept of Physiology and Vital Economics, Univ of Rochester School of Medicine and Dentistry, Rochester, N Y*

Nitrogen balances were determined on adult male rats fed diets containing various amino acid mixtures by stomach tube (ANDERSON AND NASSET *J Nutrition* 36 703, 1948). Each experiment included one week on a nitrogen-free diet, a second week in which the amino acid mixture was incorporated at about half-maintenance quantity, a third week at full maintenance quantity. In a single group of rats (series 140) a 'complete' amino acid mixture, simulating whole egg protein, was tested before and after whole egg protein. The nitrogen balance index of ingested nitrogen (K') computed from the half-maintenance and full-maintenance periods is 1.18 for egg protein and 0.99 for the 'complete' amino acid mixture. Another amino acid mixture containing only one-twelfth the DL-valine of the 'complete' mixture (9.0 mg DL-valine nitrogen per gram of total nitrogen) gave a K' of 0.44. A similar amino acid mixture containing the same total valine but all in the L-form gave a K' of 0.51. The significance of these values in relation to the utilization of D valine will be discussed.

Quantitative vitamin B₁₂ requirement of the pig R O NESHEIM* AND B CONNOR JOHNSON *Division of Animal Nutrition, Univ of Illinois, Urbana*

The synthetic 30% alpha-protein ration previously shown to be deficient in vitamin B₁₂ was fed in 2 experiments to groups of 2-day old pigs. The first experiment was designed to determine the quantitative vitamin B₁₂ requirement when the vitamin was given intramuscularly, and the second, when vitamin B₁₂ was given orally. In experiment 1, 14 two-day-old pigs were individually fed the basal diet for a 2-week depletion period. They were then allotted to 5 groups. Groups 1-5 were given by i.m. injection respectively 0, 0.1, 0.3, 0.4 and 0.6 µg of crystalline vitamin B₁₂/pig/kg of body weight daily. The gains of the pigs in group 5 were significantly greater than those in any other group and appeared optimum. Thus the requirement is not less than 0.6 µg of vitamin B₁₂/kg body weight/day. In

experiment 2, 15 two day-old pigs were allotted to 5 groups. Groups 1-4 received in their diets, respectively, 10, 15, 20 and 10 μ g of crystalline vitamin B₁₂/kg of dry matter of the diet, and group 5 was given intramuscularly 0.6 μ g of crystalline vitamin B₁₂/kg body weight daily/pig to correlate the 2 experiments. The results (weight gains, blood and bone marrow data, organ weights, and deficiency symptoms) of these experiments will be reported.

Response of *Leuconostoc citrovorum* to tryptic digests of casein CHARLES A. NICHOL* AND ARNOLD D. WELCH Dept of Pharmacology, Western Reserve Univ School of Medicine, Cleveland, Ohio

In an amino acid medium, the growth of *Leuconostoc citrovorum* SOS1 is insignificant when a tryptic digest of casein (prepared by the method of SKEGGS, *et al* J Biol Chem 176 1459, 1948) is added in amounts equivalent to 100 mg of casein/tube (10 ml). However, 12.5 mg of the hydrolyzed casein/tube potentiates greatly the response of the organism to thymidine (5-40 μ g/tube). A similar effect is exerted on the response to a highly concentrated liver fraction containing the so-called citrovorum factor (SAUBERLICH AND BAUMANN J Biol Chem 176 165, 1948). In the presence of both thymidine and the citrovorum factor, the potentiating effect of the casein hydrolysate is very striking. The substance responsible for the activity of the enzymatic digest of casein is stable at pH 12 at 100° for at least 30 minutes. Studies on the nature of the factor, its role in the growth of the organism, and its influence on the accuracy of assays for the citrovorum factor are now in progress.

Maternal nutrition and hydrocephalus in infant rats B. L. O'DELL* AND A. G. HOGAN Dept of Agricultural Chemistry, Univ of Missouri, Columbia

Female rats maintained on a synthetic diet bore a few young, less than 2%, that had hydrocephalus. The original diet, made up chiefly of casein and glucose, was recently replaced by a diet made up of Soybean oil meal 70, cerelose, 22, salts, 4, lard, 4, and the following vitamins: A, 2000 IU, D, 283 IU, E, 1 mg, K, 1 mg, thiamine, 1.6 mg, riboflavin, 1.6 mg, pyridoxine, 1.6 mg, Ca-pantothenate, 4 mg, choline, 100 mg, biotin, 20 mg. The incidence of hydrocephalus on the soybean oil meal diet was a fraction of 1%. Earlier observations had indicated that the incidence of hydrocephalus was markedly lowered when folic acid was added to the casein diet and this suggested that the incidence of hydrocephalus might be increased if the effective amount of folic acid in the diet were reduced. When the soybean oil meal diet was supplemented with a folic acid

inhibitor (crude methyl folic acid generously supplied by T. H. Jukes, Lederle Laboratories) the incidence of hydrocephalus rose sharply within 4 weeks and the results were about the same with amounts of the inhibitor varying from 1-10 mg/100 gm of diet. The total number of young born after the change was 320 and the total number of hydrocephalics was 72. The number of hydrocephalics/litter varied from 0-13.

Folic acid metabolism in normal human subjects

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The urinary excretion levels of folic acid of 33 university women consuming constant weighed basal diets and supplements of 1, 3, 5 and 10 mg of pteroylglutamic acid for a total of 313 subject-days were determined microbiologically with *Streptococcus faecalis*. On the basal diet providing 0.4 mg of folic acid daily, 29 subjects excreted a mean of 3.7 μ g (range 0.8-9.6) of folic acid/day—a mean of less than 1% of the intake. With a 1 mg test dose in 35 observations on 15 subjects, a mean of 2.7% (0.3-4.9) of the dose was excreted in the urine, a relatively small increase although this level of intake much exceeds the amounts present in ordinary diets and the provisional recommended allowance. However, with the administration of a 5 mg test dose, the percentage return rose markedly to a mean of 38% (30-46) for 59 observations, and for a 10 mg dose, to a mean of 50% (37-63) in 41 observations. Thus the rapid increase in the percentage excretion with increases in test dose size was not maintained beyond the 5 mg intake level. Human bioassays of the availability of folic acid placed 3 food sources in the following descending order: cooked frozen chicken livers, dehydrated cereal-grass tablets and dried debittered brewers' yeast, in rough correlation with the proportion of the free form of folic acid present in the test materials.

Electrolyte changes in thiamine deficiency LOUIS J. PECORA (introduced by FLOYD S. DAFT)
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Hypo- and hyperpotassemia have been reported to produce alterations in the S-T take-off and T wave in the electrocardiogram of animals and man. Thiamine-deficient rats have similar electrocardiographic changes suggesting that an electrolyte disturbance might occur in this condition. Fifteen male weanling Sprague-Dawley rats were fed a thiamine-free, otherwise adequate, purified diet. For the first 7 weeks each rat received 3 γ of thiamine daily, thereafter none. Litter mates to 10 of these rats were pair-fed the same diet containing 200 γ of thiamine per 100 gm of diet, and 5 control rats were fed the same diet *ad libitum*.

The heart body weight ratio was 0.33% for the control and pair-fed rats and 0.38% for the thiamine-deficient rats. The water content of the fat-free skeletal and cardiac muscle of each group was $77\% \pm 1$. A decreased potassium and an increased sodium level was observed in the skeletal and cardiac muscle of the thiamine-deficient rat. The pair-fed rats showed no change from the control values. The phosphate content of serum, skeletal and cardiac muscle was similar in each group. These electrolyte changes may account in part for some of the cardiac alterations observed in thiamine-deficient rats.

Effects of vitamin therapy on school children

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A study has been made of school children having one or more of the usually accepted physical signs of deficiencies of vitamin A, ascorbic acid, niacin or riboflavin for seasonal periods in each of 3 consecutive years. Children were grouped according to type and severity of lesion, sex and age (under and over 12 years of age). Four groups, vitamin A, ascorbic acid, niacin and riboflavin were established and each group was subdivided into control and therapy sections. Therapy was administered with each of the above vitamins in amounts approximately 4 times the recommended daily dietary allowances of the National Research Council. Bloods were analyzed for hemoglobin, serum protein, alkaline phosphatase, vitamin A, carotene and ascorbic acid in the spring, fall and winter according to the microchemical methods of Bessey and his associates. Vitamin A blood levels were significantly elevated by vitamin A therapy and not significantly changed by the other 3 types of therapy. Ascorbic acid therapy resulted in a significant elevation of blood ascorbic acid, but no such effect was observed with the other 3 types of therapy. The ascorbic acid therapy group had significantly lower carotene levels than control children, other types of therapy having no significant effect upon blood carotene. Hemoglobin and serum protein levels were not significantly improved by the therapy used. Children in all therapy groups showed a significantly greater increase in height and a tendency to increase more in weight than children in control groups.

Ascorbic and glucuronic acids as growth factors in chicks

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One-day old New Hampshire Red chicks were supplied a modified form of the Franklin *et al.* diet with Brigg's salt mixture at a 6% level.

The chicks were injected daily, some with ascorbic acid, others with glucuronic acid and the control groups with normal saline. Nine chicks were used in each group in the first test, conducted in summer, and 9 males and 9 females in each group in the second test, conducted in winter. In the summer test, males receiving ascorbic acid gained 31% more than controls whereas in the winter test they gained 3% less in 32 days. Females receiving ascorbic acid, however, increased markedly in weight over controls in both tests, in the first experiment the gains were 53% greater and in the second 42%. Males did not grow more with glucuronic acid in either test whereas females gained 20% more than controls in the first and 27% more in the second. In males, liver to body weight ratios increased slightly with ascorbic acid in the summer test whereas no change occurred in winter, the liver ascorbic acid concentrations remaining fairly constant. In females, the liver to body weight ratios increased in both tests with ascorbic acid and the concentration of the vitamin was also greater. These observations confirm and extend the results of other investigators and offer interesting possibilities for further research.

Effect of tryptophane depletion and repletion on enzyme activities of tissues of chick

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Deprivation of individual essential amino acids causes, in general, decreases in liver esterase and phosphatase, and in pancreatic amylase, lipase and protease, but an increase in liver catalase. When chicks were deprived of tryptophane for 2 weeks, there was a decrease in blood catalase and a rise in liver catalase. Liver esterase and phosphatase remained essentially normal but marked decreases in activity of pancreatic amylase, lipase and protease occurred. Provision of an adequate supply of tryptophane, following 2 weeks of depletion, and observations for a 4 week period, showed a gradual return to normal of blood and liver catalase, a rapid return to a normal value for pancreatic protease, and to a slightly subnormal value for pancreatic lipase. Immediately following repletion there was an upsurge of the value for pancreatic amylase to 67% above normal which gradually lowered to normal during the 4 weeks of repletion. It is of interest that during deprivation of the amino acid, values for blood catalase fall whereas those for liver catalase rise, suggesting that the enzyme either plays a different role in the 2 tissues or that the rise in the liver is at the expense of that in the blood. The data show that changes in enzyme activities caused by deprivation of tryptophane are revers-

able if an adequate quantity of the amino acid is provided

Effects of protein level, vitamin B₁₂, and folacin on utilization of methionine for transmethylation
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Weanling rats were fed choline-free diets containing 7 or 9% of casein and corn gluten meal and sufficient DL-methionine to prevent kidney lesions in a 2-week period. When 10% of gelatine was added, at the expense of sucrose no kidney damage occurred, the further addition of 0.10% DL tryptophane resulted in 100% incidence of renal damage but tryptophane without gelatine had no effect. Vitamin B₁₂ alone did not decrease incidence of kidney damage by the gelatine-tryptophane-containing diet but vitamin B₁₂ and folacin together decreased incidence 50%. A 50-100% incidence of renal injury developed in rats receiving a choline free, synthetic diet containing 60% of alcohol extracted casein. Vitamin B₁₂ and folacin, or these vitamins together with 0.64% DL methionine, did not consistently increase growth or decrease incidence of renal damage. Choline alone (0.20%) prevented kidney lesions but had little effect on growth. Choline together with vitamin B₁₂ and folacin produced normal growth and grossly normal kidney condition, ethanolamine appeared to be as effective as choline in this combination. The results indicate a significant effect of protein level on utilization of methionine and a specific relationship of vitamin B₁₂ and folacin to transmethylation in the rat.

Effect of oral administration of streptomycin on B vitamin excretion in man
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The effects of administration of streptomycin on the urinary excretion of thiamine, riboflavin, folic acid, and N'-methylnicotinamide, and on the excretion of niacin compounds following tryptophan supplements, were studied in subjects maintained for 24 to 40 days on regulated diets. After suitable control periods, streptomycin (calcium chloride complex) was administered orally for 8-12 days in doses of 1 gm 4 times daily. Within a few days, stool cultures showed either complete or partial inhibition of growth of coliform organisms. The excretion of thiamine, riboflavin and folic acid was not decreased during streptomycin administration and in one subject was increased after 6-8 days of treatment. N'-methylnicotinamide excretion was decreased in one subject and not changed in two other subjects. Excretion of pyridoxine compounds, as measured by *S. carlsbergensis*, was determined in one subject and found to be unaffected by streptomycin. The

addition of tryptophan to the control diet led to increases in excretion of quinolinic acid and N'-methylnicotinamide. This conversion of tryptophan to niacin compounds did not appear to be affected by streptomycin administration and the concomitant decrease in intestinal flora.

Interactions between ascorbic acid and plasma and amino acids
failure to find bound ascorbic acid in plasma
FREDERICK SARGENT, II AND RALPH GOLDFIN (introduced by R E JOHNSON)
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The fact that the formed elements of human blood give up their content of ascorbic acid much more slowly than does plasma led to the hypothesis that ascorbic acid was bound to a non-diffusible substance, probably a protein. Since earlier investigations had yielded conflicting data regarding the existence of bound ascorbic acid in any part of human blood, systematic physico-chemical and chemical studies were first made on plasma. The methods used were 1) measurement of rate of diffusion of ascorbic acid through a semi-permeable membrane in plasma, a mixture of amino acids, and various control solutions at 37° C, 2) ultrafiltration of plasma, 3) spectrophotometric analysis of solutions of ascorbic acid before and after addition of plasma, amino acids, glucose, and NaCl, and 4) acid hydrolysis of trichloroacetic acid filtrates of plasma after a modification of the method of Guha *et al*. The following significant findings were made 1) with gradients ranging from 0.5 to 4.5 mg % of ascorbic acid, the rate of diffusion was not significantly different in plasma, amino acids or 0.55 M NaCl, but the rate of diffusion was significantly slower in plasma than in Ringer-Locke's solution or distilled water 2) The concentration of ascorbic acid in ultrafiltered plasma was the same as that in the protein-free ultrafiltrate 3) Spectrophotometry failed to indicate the formation of a compound between ascorbic acid and plasma or amino acids 4) Plasma did not contain ascorbigen, a bound ascorbic acid described by Guha *et al*. From these investigations the authors conclude that plasma does not contain a firmly bound non-diffusible form of ascorbic acid. The presence of a loosely bound form of ascorbic acid, however, is not precluded by the methods used.

Role of vitamin B₁₂ and methyl donors in lipotropism and transmethylation in the rat and chick
A E SCHAEFER,* W D SALMON AND D R STRENGTH * *Lab of Animal Nutrition, Alabama Polytechnic Inst, Auburn*

The relation of vitamin B₁₂ to the quantitative effectiveness of monomethylaminoethanol, dimethylaminoethanol, betaine, DL-methionine and choline as methyl donors for the prevention of renal damage and fatty livers in rats and for

growth and perosis-prevention in chicks was studied. The choline requirement of weanling rats for protection against fatty livers was reduced from 0.16% to 0.10% when the diet was supplemented with vitamin B₁₂ and folacin. On the basis of methyl content monomethylaminoethanol and betaine were considerably less active for growth and prevention of renal damage and fatty livers in the rat than dimethylaminoethanol or choline when vitamin B₁₂ and folacin were omitted from the diet. In the presence of vitamin B₁₂ all the above compounds were equivalent to choline on a methyl basis. Three mols of betaine were required to replace 1 mol of choline. For the chick, betaine, DL-methionine or monomethylaminoethanol, even in the presence of vitamin B₁₂, could not replace choline for growth and protection against perosis. Monomethylaminoethanol and either DL-methionine or betaine could replace choline on a methyl basis only when supplemented with vitamin B₁₂. Vitamin B₁₂ appears to function in methylation processes.

Effect of feeding polyoxyethylene monostearate preparations on growth rate and gross pathology of weanling hamsters B. S. SCHWEIGERT,* B. H. MCBRIDE* AND A. J. CARLSON. *Division of Biochemistry and Nutrition, American Meat Inst. Fdn., Dept. of Biochemistry and Dept. of Physiology, Univ. of Chicago, Chicago, Ill.*

Weanling hamsters (4-6 weeks of age) were fed a purified diet for a 2-week preliminary period and then the effect of feeding 2 polyoxyethylene monostearate preparations (Sta-Soft and MYRJ 45) as compared to lard on the growth rate, food efficiency, mortality, gross pathology and organ weights was determined. Thirty-three animals were used in each group and the animals were kept in individual cages. The following dietary regimens were used: 5% lard, 5% Sta-Soft, 5% MYRJ, 5% lard with the food intake of the group restricted to the average amount consumed by hamsters fed Sta-Soft or MYRJ, whichever was the least, 15% lard, 15% Sta-Soft, 15% MYRJ, 15% lard with the food intake restricted as above, and 7½% lard plus 7½% CellufLOUR. The latter group was included in order to test the effect of ingesting a substance that is essentially inert nutritionally (CellufLOUR) at a level that approximated the non-fatty acid moiety of the polyoxyethylene monostearate preparations. It was concluded from these experiments that Sta-Soft or MYRJ fed at either the 5 or 15% level in the ration of weanling hamsters significantly reduced the rate of gain as compared to the results obtained with lard. This was evident after 2 weeks on experiment. A reduction in food efficiency was also observed. The retarded gains were not attributable to reduced food intake or

the caloric inertness of the polyoxyethylene fraction. Marked changes in the intestinal tract and severe diarrhea were also observed when Sta-Soft or MYRJ was fed. Mortality data, and data on the organ weights also indicated that the ingestion of these compounds was deleterious as compared to the data obtained with lard.

Amino acid intakes and excretions of primigravidae on self-selected diets BERNICE BLUM SHEFT,* HELEN OLDHAM AND THELMA PORTER. *Dept. of Home Economics, Univ. of Chicago, Chicago, Ill.*

Amino acid intakes and urinary excretions of primigravidae living at home on self-selected diets were followed for 7-day periods during each of the last 5 months of pregnancy. All food eaten was weighed, samples were saved and representative aliquots for each period analyzed for isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane, valine, arginine, histidine and total nitrogen. The intakes of other nutrients were calculated. Complete collections of excreta were made during each period. Aliquots of urine were analyzed for 'total' amino acids and nitrogen. Average daily protein intakes ranged from 59-82 gm, positive nitrogen balances were obtained when intakes were over 65 gm. For the most part, the average daily intakes of methionine were lower than the allowances recommended by Rose (*Federation Proc.* 8:546, 1949) for young men, those of the other essential amino acids were from 1-4 times those recommended. The excretions of threonine and methionine were consistently above the range found in normal adults, those of histidine were also in this category in approximately 50% of the periods of observation. The excretions of the other amino acids studied were within the normal range. No correlation was observed between the amounts of amino acids excreted and dietary intake, nitrogen balance, body weight or urine volume.

Effects of surface active agents when fed in the diet of hamsters HENRY SHERMAN,* ROBERT S. HARRIS AND WALTER W. JETTER. **Massachusetts Inst. of Technology and Boston Univ. School of Medicine, Boston, Mass.*

Sorbitan monolaurate (SL), polyoxyethylene sorbitan monolaurate (PSL), polyoxyethylene monolaurate (PL), and hydrogenated oil (HO) were fed as 5% and 15% of experimental diets to 11 groups of 36 weanling hamsters for 68 days. All diets contained casein, 24, sucrose, 61.16 or 51.16, cellufLOUR, 3, salts, 4, liver extract (1:20), 1, fortified cod liver oil, 0.3, cystine, 0.3, corn oil, 1, vitamin mixture (vitamin B₁, B₂, B₆, K, E, niacin, Ca pantothenate, choline, inositol, PGA, biotin, PABA), 0.24, and test substance, 5 or 15. One HO group was pair-fed with SL group, another re-

ceived 7.5% HO and 7.5% Celluloflour. Representative hamsters from each group were autopsied biweekly during test period, the remainder on 68th day. Experimental results are tabulated below. Hamsters fed PSL and PL developed severe diarrhea. Results of HIO pair-fed with SL indicate SL effects not due to starvation. Results of group fed 7.5% HO and 7.5% Celluloflour demonstrate inert component of test material not responsible. Results of autopsy and histological examination of animal tissues will be reported.

5% TEST COMPOUND	SL	PSL	PL	HO	HO-P
Wt increase (gm)	49	38	38	63	56
Wt incr/gm diet	12	08	07	13	15
Mortality (%)	11	40	64	17	8
15% TEST COMPOUND					
Wt increase (gm)	34			55	57
Wt incr/gm diet	12			16	18
Mortality (%)	22	100	100	8	10

The data indicate that SL, PSL, PL were toxic to hamsters when fed as 5% and 15% of a nutritionally adequate diet.

Pyridoxine and fats in metabolism HENRY SHERMAN,* LAURA M CAMPLING* AND ROBERT S HARRIS *Nutritional Biochemistry Labs, Massachusetts Inst of Technology, Cambridge*

Male weanling rats were placed on a laboratory diet consisting of Labco casein 20, corn starch 61.7, salt mixture 4 and pyridoxine-free vitamin mixture 0.3, modified by the following additions: cornstarch 14 (*diet FFPF*), plus 0.4 mg pyridoxine (*diet FF*), olive oil 14 plus 0.4 mg pyridoxine (*diet OO*), and hydrogenated fat 14 (*diet HFPF*) plus 0.4 mg pyridoxine (*diet HF*). *Diets FF, FFPF and OO* were fed concurrently for 42 days. Half those on *diets FF and FFPF* were continued on test for an additional 231 days, those on *diet FFPF* were divided into 3 subgroups and fed daily supplements of a) 0.02 mg pyridoxine, b) 30 mg linoleic acid as corn oil, or c) both. In a second series 2 groups of rats were fed *diets FF and FFPF* for 21 days, then each group was divided into 4 subgroups and fed *diets FF, FFPF, HF and HFPF* for an additional 56 days. Pyridoxine deficiency caused a decrease in appetite and markedly interfered with food utilization. Little pyridoxine was stored in rat tissues, for deficiency symptoms were evident within 5 days on the pyridoxine-free ration. Fat exerted a sparing action on pyridoxine since it retarded the development, and hastened the cure, of deficiency symptoms. Skin lesions which developed in rats on the fat-free and pyridoxine-free diet were partly due to essential fatty acid deficiency, but primarily to pyridoxine deficiency. Less fat and less arachidonic

acid were deposited in the carcasses of rats on the pyridoxine free diets. However, the percentage arachidonic acid in the carcass fat was higher. The biological synthesis of arachidonic acid was demonstrated in the groups which received pyridoxine.

Excretion of radioactive phosphorus into alimentary tract of rats on high molybdenum and copper diets RAY L SHIRLEY,* RILEY DEAL OWENS* AND GEORGE K DAVIS *Dept of Animal Husbandry and Nutrition, Florida Agricultural Experiment Station, Gainesville*

The excretion of intramuscularly injected phosphorus 32 into the various divisions of the alimentary tract of rats on rations containing added molybdenum and copper at levels of 0.0, 80.0, 0.35 and 80.35 parts/million, respectively, was determined at intervals ranging from 0.5-168 hours after administration. Fecal and urinary excretion rates were determined, as well as the occurrence of the isotope in the various alimentary tissues. Results were calculated as percentage dose and also as percentage dose/mg of total phosphorus. A maximum deposition was found in the alimentary tissues within 0.5-6 hours, in the contents of the small intestine in 1-6 hours, and in the contents of the cecum and large intestine in 24-96 hours after administration. The greatest excretion was through the small intestine and least through the stomach. All 4 groups of rats had their maximum and minimum excretion of the isotope into the various segments of the alimentary tract at approximately the same time, but the groups varied widely in the total amount of the isotope dose that was excreted. The control group excreted the most phosphorus-32, both in the feces and urine, while the high molybdenum, high copper, and high molybdenum-high copper groups excreted decreasing amounts, respectively.

Pyridoxine deficiency in the human being SELMA E SNYDERMAN, ROSARIO CARRETERO AND L EMMETT HOLT, JR *Dept of Pediatrics, New York Univ College of Medicine, New York City*

A pyridoxine-deficient diet, given for therapeutic reasons to two mentally defective infants for 76 and 130 days respectively, failed to produce clinical benefit but yielded information of nutritional interest. Both subjects developed evidences of pyridoxine deficiency, indicating that pyridoxine is a human dietary essential. The first change noted was the prompt disappearance of pyridoxic acid from the urine and a reduction of the total urinary pyridoxine to low values ranging from 0.2 to 2 μ g/day. Subsequently, both infants lost the ability to convert tryptophane to nicotinic acid, an effect which was desired in order to block a metabolic path for tryptophane that might be competing with normal tissue synthesis.

A plateauing of the weight curve occurred 33 and 73 days after the institution of the regime. On the 76th day one subject developed a series of convulsions which were promptly relieved by the administration of pyridoxine. The other subject developed a hypochromic anemia at approximately the 130th day. This responded dramatically to pyridoxine, a rise in reticulocytes was noted after 72 hours reaching a peak in 4 days after which red cell count and hemoglobin rose to normal. Both subjects gained weight normally after supplementation. In marked contrast to the excellent and prompt clinical response to the administration of pyridoxine was a delay in the reappearance of the ability to convert tryptophane to nicotinic acid.

Capillary fragility and vitamin P protective action against radiation BORIS SOKOLOFF, JAMES B. REDD AND RAYMOND DUTCHER (introduced by WALTER H. EDDY) *Southern Bio-Research Lab., Florida Southern College, Lakeland*

A clear distinction should be made between the terms 'capillary permeability' and 'capillary fragility'. The term 'increased capillary fragility' should be used only when there is an alteration in the chemical structure of the intercellular cement as evidenced by the method of tannin-ferric chloride. Using a vitamin-P compound isolated from citrus fruit, and containing 4 identified factors, a decrease in capillary fragility was found in rats and rabbits. A modified technique of Ambrose and DeEds was applied as well as the extract of inflammatory exudate (leukotaxine) with a pronounced delay in the diffusion of the dye. In another series of experiments 50 rats were submitted to X-ray irradiation. They were divided into 2 groups. One group of 20 rats served as control, while the second group of 30 rats were given vitamin P compound isolated from citrus fruit. The radiation factors were 250 kv 15 mm, with 0.5 mm Cu and 3.0 mm Bakelite filters. Target distance was 27.5 cm and 210 r/min dose rate. All the rats, both the controls and those treated with vitamin P, received 800 r total body radiation in a single exposure. Mortality rate of the control group, was 80%. All rats of this group manifested gross hemorrhages of various gravity and pronounced pathological lesions in the reticular zone of the adrenal cortex. The rats receiving vitamin P, 5 mg daily for 7 days prior to exposure and for 23 days post radiation, had a mortality rate of 10% with much less pronounced hemorrhagic diathesis. Histological investigation in the group of treated animals revealed only minor injury to the capillary wall with intercellular pores remaining intact. It appears that vitamin P factors have a specific affinity to the intercellular cement of the capillary wall by strengthening it and giving

it considerable protection against radiation ionization.

Niacin, biotin and folic acid in nutrition of the mouse EUGENE M. SPORN AND EDWARD J. SCHANTZ (introduced by HAROLD P. MORRIS) *Camp Detrick, Frederick, Md*

Extensive work on the nutrition of the rat has indicated that on a semi-synthetic ration, a dietary source of niacin, biotin or folic acid is not required for normal growth. Nieldson and Black (*J. Nutrition* 28: 203, 1944) reported biotin and folic acid deficiency in the mouse by feeding diets deficient in these vitamins. Others have obtained normal growth in mice without these factors in the ration. It was observed in his laboratory that weanling Swiss-Webster mice started at 19 days of age on a nutritionally complete diet containing 10 crystalline members of the vitamin B-complex grew at an excellent rate while mice on a diet deficient in niacin, biotin and folic acid grew at a slower rate. The difference became statistically significant after 6 weeks. The complete ration resulted in an average weight gain of 3.1 grams/week while the biotin-deficient ration resulted in 2.5 gm gain per week. Feeding rations deficient in each of the 3 vitamins demonstrated that only a biotin deficiency produced a similarly reduced rate of growth. There were no gross symptoms observed in the biotin-deficient mice aside from a decreased food intake and decreased growth rate. Bioassay of the livers of these animals indicated a slightly lesser store of biotin than in mice fed a complete ration.

Vitamin B₁₂ and folic acid for reproduction and lactation BARNETT SURE *Dept of Agricultural Chemistry, Univ of Arkansas, Fayetteville*

This work was carried out on the Wistar Strain albino rat. A ration containing 50% low-fat soybean flour furnished 25% proteins in rations, which were adequate in all dietary essentials except folic acid and vitamin B₁₂. *Ration A* was the control without folic acid and vitamin B₁₂; supplementation, *ration B* was supplemented with vitamin B₁₂; *ration C* was fortified with folic acid, and *ration D* was supplemented with folic acid and vitamin B₁₂. During a reproduction period of about 4 months the results obtained made it apparent that for optimum reproduction and lactation both folic acid and vitamin B₁₂ are essential and that folic acid plays a significant role in fecundity. Male rats from mothers on *ration B* weighed after 8 weeks growth, 109 gm more, and female rats weighed 46 gm more than rats from mothers on *ration A*.

Nutritional status of pteroylglutamic acid in persons with leukemia MARIAN E. SWENDESD, JR.

ANN L SWANSON, STANLEY MILLER AND FRANK H BETHELL (introduced by HOWARD B LEWIS)
Thomas Henry Simpson Memorial Inst for Med Research, Univ of Michigan, Ann Arbor

The clinical evaluation of metabolic antagonists of pteroylglutamic acid (PGA) in leukemia has shown that limited therapeutic benefit is obtained in many cases of acute leukemia, whereas the results of treatment in chronic leukemia are in general unsatisfactory. The possibility exists that the early development of toxic manifestations during PGA antagonist therapy in chronic leukemia may be conditioned by the nutritional status of the patient with respect to PGA itself. To determine whether PGA deficiency is associated with any specific type of leukemia, patients were given test doses of PGA and the urinary excretion of the vitamin measured. Since previous studies have indicated that untreated pernicious anemia patients, who are presumably deficient in PGA, excrete a much smaller percentage of test doses of the vitamin than normal subjects, it appears that the amount of PGA excretion following its administration may serve as a valid criterion of nutritional PGA status. Data show that low excretion rates of PGA following a 5 mg oral dose were obtained in chronic leukemia much more frequently than in acute leukemia. Seventy % of the chronic leukemia patients excreted less than 1 mg of the administered vitamin. The excretion of PGA antagonist (Aminopterin) was also less in chronic than in acute leukemia. Preliminary investigations of the converse problem, the effect of PGA antagonist administration on PGA nutrition and metabolism, have also been made. Data obtained by PGA and *L citrovorum* factor excretion studies during or after PGA antagonist (Aminopterin) therapy give some indications of PGA displacement from body tissues.

Unidentified factor for mink present in hog intestinal mucosa S B TOVE,* R J LALOR* AND C A ELVEHJEM *Dept of Biochemistry, College of Agriculture, Univ of Wisconsin, Madison*

Previous work has shown that when a regular sucrose-casein purified ration is fed to mink, loss of body weight and death will occur unless fresh liver is added to the diet (SCHAEFER, TOVE, WHITEHAIR AND ELVEHJEM *J Nutrition* 35 157, 1948). It was also demonstrated that the liver supplies 2 unidentified factors, one of which is soluble in 60% methanol, the other insoluble. Even when these animals are given rations containing 20% liver they suffer a severe weight loss and die after several months on experiment. Upon autopsy, dilation of the right side of the heart, marked fatty degeneration of the liver, kidneys and heart is noted. When 2% desiccated hog mucosa is added

to the basal ration supplemented with fresh liver an immediate gain in body weight results. No further weight loss occurs and the survival time is increased to at least 2 years. When killed at this time, the heart and kidneys appear normal, but a slight fatty degeneration of the liver is still apparent. When the hog mucosa is autoclaved at 15 pounds for 15 minutes the activity is destroyed. Unless given with fresh liver or fresh spleen the hog mucosa is inactive. Neither fresh spleen nor fresh spleen and hog mucosa is capable of replacing either of the 2 unidentified factors in liver. These results indicate that mink require a factor produced by a combination of hog intestinal mucosa and fresh liver or hog intestinal mucosa and fresh spleen.

Studies on the requirements of the mouse for some of the B vitamins JOHN J TRAVERS* AND LEOPOLD R CERECEDO *Dept of Biochemistry, Fordham Univ, New York City*

Long-term studies on growth, maintenance, reproduction, lactation and longevity in mice on high and low levels of the B complex were conducted to determine the minimum levels necessary for optimal performance. The diets used consisted of vitamin-free casein, sucrose, hydrogenated cottonseed oil, lard, ruffex, and salts, supplemented with thiamine, riboflavin, pyridoxin, pantothenic acid, folic acid, biotin, choline, a-tocopherol, and vitamins A and D. With a diet of this basal composition it was found that thiamine, riboflavin and pyridoxin, each at a level of 2.5 mg/kg of diet, allowed approximately the same overall performance as levels of 20 mg each. Swiss albino mice have been carried successfully through 4 generations on these diets. With C57 mice these diets were adequate for growth but not for reproduction and lactation. With low levels of thiamine the initial growth response in weanlings depends on the levels of the other vitamins, and there appears to be an interrelation between riboflavin and pyridoxin. When all three of these vitamins were given at 1 mg/kg of diet, approximately 50% of Swiss mice developed the typical acrodynia of pyridoxin deficiency. This is in contrast to what occurs with pyridoxin-free diets in the same strain when the levels of thiamine and riboflavin are high since under these conditions the syndrome is obtained only when desoxypyridoxin is employed.

Vitamin B₁₂ and liver protein regeneration HARRY M VARS, GORDON M KARN* AND COLIN C FERGUSON * *Harrison Dept of Surgical Research, Univ of Pennsylvania, School of Medicine, Philadelphia*

Adult male rats reared on a Purina diet were fed a nonprotein diet for 2 weeks, and subjected to a 70% partial hepatectomy. During a 2-week

postoperative period suitable groups were fed diets containing no protein, 10 or 18% casein and 10% wheat gluten, with and without the subcutaneous or intraperitoneal injection of a total of 4-10 γ of Cobione. There were no essential differences between treated and untreated groups in the N-balance, food intake, weight restoration and liver protein regeneration. A similar experiment was conducted with rats (obtained from Dr Gladys Emerson of the Merck Inst.) that had been fed a soya protein diet, free of animal protein factor for periods of 8-13 months. After 2 weeks on a non-protein diet and partial hepatectomy they were fed the same soya diet for 14 days, with and without injection of Cobione. The *ad libitum*-fed B₁₂ treated rats regained more in body weight and regenerated as much liver protein (slightly less positive N balance) than the untreated controls. The B₁₂-treated controls, pair fed to the untreated controls regained the same amount of body weight but regenerated less liver protein and had a smaller positive N-balance. Under the experimental conditions employed vitamin B₁₂ has evidenced little or no stimulatory effect in facilitating liver protein regeneration.

Protein metabolism in chronic illness effect of protein supplementation on nitrogen balance, hemoglobin, serum proteins and weight in the malnourished and effect of nutritional status on nitrogen storage S O WAIFE, MICHAEL G WOHL, JOHN G REINHOLD (introduced by JAMES H JONES) *Nutrition Project and Dept of Medicine, Philadelphia General Hospital, Philadelphia, Pa*

This study is an attempt to answer 1) Of what value is continued protein supplementation to the chronically ill patient? 2) What is the effect of a high protein intake on body weight, hemoglobin, serum albumin and globulin levels in these subjects? Twelve chronically ill, 'protein-depleted' subjects were studied by nitrogen balance methods for an average of 50 days (range 22-124 days). On a diet adequate in calories and with the protein intake supplemented by an oral casein concentrate, large amounts of nitrogen were stored. As an example, an elderly woman with rheumatoid arthritis, stored over 6 gm of nitrogen/day for 4 months. In 6 subjects nitrogen retention nearly equalled nitrogen intake. The nitrogen retention in 4 other patients was only moderately increased. This difference was undoubtedly due to a difference in degree of nitrogen depletion. There was no correlation between increased stores of nitrogen or rate of storage and changes of hemoglobin, albumin, globulin or weight. Clinically, the latter group is indistinguishable from the former except diabetic patients required significantly less insulin. Further work is needed to distinguish this

group in order to avoid useless and indiscriminate protein supplementation. The severely depleted patients showed a greater gain in weight and, to a less extent, a greater rise in globulin than the patients with a moderate nitrogen depletion. The latter group had the greater rise in hemoglobin and albumin. Protein metabolism in chronic illness differs significantly from that in acute illness in previously well subjects. The degree of repletion determines the type and degree of protein fraction which may be altered on supplementation.

Spectrographic evidence of deficiency of unsaturated fatty acids in serum and tissues of dogs on a low fat diet HILDA F WIESE,* RALPH T HOLMAN* AND ARILD E HANSEN *Dept of Pediatrics, Univ of Texas Med Branch, Galveston, and Dept of Biochemistry and Nutrition, A and M College of Texas, College Station*

Marked gross changes in the appearance of the skin and hair develop when young puppies are reared on a diet containing about 1% of the calories as fat. Lipid analyses of both the serum and skin tissue of these animals show that the fatty acids in these animals have a definitely lower degree of unsaturation compared with the fatty acids of the serum and healthy skin of dogs receiving 29% of their calories as fresh lard. The relative amount of unsaturated fatty acids having 2, 3, 4, 5 and 6 double bonds has been determined by measuring the absorption coefficient of the alkali conjugated fatty acids at 2350 Å, 2700 Å, 3025 Å, 3500 Å and 3750 Å. These values show definitely lesser amounts of diene conjugated fatty acid in the serum, skin and subcutaneous tissue of the animals exhibiting the characteristic skin changes of dietary fat deficiency than in healthy animals.

Microbiological characterization of biocytin LEMUEL D WRIGHT, EMLYN L CRESSON,* KATHERINE VALENTIK* AND HELEN R SKEGGS* *Med Research Division, Sharp and Dohme, Inc, Glenolden, Penna*

The isolation from yeast extract of a crystalline factor, termed biocytin, that is available to *Lactobacillus casei* but not to *Lactobacillus arabinosus* as a source of biotin recently has been announced (WRIGHT, CRESSON, SKEGGS, WOOD, PECK, WOLF AND FOLKERS *J Am Chem Soc* In press). Biological characterization of biocytin is extended by data with respect to 1) availability as a source of biotin to a large group of biotin-requiring organisms and unavailability as a source of biotin to only a few species, 2) stability to the action of any one of a large number of commercial enzyme preparations, 3) avidin combinability, 4) relative inactivity as compared to biotin in reversing the bacteriostatic activity of certain biotin antimetabolites, and 5) inactivity

as compared to biotin in stimulating the aspartic acid deaminase activity of bacterial cells previously exposed to pH 4 M phosphate buffer. Biocytin from chromatographic data appears to be the only complex in yeast extract capable of yielding, by acid hydrolysis, biotin or its microbiological equivalent for lactic acid bacteria.

Effects of dietary supplements on plasma tocopherol levels and vitamin E deficiency symptoms in chicks LEONA ZACHARIAS, PAUL GOLDHABER AND V. EVERETT KINSEY (introduced by FREDRICK J. STARE) *Massachusetts Eye and Ear Infirmary and Harvard Med School, Boston, Mass.*

The influence of a water-miscible vitamin preparation (Vi-Penta), iron, and other substances on vitamin E deficiency symptoms and plasma tocopherol levels of chicks fed various diets was investigated. The chief symptom of deficiency in chicks fed a vitamin E deficient base diet was encephalomalacia. The plasma tocopherol level was 0.1–0.4 mg %. Oral administration of 21 mg of α -tocopherol weekly to chicks fed the base diet

prevented deficiency symptoms and raised the plasma tocopherol level to 1.5 mg %. Chicks fed a natural diet, with or without cod liver oil, developed no deficiency symptoms, although their tocopherol level was in the same low range (0.1–0.5 mg %) as that of animals fed the base diet. Oral administration of iron supplements did not alter the plasma tocopherol levels. Although addition of Vi-Penta to the tocopherol-supplemented base diet lowered the plasma tocopherol level, it prevented deficiency symptoms when added to the unsupplemented base diet. The absence of vitamin E-deficiency symptoms in chicks raised on a cod liver oil-supplemented natural diet or a Vi-Penta-supplemented base diet (both diets containing inadequate amounts of vitamin E and significant quantities of unsaturated fatty acids) suggests the presence of protective substances other than vitamin E in both the natural diet and Vi-Penta. The use of water-miscible vitamin preparations or iron does not contribute to the production of a state of vitamin E deficiency in chicks. The relation of these findings to retrolental fibroplasia in premature infants is discussed.

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Immunochemical studies on species differences among blood group A substances HAROLD BAER,* ELVIN A KABAT, RICHARD L DAY* AND VESTA KNAUB * *Depts of Neurology, Bacteriology and Pediatrics, Columbia Univ College of Physicians and Surgeons, and the Neurological Inst and Babies Hospital, Presbyterian Hospital, New York City*

Substances showing blood group activity are found in tissues and secretions of many animals, such substances have been found in human, hog, horse and cattle stomach linings. Not every member of each species possesses all blood group activities. Isolation has revealed that materials of comparable immunochemical purity from different species showing a given blood group activity are not identical. Thus, hog substances varied in methylpentose content from 6-13%, while substances from human saliva varied from 9-18%, those from horse stomachs averaged 7% and those from cattle stomach ranged from 1.5-5.2%. The horse blood group substances contained more non hexosamine nitrogen than did substances from other species. The reactions of hog, human and horse blood group A substances with homologous and heterologous antisera formed in man as measured by the hemagglutination inhibition and quantitative precipitin methods provide further evidence of species differences. Hog and human A substances are very closely related as evidenced by their high cross reactivity while horse A substance cross reacts much less with antisera to both hog and human A substance. Analysis for hexosamine in specific precipitates of hog and human A substances with antibody to human A substance formed as a consequence of heterospecific pregnancy has shown that within experimental error all of the hexosamine of the blood group substance is precipitated. This confirms the data on purity obtained with antibody prepared against purified hog A substance.

Antibody production in nutritionally depleted patients HENRI H BALCH (introduced by A M PAPPENHEIMER, JR) *Depts of Surgery and Microbiology, New York Univ College of Medicine, New York City*

There have been reports, based largely on animal experiments, that nutritional deficiency and loss of the protein reserve may lead to a lowered resistance to infection. This lowered resistance is said to be due in part to deficient antibody formation. Antibody production has usually been measured by using the agglutination or hemolytic reactions. In the present study antibody response has been measured quantitatively in a group of patients nutritionally depleted by disease. This has been carried out by determining the anamnestic response to a single injection of 42 Lf (0.12 mg) of highly purified diphtheria toxoid in Schick negative individuals showing no hypersensitivity to Schick control. Severely ill, nutritionally depleted patients, with hypoproteinemia and extensive weight loss, who were also in negative nitrogen balance, were found capable of producing antibody as well as or better than the healthy controls. (The series included 25 depleted and 19 healthy control patients.) Furthermore, antibody production was found to continue up to the time of death from wasting disease. Antibody response could not be related to the age of the patient, the initial antibody level, total serum protein, serum albumin or serum globulin levels. No relation was found between antibody response and the development of infection. This work clearly shows that conclusions based on experiments in animals may not apply in man, with respect to the relationship of the protein reserve to antibody globulin synthesis or to the need for adequate dietary protein for antibody manufacture in order to help prevent the onset and development of infection.

Factors aiding in the demonstration of qualitative differences in the virulence-enhancing activity of mucin preparations H C BATSON AND MARTHA BROWN * *Dept of Biologic Products, Army Med Dept Research and Graduate School, Washington, D C*

Previous observations indicated that qualitative differences in the activity of immunologically active substances could not be demonstrated with any certainty, if at all, when all controlled experimental conditions favored the action of

the substances being tested. However, it appeared that differences in activity of such substances could be demonstrated by the deliberate introduction of stress factors, i.e. unfavorable conditions. The investigation reported here was a study of the effect of various stress factors on the degree and certainty of differentiation between two lots of mucin of different virulence enhancing activity. Stress factors investigated included mouse resistance, virulence of the challenge organisms (*Salmonella typhosa*), and specific immunization, both active and passive. Distinction between the lots of mucin was established with certainty when tested with moderate doses of a moderately virulent strain of *S. typhosa* in male mice of a moderately resistant strain. Distinction was not accomplished using comparable doses of a highly virulent strain of *S. typhosa* and female mice of a highly susceptible strain. Distinction between the mucins was readily accomplished by employing either actively or passively immunized mice even though they were of susceptible strain and sex and the *S. typhosa* challenge culture used was highly virulent. These observations are of critical interest in immunological assays since in determining the degree of protection conferred on susceptible mice by an active immunizing agent, the apparent immunogenic activity would be greatly exaggerated by the inadvertent use of a mucin preparation of low activity.

Immunochemical studies on cattle blood group substances SAM M. BEISER* AND ELVIN A. KABAT *Depts. of Bacteriology and Neurology, Columbia Univ. College of Physicians and Surgeons, and the Neurological Inst., Presbyterian Hospital, New York City*

Substances with either A, B, O, AO, BO, or with negligible activity as determined by hemagglutination-inhibition were prepared from individual cattle stomachs. The best cattle preparations are about 5% as active per unit weight as corresponding hog, human or horse preparations. These substances are antigenic in man resulting in the production of precipitins and some rise in hemagglutinins. Anti-cattle B formed in man precipitates all cattle preparations studied irrespective of their blood group activity, indicating that these products have bovine as well as blood group specificity. Hexosamine analyses on precipitates obtained in the region of antibody excess result in recovery of 59-90% of the bovine hexosamine added. In common with blood group substances isolated from hog and human sources, bovine substances cross-react with horse anti-S XIV. The degree of cross-reactivity is unrelated to the methylpentose content of the cattle substances, but the cross-reactivity increases on heating the preparations at 100°C for 2 hours

at about pH 1.9. Bovine blood group B substances cross-react extensively with antisera to horse B formed in man and show a much higher capacity to precipitate anti-B per unit weight than would have been expected from the hemagglutination-inhibition test. Cattle A does not cross-react with antibody to hog A or human A substances. The sera of two individuals of type B with a high natural anti-A titer precipitated with hog A but reacted only slightly with bovine A substance.

Immunochemical studies of bovine complement

DAVID T. BERMAN (introduced by M. R. IRWIN)
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Fresh unheated bovine serum agglutinates sensitized erythrocytes. The agglutinative power may be inactivated by heating at 56°C for 20 minutes, by treatment with zymine, NH₃, or by fractionation into midpiece (M) and endpiece (E). Recombination of M and E, of heated serum with NH₃-treated or zymine-treated serum, and of zymine-treated serum with NH₃-treated serum restored the agglutinative ability. Alternative hypotheses to explain these results are 1) that bovine complement (C') is primarily agglutinative, or 2) that bovine serum contains a non-hemolytic complement which is fixed by sensitized cells, which are then agglutinated by 'conglutinin'. Specific inactivation of C' prevents 'conglutination'. The agglutinative power inactivated by treating a single lot of bovine serum with heat, zymine and NH₃ to destroy all four C' components, was not restored by combining this preparation with fresh horse serum. Specifically inactivated bovine serums were recombined with specifically inactivated guinea pig, human and rabbit C'. All effective combinations of these reagents produced agglutination of sensitized cells, and some also produced lysis. Inactive combinations generally could be explained on the basis of anti-complementary action of one of the reagents, or of the combination. Bovine reagents furnished C'1, C'3 and C'4 to the other specifically inactivated complements. From these results it appears that bovine C' consists of at least 4 components analogous to, and to some extent interchangeable with, those of other species of mammals. Bovine C' is primarily agglutinative, and this activity adequately explains the so-called 'conglutination' reaction, without the necessity of resorting to an additional serum factor.

Differences in surface structure between virulent and avirulent mycobacteria HUBERT BLOCH
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It was found recently (H. Bloch, *J. Exper. Med.* 91: 197, 1950) that a lipid material could be extracted from virulent but not from avirulent

tubercle bacilli, and that several biological functions of the bacilli related to virulence could be attributed to this material which is most likely coating the cellular surface of virulent organisms. Further investigations revealed considerable differences in the permeability of the cellular surface of virulent and avirulent mycobacteria. Using the Thunberg methylene blue method, it could be shown that avirulent variants of tubercle bacilli as well as saprophytic mycobacteria decolorize methylene blue in the presence of a substrate, as most bacteria do, whereas virulent organisms do not reduce the dye. This lack of reactivity is due to a considerable impermeability of the cellular surface which distinguishes virulent organisms. Removal of the lipid coating mentioned above by the action of organic solvents or of certain enzymatic factors promptly renders virulent organisms methylene blue decolorizing. Whereas avirulent mycobacteria reduce methylene blue at any age of the culture, virulent bacilli may acquire this property by aging. The time required for a virulent culture to become methylene blue-decolorizing is inversely proportional to its virulence.

Second attacks of poliomyelitis in human beings, in relation to immunity DAVID BODIAN AND HOWARD A. HOWE *Poliomyelitis Research Center, Dept of Epidemiology, Johns Hopkins Univ., Baltimore, Md*

It was shown that second paralytic attacks of poliomyelitis in monkeys result only from inoculation with strains of different antigenic type (BODIAN *Am J Hyg* 49 200, 1949). Even 3 attacks may be produced by viruses of the 3 known types, Brunhilde, Lansing, and Leon (BODIAN, MORGAN AND HOWE *Am J Hyg* 49 225, 1949). Leon virus heretofore was represented by one strain (Los Angeles, 1937), although there is evidence for a wider distribution of this type in the U.S. (BODIAN *Proc Soc Exper Biol & Med* 72 259, 1949). In September 1944, in Baltimore, we isolated fecal virus from a boy of 15 who was suffering a second attack, and whose first attack occurred at 9 months, leaving severe residual muscle atrophy. The virus has been shown to be of the Leon type. Antibody against Lansing, Brunhilde, and Leon viruses was absent from his serum at the time of the second attack. His serum 3 months later neutralized Leon, but not Lansing or Brunhilde. His serum neutralized his own virus somewhat, during the second attack, and had greater potency 3 months later. From the same outbreak, we isolated a virus of the Brunhilde type (Frederick strain, reference 2, above), so that more than one antigenic type was prevalent. Whether paralytic reinfection in man, as in monkeys, is due to infection with a second antigenic

type is not answered. Antibodies against the virus type of the first attack may have disappeared, or his first attack could have been due to a type not yet identified.

Relationship between serum antibodies and carrier state to poliomyelitis GORDON C. BROWN AND JOHN D. AINSLIE * *Dept of Epidemiology and Virus Lab, School of Public Health, Univ of Michigan, Ann Arbor*

Three families in which cases of poliomyelitis occurred were studied for the presence of virus in stools and serum antibodies. Eight adults and 14 children were represented. The specimens had been obtained shortly after onset and again 3 weeks later. Virus was isolated from stools by intracerebral inoculation of monkeys. Serum antibodies were demonstrated by virus neutralization techniques in mice against the Lansing strain and in monkeys against the virus isolated from the family under test. Poliomyelitis virus was isolated from all 5 cases and from 8 of 17 familial associates. With two exceptions, the positive isolations were from persons under 12. The individuals from whom virus was not recovered were all over 15. Two people remained carriers for 3 weeks. Only 3 of 12 carrying virus in stools were found to have Lansing antibodies. Two of these were cases. Of 9 associates with no virus in their stools, all had demonstrable serum antibodies, suggesting a close correlation between the presence of Lansing antibodies and the absence of virus in stools. This same correlation was observed with the later specimens. Sera of the cases and their associates were tested for neutralizing antibodies to the specific strains of virus recovered. Most of these sera contained antibodies to the specific 'family' virus as early as 3 days after onset of illness in the case, suggesting an early experience with the agent. These antibodies appear to exist regardless of the presence or absence of Lansing virus antibodies or of virus in the stools.

Further studies on the role of activated protease and protease-inhibitors in allergic reactions KENNETH L. BURDON *Dept of Microbiology, Baylor Univ College of Medicine, Houston, Texas*

An improved fibrinogenolytic technique which serves as well for the quantitative titration of activated serum proteases as for testing the activity of various protease-inhibiting substances, has been applied, in addition to the film-gelatin digestion procedure previously used, in the further study of the protease-antiprotease system in normal, sensitized and desensitized guinea pigs, rabbits and human beings. Critical tests on serum samples from guinea pigs sensitized to a pure, non-antitryptic antigen (crystalline egg albumin) using in parallel the highly sensitive fibrinogeno-

lytic technique and the fully standardized film method, with crystalline trypsin, have corroborated earlier findings, which indicated a marked disturbance of the protease-antiprotease balance during anaphylactic shock, and the regular association of the refractory state with a relatively high average antitryptic titer in the blood. Results of studies on the activation and titration of the blood proteases of normal and sensitized animals, and human beings, and of attempts to prevent systemic anaphylaxis and local allergic reactions by the administration of highly antitryptic substances are presented.

Effect of x-irradiation on enteric cholera and coproantibody response in the guinea pig WILLIAM BURROWS *Dept of Bacteriology and Parasitology, Univ of Chicago, and Biology Division, Argonne Natl Lab, Chicago, Ill*

Experimental enteric cholera in the guinea pig was significantly altered by x-irradiation in doses of 100-200r. Animals were infected by intragastric inoculation immediately following irradiation, and 4-5 were killed on alternate days thereafter for a period of 20 days, and examined for bacterial infection of the tissues. Eighty % were infected after the 5th day, and of these hemolytic streptococci were found in 24%, staphylococci in 24%, and cholera vibrios in 52%. Assay of the cholera infection by count of vibrios excreted in the feces showed a marked suppression in the first 1-3 days, followed by a sharp increase in the intensity of the infection by the 5th-10th day which was 5-fold or more than that in the non-irradiated animal and corresponded in time with the spread of infection from the bowel. Serum antibody response was negligible but titration of coproagglutinin showed an accelerated immune response immediately following infection, reaching a peak at the 2nd-3rd day, and its disappearance by the 5th day associated with the increase in numbers of vibrios excreted. The results suggest that x-irradiation produces a transitory acceleration in coproantibody response, that the relation between coproantibody titer and intensity of enteric infection is inverse, that x-irradiation results in generalized spread of enteric cholera, and that disappearance of coproantibody is associated with invasion of the tissues by bacteria present in the bowel in radiation sickness.

A method for the enhancement of hypersensitivity to a simple chemical substance (picryl chloride) MERRILL W CHASE *Rockefeller Inst for Med Research, New York City*

While the methods for inducing cutaneous sensitivity towards chemical allergens usually necessarily involve application of the simple chemicals to the skin, typical contact-type hypersensitivity has been elicited in guinea pigs by means of the

intraperitoneal injection of special conjugates (prepared by coupling the simple excitants to homologous erythrocyte stromata) along with dead tubercle bacilli and paraffin oil (LANDSTEINER, K AND M W CHASE *J Exper Med* 73 431, 1941). The described method may be simplified technically by making at one time several small intramuscular injections of an emulsion of conjugate, Aquaphor or Falba, and paraffin oil containing killed mycobacteria (CHASE, M W *J Exper Med* 86 489, 1947). When picrylated stromata are thus employed as sensitizer, a large proportion of the animals develop sensitiveness to picryl chloride, the limiting reactive concentrations in olive oil varying between 1:100 and 1:500, infrequently 1:1500. This degree of sensitiveness may be further increased between 10- and 30-fold, approaching the drug-sensitiveness (by contact) of human beings, when applications of picryl chloride in dibutyl phthalate or almond oil are made to the skin at a few appropriately spaced times following the initial intramuscular sensitizing injection. Distinct reactions occurred at 1:15000 concentration in 45% of 283 animals so treated and at 1:45000 in 14.2% of 147 animals. Neither of the two types of treatment alone, nor restimulation with the conjugate, sufficed.

A precipitin test for carbohydrate antibodies in human tuberculosis and in leprosy NINE CHOU-CROUN *Dept of Public Health and Preventive Medicine, Cornell Univ Med College, New York City*

A precipitin test for carbohydrate antibodies in human tuberculosis has been obtained. The precipitating agent is the water soluble carbohydrate, which is split, by hydrolysis, from the antigenic lipo-carbohydrate that we have extracted from the tubercle bacillus. This same polysaccharide gives also a precipitin reaction with the sera of leprosy patients. More than 300 cases of active and inactive tuberculosis have already been studied on a qualitative basis. The clinical classification of these cases seem to indicate that carbohydrate antibodies are more prevalent in tuberculous patients that are successfully fighting against the disease, than in those patients in whom the infection is progressing rapidly. They also show that antibodies remain present, for a certain period of time, in the sera of patients who have apparently overcome the disease (from the clinical point of view). In leprosy, where more than 100 cases have been studied, these carbohydrate antibodies seem to be more prevalent than in tuberculosis. As a control for the specificity of the test, a large number of sera from a variety of other infections have been tested. The only significant crossreaction that has been obtained is with leprosy and this reaction is

demonstration that the tubercle bacillus and the leprosy bacillus are closely related chemically

Use of I^{131} label for measurement of serum antibody SIDNEY COHEN (introduced by MONROE J SCHLESINGER) *Yamms Research Lab, Beth Israel Hospital and Dept of Medicine, Harvard Med School, Boston, Mass*

Pressman (*J Immunol* 59 141, 1948) using I^{131} has iodinated antisera with 4 iodine atoms per molecule of protein without obvious loss of reactivity of the serum. The application of such a procedure to the determination of serum antibody by measurement of radioactivity is being studied. A preliminary investigation has been made using the diphtheria toxin horse antitoxin system. Equine antitoxic sera have been labelled in the proportion of one iodine atom to 4 or 5 molecules of globulin. After adequate dialysis all of the residual iodine is precipitable by trichloroacetic acid. The I^{131} content of washed toxin antitoxin floccules has been determined and the antibody nitrogen calculated using the ratio of I^{131} to protein nitrogen obtained for the iodinated serum. Results obtained in this way fall 6-8% below those obtained by standard Kjeldahl methods. The zone of flocculation of iodinated antitoxin is very nearly the same as the original serum. Iodinated normal (non-immune) serum protein is not appreciably adsorbed by toxin-antitoxin floccules.

Enumeration of elementary particles of the virus of meningo-pneumonitis T TIMOTHY CROCKER (introduced by JOSEPH L MELNICK) *Dept of Physiological Chemistry and Section of Preventive Medicine, Yale Univ School of Medicine, New Haven, Conn*

Direct counting of elementary particles of the virus of meningo pneumonitis (a member of the psittacosis lymphogranuloma venereum group) within a standard error of the determination of about 10% has been accomplished by application of the technic of Williams and Backus. These workers introduced the use of latex spheres of nearly uniform diameter in suspensions of known concentration in making similar determinations for certain plant viruses. The method includes counting of latex particles and virus particles in limited fields in the electron microscope. Calculation of virus particle concentration is made from the ratio of the two types of particles counted and the known concentration of the latex spheres in a mixture of suspensions of the two particles. This method permits morphologic identification of the virus during counting and yields determinations of virus concentration more accurately than does biologic titration. Problems arising from application of this technic to an animal virus infectious for laboratory workers have been taken

into account. An investigation of morphologic and biologic alterations produced in the virus by heat, ultraviolet radiation, and chemical fixation has been directed toward development of safety without loss of accuracy in these determinations.

Chemical determination of histamine in blood of infants and children with special reference to rheumatic fever PAUL F DE GARA *New York Hospital and the Dept of Pediatrics, Cornell Univ Med College, New York City,*

Increase in the histamine level has been noted in connection with immunological reactions. Some observers have also reported an increase during certain phases of acute allergic conditions. These observations were made using various modifications of a bioassay procedure which is not readily adaptable to serial studies. Since an allergic factor in the etiology of rheumatic fever has been stressed by some observers, a study of blood histamine levels during the various phases of this disease and a comparison of the results with those found in normal individuals and in patients with allergic conditions was undertaken. A chemical method for the determination of the small amounts of histamine present in normal human blood has been described by Lubschez (*J Biol Chem* In press). The histamine in 5-10 ml of whole blood is isolated, and the solution is concentrated and analysed colorimetrically using a diazonium reagent. Using this method, blood from 10 normal children and 50 patients with active or inactive rheumatic fever, bronchial asthma, eczema, rheumatoid arthritis and miscellaneous other diseases was analysed for histamine. The influence of ACTH upon the blood histamine level was also investigated. The results obtained are presented and discussed.

Effect of sensitization and irradiation on the fate of I^{131} -labelled bovine gamma globulin in rabbits and mice F J DIXON,* G J DAMMIN* AND S C BUKANTZ *Depts of Pathology and Internal Medicine, Washington Univ School of Medicine, St Louis, Mo*

This study was undertaken to determine the effect of sensitization and irradiation on the fate of antigenic protein injected into rabbits and mice. Bovine gamma globulin was iodinated and traced according to a modification of the technique previously described by Warren and Dixon (*Am J M Sc* 216 136, 1948). Three groups of rabbits were injected intravenously with 500 mg of I^{131} labelled bovine gamma globulin. The first group had been sensitized 20 days earlier by intramuscular injection of 350 mg of alum precipitated bovine gamma globulin, the second had been exposed two days before injection to 500r of x-ray, and the third group consisted of non-sensitized controls.

Radioactivity levels of blood, tissues, and excreta were determined during a nine-day period. The most significant changes after injection of the I^{131} labelled protein were: 1) A loss of radioactivity from the blood stream of all animals, most rapid in sensitized. At 96 hours less than 1% of the original injected activity was still present in the blood of sensitized animals, while controls and γ -rayed animals had 8% and 7% respectively. 2) Liberation of I^{131} from the protein-bound state, most rapid in sensitized animals. This liberation resulted in the appearance of non-protein bound I^{131} in the blood. 3) Appearance of non-protein bound I^{131} in the urine, in 96 hours accounting for approximately three-fourths of total injected activity in sensitized animals, and approximately one-half in γ -rayed animals and controls. 4) A higher level of tissue-bound radioactivity in sensitized animals than in other groups.

Specific localization of anti-rat-lung serum as determined by the use of radioactive tracers
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Anti-rat-lung serum and antiovalbumin serum, prepared in rabbits, were fractionated and the globulin fractions labelled with radioiodine (I^{131}). These materials were each injected into rats and the distribution of radioactivity served as an indication of the distribution of antibody. It was found that rats injected with radioactive anti-lung serum exhibited specific localization of radioactivity in their lungs, and also in their kidneys. Although the localization in kidney of anti-lung serum was quite similar in extent to the localization of anti-kidney serum, the localization in lung was quite different for the two antisera. It appears from these results that there are substances in lung which are different serologically from any components in kidney, and that there are other substances in lung and kidney which are similar, or perhaps identical, and which are the antigens responsible for the development of kidney-localizing antibody. Since we previously showed that nephrotoxic factor in anti-kidney serum is probably identical with the kidney-localizing antibody of this antiserum, the kidney-localizing activity of anti-lung serum was compared with that of anti-kidney serum. The former, like the latter, localized clearly in glomeruli (radioautography), decayed in kidney tissue with a 'half-life' of about 20 days, and was absorbed *in vitro* by a fraction of kidney homogenate which was insoluble in NaCl solution and in alcohol, acetone and ether. In light of these extensive similarities it is interesting to know that anti-lung serum has been reported to produce, in rabbits, a nephritis which is indistinguishable from that induced by anti-kidney serum.

Serological studies on infectious mononucleosis and viral hepatitis with human erythrocytes modified by different strains of Newcastle disease virus
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Serological reactions with human erythrocytes modified by adsorption and elution of a Connecticut or an Australian strain of Newcastle disease virus (NDV) have been studied to investigate the effect of strain differences in NDV on the results of agglutinative tests performed with serum from patients with various infectious diseases. Serum from 13% of 59 cases of infectious mononucleosis agglutinated Rbc modified by the Connecticut strain of NDV in higher titer than found in serum of normal individuals whereas serum from 74.5% of these same cases agglutinated Rbc similarly modified by an Australian strain. Individual titers were often much higher with the Australian strain. Adsorption experiments indicate that Rbc modified by either strain react with the same component of serum. Elevated titers were found in serum from 32% of 40 patients with viral hepatitis using Rbc altered by the Connecticut strain of NDV and in 26% of 90 cases studied with the Australian strain. Thirteen per cent of serum from patients with other infectious diseases had elevated titers with the Connecticut strain and 10% using the Australian strain. The capacity of virus modified Rbc to agglutinate fresh Rbc, the hemagglutination titer against chicken and human Rbc, and the infectivity titer for eggs were all closely comparable for these strains as well as for 2 other strains of NDV. When subjected to elevated temperatures differences in the stability of the hemagglutinin of these 4 strains were demonstrable.

Observations on factors influencing the diagnostic agglutination titration in brucellosis
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The method of antigen preparation and the time and temperature of incubation were observed to influence specificity and sensitivity in diagnostic brucella agglutination titrations. Serums from patients with brucellosis and cross reacting serums from persons recovered from tularemia and from persons immunized against cholera were used. The agglutination titers of these serums were similar when tested with 22 smooth brucella strains of different origin and characterized by different dissociation indices. Five methods of tube test incubation were studied. Tests were performed with heat, phenol and formalin killed antigens incubated at 24° C, 37° C, 45° C, 53° C, and 60° C for 4, 8, 16, and 32 hours. Phenol killed and heat killed antigens gave identical titers in

cases of active and convalescent brucellosis. In cross reacting serums, however, the titers were much lower at 53° C or 60° C than at lower temperatures of incubation. Experiments in which the antiserums and bacterial suspensions were heated separately at 53° C before titration suggested that inactivation of antigen or antibody did not occur at this temperature and therefore was not responsible for the reduction of cross reacting titers. With formalin killed antigens the cross reacting serums failed to show any difference in agglutination titer at different incubation temperatures. Prozones with serums from recovered persons were enhanced by the use of formalin-killed antigens. Serums from certain recovered persons agglutinated phenol and heat killed antigens at all temperatures studied but titers appeared to be temperature-dependent if formalin-killed antigens were used.

Relationship between serum protein fractions and antibody nitrogen response in diabetes mellitus

JOHN A. FLICK, SIGMUND KETTERER,* MICHAEL G. WOHL* AND S. O. WAIFE* (with technical assistance of GEORGE CLOUGH) *Dept. of Bacteriology, Univ. of Pennsylvania School of Medicine and Philadelphia General Hospital, Philadelphia*

The object of this study was to determine whether or not any relationship exists between the antibody response following typhoid vaccination and the concentration of some serum protein fraction in diabetic patients. Twenty six hospitalized diabetic patients were studied chemically and immunologically for a period of 30 days. Various serum protein fractions and the typhoid antibody concentration were determined chemically at intervals. The results were correlated and statistically analyzed. The data suggest the following conclusions: 1) Diabetic patients with hypoalbuminemia of less than 4 grams % showed a lower typhoid antibody response as measured by the antibody nitrogen concentration than a comparative group of normo-albuminemic diabetics having serum albumin levels above 4 grams %. 2) Diabetic patients with a serum globulin value of over 3 grams % had a slightly higher antibody response than patients showing a globulin level of below 3 grams %. 3) No significant difference in antibody response was found in patients with high gamma globulin as compared with those having normal gamma globulin values. 4) The highest antibody responses were found in patients with normal albumin and high gamma globulin values.

High-intensity electrons as a tool for preparation of vaccines. I. Preparation of rabies vaccine

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The experiments presented are a first attempt to use corpuscular radiation in the preparation of vaccines. High intensity ultra-short time electron bursts produced by a 3 MeV Capacitron were used for the inactivation of rabies virus. The Capacitron releases intensities of several million 'rep' (Roentgen Equivalent Physical) during one micro-second. The selective effect of this type of radiation on nucleic acids and the independence of the radiation treatment from temperature and type of medium, make it particularly attractive for the preparation of potent and stable vaccines. The rabies vaccine was prepared as a 5% suspension of mouse brains in nutrient broth and irradiated with 1,500,000 rep in the frozen state. The phenol vaccine was prepared from the same virus in the usual way. Potency tests were performed on mice, using the homologous strain as challenge virus. The results were expressed as 'protection' in LD₅₀. A number of irradiated vaccines prepared under various conditions protected against 2,540-250,000 LD₅₀, while the phenol vaccines protected against 61-3,120 LD₅₀. It is therefore apparent, that a potent antirabies vaccine can be prepared with the aid of corpuscular radiation.

Observations on agglutination of polysaccharide-treated erythrocytes by human tularemia antisera

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Human Type O erythrocytes treated with a polysaccharide obtained by phenol extraction of *Pasteurella tularensis* become specifically agglutinable by sera from human tularemia. The reaction resembles virus hemagglutination and is most easily detected by observing the pattern of settling in the bottom of the tube. Hemagglutination titers reached 1-10,000 in sera from recently recovered tularemia patients. Sera from persons immunized with tularemia vaccines also show the reaction, but to a lower titer. The method is simple and convenient and requires only a small amount of serum. The hemagglutination titer appears to be correlated with the amount of antibody as measured by the quantitative precipitin reaction with polysaccharide, but not with the bacterial agglutination titer. In the tularemia cases studied hemagglutination titers increased more rapidly and abruptly than bacterial agglutination titers. The hemagglutination reaction also showed less tendency toward cross reaction with sera from brucellosis than did the bacterial agglutination titration.

¹ Deceased

Hemagglutination by meningopneumonitis virus and its inhibition by immune sera D A HAIG* AND M R HILLEMANN *Dept of Virus and Rickettsial Diseases, Army Med Dept Research and Graduate School, Washington, D C*

Allantoic fluid from embryonated eggs infected with meningopneumonitis virus contains a specific hemagglutinin which aggregates mouse erythrocytes but not the red cells of a number of other species of mammals and birds. Agglutination occurs when the tests are incubated at 26–37° C but not at 4° C. This hemagglutinin found in allantoic fluids infected with meningopneumonitis virus is specifically inhibited by convalescent or immune sera of chickens, pigeons, rabbits or human beings infected with meningopneumonitis, ornithosis, feline pneumonitis or mouse pneumonitis viruses. Thus, the hemagglutination-inhibition test, like the complement-fixation test is specific for the psittacosis-lymphogranuloma venereum group of agents but is not type-specific for the individual viruses. Preliminary studies suggested that the infectivity and hemagglutinating activity found in allantoic fluids from inoculated eggs are not identical. Fluids which contain hemagglutinin and elementary bodies and possess high infective titers have a greatly reduced number of intact virus particles after centrifugation at about 13000 RPM but still possess essentially undiminished hemagglutinin. The nature of this hemagglutinin is being investigated. The hemagglutination test with the members of the psittacosis-lymphogranuloma venereum group should be useful in studies on the sera of domestic fowl which fail to react in the ordinary complement-fixation test.

Anti-reaginic action of normal rabbit serum MARGO HASSON* AND M SCHERAGO *Dept of Bacteriology, Univ of Kentucky, Lexington*

In the course of an investigation of the antigenicity of human atopic reagin from two ragweed sensitive hay fever patients we found that although the sera from the rabbits that were injected with the reaginic sera prevented the reagin to some extent from passively sensitizing the skin of non-allergic recipients, sera from normal rabbits had a similar, though not as potent, antagonistic action. A more detailed study of this antagonistic action of normal rabbit serum on two additional human reaginic sera (one from a ragweed sensitive hay fever patient and the other from a rabbit dander sensitive hay fever patient) was made, using unheated and heat inactivated rabbit sera. Samples of rabbit sera from 2 months old, one year old, and 1½-year-old males and from 1½-year-old females were used. Although both samples of the reaginic sera were inhibited by the normal rabbit sera the one from the ragweed

sensitive patient was completely inhibited only after it had been diluted 1–80, whereas the one from the rabbit dander sensitive patient was completely inhibited in even as low a dilution as 1–10. Inactivation of the rabbit sera at 56° C for 30 minutes had no significant effect on their antireaginic action.

Serological reactivity of dextran plasma substitute EDWARD J HEHRE* AND JOHN Y SUGG *Dept of Bacteriology and Immunology, Cornell Univ Med College, New York City*

Specimens of partially hydrolysed dextran, prepared in Sweden for use as a plasma substitute, were found to possess *in vitro* serological activity and also the capacity to produce anaphylactic shock in guinea pigs passively sensitized with an appropriate antiserum. We had previously shown (*J Immunol* 43 119, 1942) that unhydrolysed dextrans synthesized by *Leuconostoc mesenteroides* give precipitation and complement fixations reactions not only with leuconostoc antiserum but also with antisera of type 2 and type 20 pneumococci. Swedish (Pharmacia) dextran for infusion obtained in 1946 and 1949 likewise reacted with types 2 and 20 pneumococcus antisera. Precipitation occurred over a somewhat more narrow range of polysaccharide and serum dilutions than in the case of a native dextran, but complement fixation was obtained with definitely higher dilutions of the partially hydrolysed than of the native polysaccharide. Intravenous injection of 0.1 mg or 0.2 mg of any of the samples of Swedish dextran into guinea pigs previously sensitized with a potent type 2 pneumococcus antiserum invariably caused acute fatal anaphylactic shock. Intravenous administrations of large amounts of dextran into many people without untoward reaction have been reported. Nevertheless, the antibody-combining properties of dextrans should be recognized as a theoretical source of danger in the case of persons who might possess a high titer of appropriate antibodies at the time of injection.

Interrelationships of crystalline egg albumin and its deaminated and denatured derivatives MICHAEL HEIDELBERGER AND PAUL H MAURER* *Depts of Biochemistry and Medicine, Columbia Univ College of Physicians and Surgeons, New York City*

When crystalline egg albumin (Ea) is partially deaminated at pH 4.0 and 0° C 2 fractions are formed. Fraction A, insoluble, and Fraction B, soluble, at the isoelectric point. In both the oxidized (–S–S–) and reduced (–SH) states fraction B has physical, chemical and immunological properties similar to those of Ea. Homologous and cross reactions of Ea and Fraction B with calibrated anti-Ea and calibrated anti-B sera were indistinguishable up to the region of moderate antigen

excess Differences, possibly significant, were noted only in the inhibition zone Fraction A, although 'denatured' was serologically different from both Ea and acid denatured Ea (DnEa) (*JACS* 67 574, 1945) Acid DnEa was partially deaminated at 0° C with HNO_2 at pH 3-3.5 The resultant DnEa Deam (-S-S- form) was reduced to the -SH form with thioglycolic acid at pH 7.5 Although there were small differences in physical and chemical properties, there were no significant immunochemical differences in a calibrated anti-acid DnEa serum The action of free HNO_2 on crystalline Ea gave a product similar to Fraction A In the Ea-anti-Ea and acid DnEa-anti-acid DnEa systems serological specificity appears to be unaffected by partial removal of amino groups and is uninfluenced by the state of the -S- groups as -S-S- or -SH (cf also *JBC* 110 343, 1935)

Reciprocal neutralization tests with four strains of poliomyelitis virus HOWARD A. HOWE *Poliomyelitis Research Center, Dept of Epidemiology, Johns Hopkins Univ, Baltimore, Md*

Reciprocal neutralization tests have been done in rhesus monkeys with 4 strains of poliomyelitis virus which were previously typed by the method of reinfection (Bodian *Am J Hyg* 49 200, 1949) and of immunization by vaccination (Morgan *ibid* 225) The Lansing, Brunhilde, Kotter and Frederick strains were tested with ten fold serial virus dilutions in the presence of distilled water, normal monkey serum and specific hyperimmune sera The numbers of animals receiving any given dilution ranged from 3-34 but averaged 9 The Lansing strain was also set up against 60 PD₅₀ of virus and half log dilutions of immune serum in groups of 3 monkeys and against 10 PD₅₀ of virus with ten fold serum dilutions in groups of 8 mice The homologous neutralization index of Lansing virus in monkeys was 4.5 by the method of virus dilution and 3.8 by serum dilution It was 4.0 by the serum dilution in mice All other homologous neutralization indices lay around 3 Sharp separation of the Lansing type from the other strains was demonstrated As in the studies cited above, the Brunhilde, Kotter and Frederick were found to be of the same type despite marked differences in clinical behavior in experimental animals

Universal serologic reaction in the differentiation of syphilis and yaws REUBEN L. KAHN *Univ of Michigan Hospital, Ann Arbor*

The reactions in syphilis and yaws given by the universal precipitation technique with lipid antigen are not identical in their serologic patterns This technique consists of quantitative precipitation set-ups with NaCl solutions of reduced (0 to 0.3%) and increased (1.5 to 2.1%) concentrations

compared with physiologic salt solution and also of moderate (0.6 to 1.2%) concentrations close to the physiologic level The precipitation results in all the set-ups are read on mixing the reagents and after 4 and 24 hours at ice box temperature It was found that precipitation given by the quantitative set-ups with reduced and increased salt concentrations is increased to a greater extent on cold incubation in syphilis than in yaws Precipitation given by the quantitative set-ups with moderate salt concentration is generally not increased by cold incubation in syphilis and may be increased by cold incubation in yaws, especially when untreated (Kahn, R. L. *Am J Clin Path* 19 347, 1949)

Structural changes produced in Brown-Pearce carcinoma cells by means of a specific antibody BERNARD KALFAIAN* AND JOHN G. KIDD *Dept of Pathology, The New York Hospital, Cornell Med Center, New York City*

Brown-Pearce carcinoma cells lose their viability when incubated with the antibody that reacts specifically with a distinctive constituent of them (*J Exper Med* 83 227-250, 1946) Furthermore, contrary to previous observations, they regularly manifest characteristic structural changes, evident both in fresh and in fixed preparations When the carcinoma cells are suspended as individuals in isotonic saline solution and incubated at 37°C with rabbit serum containing the specific antibody and complement, their cytoplasm immediately begins to swell and the cells become spherical The cytoplasmic basophilism rapidly diminishes and within 10-15 minutes but little remains Soon thereafter the cytoplasm in the fixed and stained preparations appears empty except for a moderate amount of amorphous acidophilic material together with a few rod-like, granular and vesicular bodies, in fresh cells the formed materials appear to be suspended irregularly in a transparent fluid that distends the cytoplasm Meanwhile, the plasma membrane is converted into an irregularly thickened acidophilic structure with swollen cytoplasmic particles adherent to it, 'blisters' do not develop from its outer surface as they regularly do within a few minutes from that of the viable cells suspended in saline or in rabbit serum devoid of antibody The nuclei often remain intact for some time after the cytoplasmic changes have become advanced, but within 30-60 minutes they and their nucleoli begin to shrink and the nuclear membrane becomes wrinkled, though the chromatin often remains unchanged in appearance for several hours before the nuclei finally become pyknotic These alterations do not develop spontaneously while the cells remain for long periods in saline solution or in normal rabbit serum

Effects of 'sensitized' lymphocytes on transplanted cancer cells JOHN G KIDD AND HELENE WALLACE TOOLAN * *Dept of Pathology, New York Hospital, Cornell Med Center, New York City*

Transplanted cancer cells proliferate for a time when implanted in racially-resistant mice but then regress, as is well known, histological observations indicate that the tumor cells are overcome by 'sensitized' lymphocytes—lymphocytes, that is to say, of hosts that have reacted against the alien elements, (*Federation Proc* 8 360, 373, 1949) In further experiments lymph nodes have been procured from A mice that had previously overcome the cells of the lymphosarcoma 6C3HED and hence were immune to them The nodes were minced and incubated at 37°C for 1-2 hours *in vitro* with suspensions of the lymphosarcoma cells This treatment regularly inhibited or abolished the ability of the lymphosarcoma cells to proliferate when the incubated mixtures were subsequently implanted into susceptible hosts Under identical conditions the serum of the same immune hosts had no effect on the lymphosarcoma cells and this was also true of minced lymph nodes procured from normal A mice By contrast, the host elements (predominantly lymphocytes) that had accumulated about sites where the lymphosarcoma cells had recently been implanted in the immune A mice proved notably active against the tumor cells in similar tests

Plate complement-fixation test and its application to the Cocksackie viruses LISBETH M KRAFT* AND JOSEPH L MELNICK *Section of Preventive Medicine, Yale Univ School of Medicine, New Haven, Conn*

Complement fixation tests may be performed on lucite plates using microdrops delivered by calibrated dropping pipettes, as recommended by Fulton and Dumbell Since the final total volume of the test is 0.1 ml, precautions must be taken against evaporation during the periods of fixation and hemolysis Ten lucite plates may be used in a single test, this is equivalent to 1000 tubes of a conventional test The technique has been applied to the identification of 7 strains of the Cocksackie virus group Serum and complement were varied on a single plate against a standard dose of antigen For antigen standardization a single dilution of hyperimmune serum was used while varying antigen and complement Results were obtained in terms of the amount of complement fixed A standard amount of each of the 7 strain antigens was tested with all 7 hyperimmune sera prepared against them The amounts of complement fixed at each serum dilution were then plotted graphically and the areas under the curves determined for each antigen From a comparison

of these areas, it was found that two strains were closely similar (Texas and High Point), one was partially related to another (Connecticut and High Point) and the remaining four (Ohio, Dillard types 1, 2 and 3) were distinct from each other and from the Connecticut, High Point and Texas strains

Immunological studies on Vi antigen from V form species of *Salmonella* MAURICE LANDY* AND H C BATSON *Dept of Biologic Products, Army Med Dept Research and Graduate School, Washington, D C*

Vi antigen originally was discovered in *Salmonella typhosa* and as a consequence, much of our knowledge of the immunological activity of this antigen has been established through the study of typhoid cultures However, protective potency data thus obtained are difficult to evaluate since Vi antigen preparation from such cultures generally contain the typhoid somatic factors IX and XII, which are known to possess immunogenic potency The discovery of Vi antigen in *Salmonella* species other than *S typhosa*, viz, *S ballerup*, *S coli* and *S paratyphi C* provided a means for the study of the immunological properties of Vi antigen in the absence of somatic factors common to *S typhosa* Vi antigen extracts were prepared by extracting acetone dried mass cultures of these V form organisms with 1% HCl The Vi antigen content of these extracts was estimated by both *in vitro* (precipitin and colodion particle agglutination tests with heterologous Vi antisera) and *in vivo* (mouse protection against *S typhosa* challenge) tests Employing these techniques it was found that V form cultures of *S ballerup* and *S coli* were the richest sources of Vi antigen, the Vi content of representative *S typhosa* cultures was somewhat less and that of *S paratyphi C* (East Africa) was least Active immunity tests yielded impressive evidence that these Vi antigen preparations were highly effective in protecting mice against challenge with V form typhoid cultures but were only slightly effective against W form typhoid cultures In quantitative assays the determined ED₅₀ values of the Vi extracts were found to be relatively independent of the virulence of the challenge strain employed Extracts of the V form cultures stimulated the production of Vi antibody in both rabbits and mice These results are indicative of the immunogenic identity of Vi antigen from the four known V form *Salmonella* species and of its marked effectiveness as an immunizing agent in experimental typhoid infection in mice

Stability of egg white inhibitor of influenza virus hemagglutination in relation to preparation of local standard of inhibitory activity FRANK LANNI, YVONNE THÉRI LANNI* AND J W

BEARD *Dept of Surgery, Duke Univ School of Medicine, Durham, N C*

To facilitate comparison of the activities of inhibitor preparations, measured on different days, efforts were made to establish a standard. Frozen samples of 10% egg white in buffered saline of pH 7.3, lyophilized samples, and samples stored with various preservatives in the cold were assayed periodically with heated swine influenza virus. The frozen and the lyophilized samples gave irregular results. Of the others, the most satisfactory were those preserved with 0.02% merthiolate or 0.25% phenol. With these, no significant change in activity could be demonstrated over a period of 6 months, except, possibly a slight drop in the first weeks. This initial drop may have been related to a slight precipitate which developed and settled in the early period. Except for this precipitate and a few small mold colonies appearing in some merthiolate-preserved samples, the physical character of the solutions was excellent. A sensitive criterion of degradation of the inhibitor is a change in the slope of the inhibition curve. For untreated inhibitor the slope is characteristically steep, but when inhibitor is treated with active influenza viruses, proteolytic enzymes, or certain protein-denaturing agents, or is exposed to elevated temperatures for long periods, weak inhibitor, characterized by a shallow slope, appears. Comparison of aged (6 months at about 5°) and fresh samples of egg white failed to show significant degradation in the former. It is concluded that an aged solution of egg white, preserved with merthiolate or phenol, is satisfactory for use as a local standard of activity.

Influence of ultraviolet irradiation on antibodies of pollen-allergic man MARY H. LOVELESS
Cornell Univ Med College, New York City

A student who had been used repeatedly over a period of a year for purposes of passive sensitization (Prausnitz-Kustner tests) developed jaundice which was diagnosed as being either homologous serum jaundice or infectious hepatitis. Because of the implication, it was deemed necessary to devise a method which would destroy the virus of homologous serum jaundice without injuring the sensitizing antibodies of the allergic patient. Accordingly, a large batch of serum containing the sensitizing antibodies for ragweed pollen was subjected to ultraviolet irradiation in a manner known to destroy the virus of homologous serum jaundice. Titrations were then carried out by the serum-dilution method as well as the antibody-neutralization procedure, in order to see whether irradiation had altered the activity of the serum. No differences between the original and the irradiated sample were perceived. It is advocated that all serum to be employed for passive sensitiza-

tion studies in the future be subjected to this type of ultraviolet irradiation.

Response to BCG as an index of genetic resistance to tuberculosis MAX B. LURIE AND PETER ZAPPASODI * *Henry Phipps Inst, Univ of Pennsylvania, Philadelphia*

Five highly inbred rabbit families of different known genetic resistance to tuberculosis were given a single intracutaneous inoculation of 1 to 3 million of finely dispersed viable BCG. The growth of the lesion at the site of inoculation and its healing, as well as rate and intensity of development of tuberculin sensitivity were measured and correlated with the varying genetic resistance of these rabbits to virulent bovine tubercle bacilli. It was found that in resistant rabbits the lesion at the site of inoculation grows rapidly, reaches its peak quickly and heals promptly. In susceptible rabbits this lesion grows more slowly, achieves its height less quickly and heals much more tardily. Associated with these differences there is a more rapid and, usually, more intense development of tuberculin skin sensitivity in the natively resistant animals. Since even fully virulent human type tubercle bacilli are more rapidly destroyed in the tissues of natively resistant than in those of naturally susceptible animals, it may be inferred that the more rapid development of allergic sensitivity by the more resistant animals is due to the more rapid disintegration of the BCG in the tissues of these animals, and hence, to a more rapid release of the sensitizing antigens in their tissues. Since BCG causes a typical but evanescent tuberculosis, the procedure offers a simple and adequate test for selecting breeding rabbits of the desired genetic resistance and susceptibility to the infection. Furthermore, since the resistance acquired from this vaccination by the parents is not transmitted to the offspring, there is no inherent objection to using the latter in studies on the nature of the genetic resistance to this disease. Finally, the relative, graded resistance of the vaccinated rabbits of the different families is not altered by this BCG inoculation, for on reinfection with virulent bovine tubercle bacilli the rabbits retain their original, varying resistance.

Complement-fixation tests as an aid in the differential diagnosis of extra-intestinal amebiasis SARA C. McDEARMAN AND WOLCOTT B. DUNHAM (introduced by DOUGLAS H. SPRUNT)
Veterans Admin Med Teaching Group, Kennedy Hospital, Memphis, Tenn

Intestinal infections with *Endameba histolytica* usually can be diagnosed by the demonstration of amebae in the stools, but the frequent inability to find amebae in other organs has presented difficulties in the diagnosis of extra-intestinal

involvement The present study indicates that complement-fixation tests are of especial value in drawing attention to the possible presence of amebic infections other than intestinal The technique employed was the quantitative complement-fixation test of Kent and Rein using a commercial antigen prepared according to a modification of the method of Rees and associates The patients on whom tests were performed were classified as follows extra-intestinal amebiasis, 20 positive, 3 negative, intestinal amebiasis, only, 27 positive, 183 negative, equivocal or no evidence for amebiasis, 22 positive, 2337 negative Though some cases of intestinal infections without apparent extension to other viscera gave positive reactions, the fact that $\frac{1}{8}$ of all cases with positive tests had extra-intestinal infections indicates that the chief value of the test is in the differential diagnosis of this form of infection This interpretation is strengthened by the finding that 20 out of 23 cases of extra-intestinal infections gave positive tests

Persistence of antigen PHILIP D McMASTER AND HEINZ KRUSE (introduced by JULES FREUND)
Rockefeller Institute for Medical Research, New York City

A 'tagged' antigen (a blue azoglobulin), intravenously injected into mice, can be identified later in reticulo-endothelial cells throughout the body The subsequent retention of some sort of blue dye-protein in these cells for several months has raised the question, how long does the injected antigen persist as such? To test the point, immunological observations recently obtained in this laboratory have been used Characteristic constrictions of the smaller blood vessels, seen under the microscope in the ears of passively sensitized mice when injected with antibody, serve as an exceedingly delicate test for anaphylactic sensitivity, so delicate that the absorption of only 0.5% of bovine γ -globulin, from the peritoneum of a mouse can be detected, 1-2 days later, by an intravenous injection of anti-globulin serum The persistence of bovine γ -globulin (as antigen) in mice, injected intravenously with 0.1 cc of a 5% solution of it, was sought, 1-70 days later, by injecting ground liver and blood from these animals into the peritoneal cavities of normal mice Two days later, the recipients, given intravenous injections of anti-globulin serum, showed the specific vascular reactions, just mentioned, indicating the absorption of transferred antigen Suitable controls yielded negative results Apparently some of the antigen, as here employed, can persist in mice for at least 70 days

Sorption of influenza virus by chicken erythrocytes THOMAS P MAGILL *Dept of Microbiol-*

ogy and Immunology, Long Island College of Medicine, Brooklyn

The mechanism involved in the sorption and elution of influenza virus by erythrocytes is of importance because it may help clarify the physiology of virus infections It has been generally assumed that in infectious processes the virus is the aggressor, but no satisfactory explanation for the aggressiveness had been offered until Hirst suggested on the basis of his own observations that the influenza virus possesses an enzyme activity with an affinity for certain receptors on the cell surface He hypothesized that the virus attaches itself to specific receptors and is subsequently eluted because of the destruction of the receptors by the enzyme activity That the hemagglutinating activity of influenza virus is not entirely a specific phenomenon is clear from data previously presented Suspensions of some strains of influenza virus which do not agglutinate sheep erythrocytes under usual test conditions, readily agglutinate those erythrocytes when the test systems are adjusted with suitable buffer mixtures Moreover, addition of suitable buffers will eliminate certain obvious differences between hemagglutination which resembles the 'O' kind and of the 'D' kind That is, hemagglutination by allantoic fluid containing influenza virus is in part, at least, influenced by conditions effected by buffer mixtures (pH?) Hirst and others have reported that chicken erythrocytes will sorb most of the influenza virus from infected allantoic fluid But, little attention has been given to the small amount of virus remaining in the allantoic fluid after sorption

Further studies on kinetics of immune hemolysis

MANFRED M MAYER, CHARLES C CROFT* AND WALTER M BOWMAN* *Dept of Bacteriology, Johns Hopkins School of Hygiene and Public Health, Baltimore, Md*

Various factors influencing the kinetics of the lysis of erythrocytes by antibody and complement have been studied Emphasis has been placed on the dissociation of antibody from sensitized erythrocytes or red cell stromata This dissociation, which has been found to vary in extent with different hemolytic antisera, can lead to continuous transfer of antibody from cell to cell, producing a marked effect on the kinetics of hemolysis

Social serology antibody levels in a normal young population during an epidemic of poliomyelitis JOSEPH L MELNICK AND NADA LEDINKO* *Section of Preventive Medicine, Yale Univ School of Medicine, New Haven, Conn*

An attempt has been made to relate socioeconomic conditions to the development of certain antibodies in a normal urban population

and to follow the level of these antibodies during the course of an epidemic of poliomyelitis. The antibodies studied included 1) neutralizing antibodies to the Lansing strain of poliomyelitis virus, 2) neutralizing (and complement fixing) antibodies to a local strain of Coxsackie virus isolated during the epidemic, 3) antihemagglutinins to Newcastle disease virus, 4) antihemagglutinins to Type A influenza virus, 5) agglutinins to Group A Type 4 hemolytic streptococcus and 6) total 'gamma globulin' determinations. During the 1948 epidemic of poliomyelitis in North Carolina, 250 samples of serum of normal children in Winston-Salem were obtained at the beginning of the epidemic and again at the end in the late fall. Specimens were obtained from both negro and white children from 6 months to 15 years of age living under favorable and unfavorable economic circumstances. As a baseline for the newborn, 19 placental bloods were collected in the same area. Marked differences in levels of gamma globulin, Lansing poliomyelitis antibodies, and streptococcal antibodies were present in the groups living under different social and sanitary conditions in the same city. Children in the more favored group developed antibodies at a significantly older age than those in the less favored group. The only antibodies which increased during the epidemic period were the Lansing poliomyelitis and Coxsackie virus antibodies.

Lymphocyte origin of a plasma factor responsible for *in vitro* tuberculin type hypersensitivity

JOSEPH M. MILLER AND CUTTING B. FAVOUR (introduced by J. MUELLER) *Med Clinics, Peter Bent Brigham Hospital and Dept. of Medicine, Harvard Med. School, Cambridge, Mass.*

One feature of the *in vitro* tuberculin effect is the affinity of leukocytes from tuberculin negative as well as tuberculin sensitive hosts for tuberculin. When white cells are sensitized with tuberculin and exposed to a factor in the serum from tuberculin sensitive subjects, they undergo lysis. This lytic plasma factor is present in the euglobulin portion of blood, is heat labile and requires complement for its specific effect. The present aim was to demonstrate that the plasma factor could be derived from the lymphocytes of the circulating blood of an appropriately sensitized host. As tuberculin sensitized subjects, humans hospitalized with active tuberculous infection and guinea pigs sensitized with heat killed tubercle bacilli were chosen. Normal tuberculin-negative humans and uninoculated guinea pigs served as controls. White cells were obtained from the blood of these subjects by a method using Fraction I of Cohn and the white cells were further separated into suspensions rich in lymphocytes alone and neutrophils alone. These cells

were washed free of parent plasma and resuspended in the plasma of unsensitized subjects. By prolonged incubation of these cell-plasma suspensions it was possible to 'activate' plasma from an unsensitized host so that it became rich in the cytolytic factor previously found only in plasma of strongly sensitized subjects. By further dialysis procedures, it became apparent that the factor in such 'activated' plasma is the same in many chemical properties as fresh plasma factor. Differential studies of lymphocytes and neutrophils suggest that the origin of this plasma factor is the lymphocyte of the tuberculin sensitive host.

Complement-fixing brain antibody in experimental disseminated encephalomyelitis in mice P. K. OLITSKY, J. CASALS* AND L. C. MURPHY* *Rockefeller Inst. for Med. Research, New York City*

Murine encephalomyelitis (DE) was produced by intramuscular injections of homologous brain and adjuvant (*J. Exper. Med.* 90:213, 1949) to study development of complement-fixing (CF) antibody against brain tissue in response to the injections, and in relation to visible signs of DE. An experiment was performed with pooled blood sera from 23 mice in 3 series of 6-10 in each, also with that of 19 mice in 3 series of 6-7 each which received 3 injections of brain plus adjuvant and were bled 20 days after the first inoculation, before DE signs were manifested. Included were pooled 13 sera from 7 mice collected during the height of DE; they received 3-6 injections. Also pooled sera from 3 mice obtained during quiescent stage between relapses and bled 3-6 days after recovery from the last one, also, sera from 7 mice bled after 83 days and 6 times injected, which had not shown signs of illness. A marked CF reaction resulted with sera derived from animals having DE signs, or in quiescent stage, all others were negative even in 1:2 or 1:4 dilutions. Moreover, positive reactions were obtained only with saline extracts of normal mouse, beef, sheep, guinea pig and rabbit brain used as CF antigens, acetone-ether extracted ('dehpinized'), infant mouse (myelin-poor) brain, and mouse liver, testis, kidney, muscle and lung CF antigens gave completely negative results. Further studies may reveal any correlation between such antibody and DE, also the particular component in brain that induces the affection.

Diagnostic tests with Albany, type I and II Coxsackie (Dalldorf) virus P. K. OLITSKY, J. CASALS* AND L. C. MURPHY* *Rockefeller Inst. for Med. Research, New York City*

Antigenic differences among Coxsackie viruses have been demonstrated by means of the neutralization test. An investigation was undertaken

of the possible application of the complement-fixation (CF) test as a diagnostic method in man as well as a means for detecting immunological differences among these viruses CF antigens prepared from infected mouse-limb tissue were found satisfactory especially when treated with acetone-ether, no antigen was found in brain tissue Immune sera were obtained from mice bled after 2-4 injections of infected limb tissue, brain tissue, on the other hand, yielded no complement-fixing antibody With these materials, Albany (Dalldorf) virus types I and II were found unrelated, the specific titers of the sera were between 1:16 and 1:128, and that of the antigens between 1:4 and 1:16 No cross-reactions occurred, even when sera were used in dilution of 1:2 and when undiluted antigens were added Tests with additional strains, obtained from other laboratories, are in progress Human sera thus far collected from patients in widely different areas have reacted with one of the Albany types (type I) but not with the other Hemagglutination tests, using either infected mouse limb or brain tissue as source of virus, and sheep, chick, guinea pig, and human O erythrocytes, have yielded no positive results under varied conditions of incubation time and temperature, and with different media for suspending the virus

Amino acid inhibition of Theiler's GD VII virus in mouse brain mince HAROLD E PEARSON AND RICHARD J WINZLER * *Depts of Microbiology and Biochemistry, Univ of Southern California School of Medicine and Laboratory Division, Los Angeles County Hospital, Los Angeles, Calif*

As reported previously (*Federation Proc* 8:409, 1949), L-lysine monohydrochloride was the only naturally occurring amino acid in concentration of 1 mg/ml found to inhibit the propagation of Theiler's GD VII strain of mouse encephalomyelitis virus in cultures of minced one-day mouse brain contained in flasks of Simm's solution and incubated at 35° C for 2 days L- α -aminoadipic acid and α -ketoadipic acid but not α -ketoglutaric acid also inhibit virus In concentrations of 3 mg/ml the following amino acids, tested individually, inhibit virus production L-arginine monohydrochloride, L-cysteine hydrochloride, L-cystine, L-histidine monohydrochloride, DL-ornithine, DL-serine, DL-threonine and L-tryptophane In concentrations of 3 mg/ml the following do not inhibit virus DL-alanine, L-glutamic acid, glycine, L-leucine, DL-methionine, L-proline or DL-valine Attempts were made to reverse the inhibiting action of L-lysine monohydrochloride (1 mg/ml) by addition of the various, individual amino acids DL-methionine, L-leucine (3 mg/ml) and L-tyrosine (1 mg/ml)

partially prevent the inhibition Methionine does not reverse inhibition by histidine or by tryptophane Virus inhibition by lysine and the protective action of methionine on virus propagation are paralleled by the effects of this amino acid on the $P^{32}O_4$ uptake by minced, one-day old mouse brain

Relation between propagation of a mouse encephalomyelitis virus and uptake of radiophosphate by minced brain from mice of different ages HAROLD E PEARSON, RICHARD J WINZLER,* AND MAX E RAFELSON * *Depts of Biochemistry and Microbiology, Univ of Southern California School of Medicine and Laboratory Division, Los Angeles County Hospital, Los Angeles, Calif*

It has previously been shown that minced one-day old mouse brain was capable of supporting the propagation of Theiler's GD VII mouse encephalomyelitis virus *in vitro* Virus-infected cultures incorporate inorganic phosphate into their phospholipids and nucleoproteins at rates significantly greater than uninfected controls Minced brain from mice older than 10 days is unable to support virus growth A study of the rates of oxygen consumption and of P^{32} uptake by minced brain tissue from mice of different ages in the presence and absence of virus shows that older mouse brain has a higher initial Q_{O_2} than one-day old mouse brain, but that the Q_{O_2} declines at a very much more rapid rate than one-day old tissue The incorporation of inorganic phosphate into the lipids and proteins in a 24-hour incubation period is much greater in the one-day old brain than in older brain The stimulating effect of the virus on these processes is no longer apparent in brain from mice older than 7 days, and corresponds to the ability of the tissue to support virus growth

Specific protection of the antibody-combining site during iodination of antibody DAVID PRESSMAN AND L A STERNBERGER * *Sloan-Kettering Inst for Cancer Research, New York City*

Although it is known that antibody specificity can be destroyed by extensive iodination, we have found that it is possible to protect the antibody specific region so that iodination, extensive enough to cause otherwise complete destruction of specificity, can be carried out with essentially complete retention of antibody specificity The specific protection was made in the case of antibody specific to the p-azobenzoate ion or the p-azobenzenearsonate ion by adding to the antiserum, before iodination, the specific hapten, either benzoate ion or benzenearsonate ion Subsequent iodination intense enough to destroy the antibody activity of the unprotected serum did

not alter appreciably the antibody activity of the protected sera, since almost full antibody activity, as measured by specific precipitation, was recovered by dialyzing out the protecting hapten after iodination. The mechanism of protection is the steric blocking of an attack by the iodine on the antibody specific combining region since that region is already combined with the hapten.

Chemical and immunological studies on lecithin and cardiolipin **FREDERICK A H RICE*** AND **ABRAHAM G OSLER** *Johns Hopkins Univ and USPHS Research and Post Graduate Training Center, Baltimore, Md*

Beef heart lecithin prepared according to Pangborn for use with cardiolipin in serologic tests for syphilis has been fractionated into 3 components on a mixture of celite and magnesol. Only one of these components is active as a constituent of the lecithin-cardiolipin-cholesterol test antigen. Cardiolipin has also been partitioned chromatographically into 2 components, both of which are active in flocculation tests for syphilis. It has been found that of the 3 constituents of the test antigen, cardiolipin alone reacts with antibody present in syphilis sera, while lecithin and cholesterol perform auxiliary functions.

Inhibitory and enhancing effects of secretions of human respiratory tract on influenza virus **HARRY M ROSE** *Depts of Medicine and Bacteriology, College of Physicians and Surgeons, Columbia Univ, New York City*

Mucoid secretions of the human respiratory tract (sputum) contain factors other than specific antibodies which will either inhibit or enhance infection by the PR8 strain of influenza virus and which will also inhibit the agglutination of erythrocytes by the virus. One of these factors is spontaneously labile and is rapidly destroyed by heating to 100°C, it appears to be responsible for the ability of certain sputum specimens to inhibit viral hemagglutination in high dilution, to reduce infectivity of virus inoculated intranasally in mice and to restrict multiplication of the virus in chick embryos. A second factor is present which is relatively stable and resembles in many respects the Francis inhibitor (*J Exper Med* 85:1, 1947) found in normal serum. This factor inhibits viral hemagglutination but seems to have little or no effect on infectious properties of the virus. Heating to 100°C for 10-30 minutes does not destroy this factor and actually increases its inhibition titer against some strains of virus, particularly Lee. There is evidently a third factor which markedly enhances the infectivity of the PR8 strain when injected intraperitoneally in mice. A similar type of enhancement is obtained with hog gastric mucin but not with purified

blood group A substance, histamine, hyaluronic acid, hyaluronidase or pneumococcal polysaccharides. When preparations of sputum are progressively heated to 100°C their ability to reduce infectivity of the virus is lost and enhancement of infectivity is correspondingly increased. These results suggest that substances in the secretions of the human respiratory tract may play a significant role in determining either resistance or susceptibility to infection by influenza virus, possibly depending upon the equilibrium of these substances.

Streptococci and related filtrable infective agents in outdoor air during epidemics of influenza and of milder respiratory infections **EDWARD C ROSENOW** *Bacteriologic Research, Longview Hospital, Cincinnati, Ohio*

The study was made to determine perhaps the importance of aerial spread of pneumotropic streptococci and a possible related pneumotropic virus during epidemics of respiratory infections. Non-hemolytic streptococci were isolated from each of 17 samplings made on trains and automobiles in transit totalling 7000 miles and atop 12-foot poles and on tall buildings during and following epidemics. Mice were inoculated cerebrally and/or nasally and 11-day embryonated eggs into the allantois or yolk sac with saline washings or Seitz filtrates and with streptococci as isolated in dextrose-brain broth and with emulsions and filtrates of emulsions of pneumonic lungs and of allantoic fluid of inoculated eggs in which the embryo died on successive mouse and egg passage. The incidence of pneumonitis in mice and death of embryo and isolations of streptococci from emulsions of pneumonic lungs and of embryos that died were far greater following inoculation of material from samplings made during epidemics than from samplings made after epidemics had subsided. Isolations of the streptococcus from pneumonic lungs and allantoic fluid of inoculated embryonated eggs following inoculation of filtrates were far higher than isolations from the filtrates inoculated, indicating that the vital tissues for which the streptococcus had predilection furnished more favorable conditions for growth of filtrable forms into the streptococcus than dextrose-brain broth. The streptococci isolated from saline washings, from pneumonic lungs and inoculated eggs were agglutinated specifically by antisera and thermal antibody prepared from streptococci isolated from persons having respiratory infection and by convalescent serum.

Antibody in yolk of eggs and in serum from hens inoculated with viruses of influenza and poliomyelitis **JONAS E SALK AND J S YOUNGNER*** *Virus Research Lab, Dept of Bacteriology, Univ*

of Pittsburgh School of Medicine, Pittsburgh, Pa

It is known that antibody to certain antigens is present in egg-yolk of laying hens with high serum antibody titers. It seemed of interest to investigate this phenomenon further in regard to the mechanism whereby antibody appears in the yolk, and to explore the use of eggs of immunized hens as a possible source of antibody in large quantity. Groups of laying hens were inoculated into the breast muscle with either the PR8 strain of Type A influenza virus or the Lansing strain of poliomyelitis virus, with and without combination with an adjuvant (Bayol F and Arlcel A, described by FREUND). Blood samples were obtained at intervals and all eggs laid were tapped. Antibody determinations were made on serum samples and on aqueous extracts of yolk freed of the major portion of the lipid components by extraction with ether and ethylene dichloride. Only in animals given Lansing virus with adjuvant did antibody appear in serum and yolk. However, in hens inoculated with influenza virus with or without adjuvant, antibody in high titer developed, with adjuvants the levels reached were considerably higher. Antibody appeared in yolk later than in serum. Subsequently, in adjuvant vaccinated hens, titer of antibody/unit vol of yolk was equivalent to that in serum and was consistent in successively laid eggs. From time to time, however, in hens given virus alone, eggs were laid which had no demonstrable antibody despite moderate serum levels. The mechanism of the phenomenon, which does not appear to be one of passive transfer, is under investigation.

Active immunization of mice against experimental infection with type I meningococci H W SCHERP Dept of Bacteriology, Univ of Rochester School of Medicine and Dentistry, Rochester, N Y

Four materials were tested for their ability to immunize mice against experimental infection with type I meningococci: a vaccine of formalinized virulent type I meningococci, as a control, the soluble portion from cells of the same strain autolyzed with chloroform, as a source of soluble antigens, the insoluble portion of the autolyzate, as a source of antigens stable to autolysis, and purified specific polysaccharide from the same strain. Groups of mice received 3 or 4 injections of these materials intraperitoneally during a period of one or 2 weeks. One week after the last injection, the mice were challenged with the homologous meningococcus suspended in gastric mucin. Mice receiving 4 doses of 0.01 mg (= 20,000,000 cells) or more of the vaccine resisted from 100,000 to 200,000 LD₅₀. Mice receiving 4

doses of 0.001 mg resisted 5,000 LD₅₀. Three or 4 doses of from 0.001-0.1 mg of the soluble portion of the autolyzate induced essentially no immunity. Initially, 4 doses of 0.1 mg of the insoluble portion of the autolyzate were as effective as 4 doses of 0.001 mg of the vaccine, but this capacity decreased progressively in successive tests of the same preparation. Three or 4 doses of from 0.0001-0.01 mg of the polysaccharide engendered no demonstrable immunity. The test organism evidently possessed an adequate complement of immunizing antigen, which was largely decomposed during autolysis since neither the soluble portion nor the polysaccharide immunized, and the small fraction of immunizing antigen remaining in the insoluble portion decreased with time.

Immunization experiments with *Ascaris lumbricoides* in rodents J F A SPRENT Ontario Research Fdn, Toronto, Canada

Attempts to immunize mice to infection with the larvae of *Ascaris lumbricoides* by injection of metabolic products of adult and larval worms and with homologous antiserums were not successful. The relationship between the antibodies produced by ascaris infected guinea pigs and substances present in the tissues and fluids of the worm was studied using anaphylaxis as an indication of antigen-antibody reaction. All isolated tissues and fluids of *A. lumbricoides* and whole worm extracts of certain other nematodes caused anaphylaxis in ascaris infected animals. *Ascaris* extracts from which protein had been removed by trichloroacetic acid and ammonium sulphate were active in this way and precipitation of polysaccharides by alcohol failed to alter the effect. Guinea pigs infected with *Trichinella spiralis* were also shocked by ascaris extracts. The presence of a common antigen among helminths, functional in promoting resistance, was suggested by the observation that previous infection with nematodes other than *A. lumbricoides* resulted in increased resistance to *A. lumbricoides*.

Specific purification of antiprotein antibody L A STERNBERGER* AND DAVID PRESSMAN Sloan-Kettering Inst for Cancer Research, New York City

A procedure has been developed which is probably of general applicability for the specific purification of antiprotein antibody. The principle involved is the alteration of the specific antigen so that it can be separated from the antibody brought down in the specific precipitate. The alteration was accomplished by coupling the specific antigen with diazotized arsanilic acid. It was shown that such an antigen can be removed from an alkaline solution by a suspension of calcium aluminate while rabbit γ -globulin remains

in solution under these conditions. The coupled antigen retained the ability to precipitate specifically the antiprotein antibody. The purified antibody was obtained from the specific precipitate with the coupled antigen by dissolving the washed precipitate in calcium hydroxide solution and removing most of the antigen on a suspension of calcium aluminate, leaving purified antibody in solution. By this method solutions of antibody to bovine serum albumin and to bovine serum globulin have been obtained with essentially all of the protein precipitable as specific antibody. The yields were above 30%.

Properties of partially purified scarlet fever (erythrogenic) toxin prepared by various methods AARON H. STOCK AND ETHEL VERNEY *
Dept. of Pathology and Bacteriology, Children's Hospital of Pittsburgh, Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.

The NY5 strain of hemolytic streptococcus was grown in the dialyzable portion of (Difco) proteose peptone by methods of mass culture. From the filtrates, purified scarlet fever toxin was prepared by 4 different methods: 1) precipitation of concentrated filtrate with alcohol, 2) adsorption on aluminum silicate followed by elution and salting out, 3) salting out with ammonium sulfate, and 4) prolonged dialysis of concentrated filtrate. The 4 types of concentrated toxin had these properties in common: a) all flocculated at the same level with most commercial antitoxins, b) all contained small amounts of acid-precipitable protein, and varying amounts of non acid-precipitable protein with which scarlet fever toxin has been associated in previous studies, and also other nitrogenous material, c) when the acid-precipitable protein was separated at the isoelectric point, varying amounts of the flocculating (and presumably erythrogenic) substance were concomitantly removed, d) all contained nucleases. The purified toxins differed as follows: a) streptococcal hyaluronic acid in large quantities was found only in concentrates produced by methods 1 and 4, b) the proteins in concentrates by methods 2 and 3 varied in heat coagulability. Only 1 lot of the many prepared concentrated toxins contained a high titer of skin test doses (STD)/mg of total nitrogen or protein similar to those described by Stock, and Krejci, Stock, *et al.* (1942). Inasmuch as more streptococcal protein/STD was found in the present purified toxins than previously reported by one of us, attempts were made to explain this difference by resolving the mixture via fractionation, electrophoresis or ultracentrifugation. These experiments met with limited success. The significance of the difference in data from those previously reported will be discussed.

Toxic Component of *Shigella paradysenteriae*

CHLOE TAL* AND WALTHER F. GOEBEL, *Rockefeller Inst. for Med. Research, New York City*

The component responsible for the toxicity of the somatic antigens of *Shigella paradysenteriae* is resistant to a variety of enzymes. It should be possible, therefore, to degrade these substances by enzymatic procedures without impairing the toxic component itself. The somatic antigen of Type Z *Shigella paradysenteriae* is a lipocarbohydrate-protein complex as are those of the other Flexner types. Digestion of the Type Z antigen with pancreatin reduces the nitrogen content from 6.5-2.7%. Subsequent treatment with acid formamide, and precipitation with alcohol, yields a product still as toxic as the original material and containing 2.1% nitrogen and 1.2% phosphorus. This substance is antigenic and still shows an absorption band at 2550 Å, as did the untreated antigenic complex. Digestion of the former with intestinal alkaline phosphatase brings about a further loss of 10% of the total phosphorus and a loss of the absorption band, but there is no diminution in toxicity. Color tests for amino acids are now negative. This material contains 22.3% glucosamine which agrees with the total nitrogen content, 2.1% of it is still fully toxic and antigenic. Extraction with chloroform-methyl alcohol removes all remaining lipid (5%) and 10% of the remaining phosphorus without diminishing toxicity. It appears, therefore, that both the protein and the lipid components can be dissociated from the complex without impairing the toxic component. The latter still unidentified remains in chemical combination with a non-toxic carbohydrate from which it has not yet been separated.

Role of spleen in hemolysin production WILLIAM H. TALIAFERRO AND LUCY G. TALIAFERRO *
Dept. of Bacteriology and Parasitology, Univ. of Chicago, Chicago, Ill.

Following a single intravenous injection of 2.5×10^9 sheep erythrocytes/kg into rabbits, serum hemolysins (determined photometrically) generally rose sharply around the 4th day, reached a peak from the 7th through the 10th day and then declined. The titers of serums from such immunized rabbits and of splenic extracts, prepared by mincing spleens in a Waring blender with distilled water, showed 3 stages: 1) For the first 4 days, splenic and serum titers were low and approximately the same. 2) From the 5th to the 8th day, splenic titers rose more slowly than serum titers. 3) From the 11th to the 18th day, splenic titers decreased rapidly while serum titers declined more slowly. Splenectomy significantly depressed antibody formation when performed on the day of immunization through the 4th day,

was sometimes effective when performed on the 5th day, and was practically ineffective when performed on the 6th and 8th day. The results indicate that, under the conditions of these experiments, most of the antibody is formed by the spleen and that active formation is limited to a comparatively short period.

Studies on allergic encephalomyelitis in dogs

LEWIS THOMAS, F Y PATERSON AND ELIZABETH SMITHWICK (introduced by JULES FREUND) *Division of Infectious Disease, Dept of Medicine, Tulane Univ School of Medicine, New Orleans, La*

Allergic encephalomyelitis has been produced in dogs following immunization with homologous brain plus adjuvants. The disease occurs with less frequency than in monkeys or guinea pigs, and requires a greater number of injections over a longer period of time. Following immunization of dogs with homologous brain, a complement-fixing antibody is demonstrable in the majority of animals. The antigen involved is demonstrable in the brains of other species, is not present in new born brain tissue, and is present in peripheral nerve but absent in other organs. It is obtainable from brain extracts following boiling and formalinization. The antigen is soluble in acetone, alcohol, and ether. It is abundantly present in acetone extracts of purified white matter lipids. Its activity is not diminished by saponification. It is distinct from cholesterol and is not precipitated by digitonin. The activity of the antigen is such that complement fixation occurs with quantities of 1 γ or less/cc. Serum titers of immunized dogs range from 1:32 to 1:512. The possible nature of the antigen and its role in allergic encephalomyelitis will be discussed.

Antigenic extinction principle in potency-testing rabies vaccines

F W TRADER* AND I W MCLEAN, JR *Research Labs, Parke Davis & Co, Detroit, Mich*

The antigenic extinction principle consists of vaccinating groups of animals with serial dilutions of the preparation to be assayed and then determining, indirectly by serum assays for the presence or absence of an arbitrary level of antibody, the limiting dilution of the preparation containing sufficient antigen to give the required antibody response. This procedure has been used in testing influenza virus vaccines and the results reported here demonstrate its applicability to rabies vaccines. The tests are conducted in white Swiss mice, are relatively economical and give highly reproducible results. It was found that 1) Indirect serum neutralization gives a more reliable and sensitive test than direct challenge, but correlation between the 2 methods is good. 2) The total amount of 'brain tissue-virus'

administered was the most important variable, with number of injections, schedule of injections, volume or concentration administered in the vaccination, time of bleeding (after 10 days), number of virus MLD's utilized in the neutralization (10-1000), time allowed for neutralization (0.5-18 hours), and size of mice used in testing for neutralization (11-20 gm) producing only minor variations in the antigenic titer. The data accumulated have resulted in a reliable test method for antigenicity of rabies vaccines either by direct comparison with a reference standard vaccine or by setting an arbitrary minimum level for the antigenic extinction titer when tested under standardized conditions.

Quantitative studies of complement fixation

Complement-fixing potency of immune sera in relation to antibody nitrogen content. A G OSLER, A L WALLACE,* AND M M MAYER *Dept of Bacteriology, Johns Hopkins School of Hygiene and Public Health, Baltimore, Md*

Quantitative complement fixation studies with crystalline bovine serum albumin and homologous rabbit antisera indicate that the extent of fixation in terms of the number of 50% units of complement fixed is not a direct linear function of the amount of antibody, but follows a sigmoid curve. The use of a 50% fixation unit ($C'F_{50}$), based on absolute antibody weight or on serum dilution, is proposed as a means of characterizing the complement-fixing potency of an antiserum. The number of $C'F_{50}$ per μ gm of antibody N varies with the length and intensity of immunization but is not a function of the total antibody content.

Formation of neutralizing antibodies in monkeys after injection of poliomyelitis virus and adjuvants

ROBERT WARD, DORA RADER, MURRAY M LIPTON AND JULES FREUND *Dept of Pediatrics, New York Univ Med Center and Public Health Research Inst of The City of New York, Inc, New York City*

Rhesus monkeys received 5 simultaneous intramuscular injections of monkey spinal cord containing Lansing poliomyelitis virus suspended in physiological saline (A), or emulsified in paraffin oil (B), or emulsified in paraffin oil containing killed *Myco butyricum* (C). Five weeks later all animals received preparation A in the same manner. At each of the 2 occasions, the monkeys were given 1 gm of infected cord. Serum neutralizing antibodies were titrated in mice against about 20-50 LD₅₀ units of virus. The titers, 4 weeks after the first injection and 2 weeks after the second injection were several times higher in the groups receiving adjuvants (B and C) than in the group with virus alone (A). Some of the monkeys in group C with *Myco butyricum* died of

allergic encephalomyelitis In another experiment in which monkeys were vaccinated with virus incorporated in paraffin oil and given a booster injection of virus alone, similar results were obtained Morgan and her associates (*J Exper Med* 85 131, 1947, *Am J Hyg* 45 379, 1947, *J Immunol* 62 301, 1949) found that repeated i.m injections of Lansing virus in monkeys resulted in high neutralizing antibody titers When Morgan injected subcutaneously 2 or 3 times a small amount of virus emulsified in paraffin oil with or without killed *Myco tuberculosis*, antibody formation was scant

Applications of protamine precipitation in purification of certain viruses J WARREN, M L WEIL,* S B RUSS* AND M JEFFRIES * *Dept of Virus and Rickettsial Diseases, Army Med Dept Research and Graduate School, Washington, D C*

The addition of protamine sulfate to virus infected tissue suspensions will cause an immediate flocculation of considerable tissue material and if the mixture is centrifuged at low speeds a clear supernate is separated from a tenacious sediment We have studied the behavior of a number of mammalian viruses in this reaction and they appear to fall into 2 groups, those which remain in the supernatant and those which are precipitated in the sediment along with tissue components Viruses in the protamine supernatant can be further purified by the removal of the excess protamine with heparin or Paritol In the case of Japanese encephalitis and encephalomyocarditis viruses, we have followed protamine clarification with other procedures as ether extraction, enzymic digestion, ammonium sulfate precipitation or ultracentrifugation As the result of such treatment it is possible to obtain virus concentrates which on the basis of nitrogen content, infectivity and electron micrography appear to be of high purity For example, preparations of encephalomyocarditis virus have been obtained with $10^{15.9}$ LD₅₀/gm of nitrogen In the case of those agents which are precipitated by protamine along with other tissue proteins, it has been found that resuspension of the sediments in high concentrations of sodium chloride will free the virus from the bulk of the precipitate It may then be subjected to any of the above procedures

Inhibition of LA-peptidase by tuberculo-carbohydrates and its possible significance in the mechanism of the cytotoxic action of tuberculin CHARLES WEISS, MARIAL L BOYAR* AND CHARLOTTE H SCHARF * *Laboratories, Jewish Hospital, Philadelphia, Pa*

In order to throw light on the mechanism of the cytotoxic action of tuberculin, the properties of its components (proteins, lipids and carbohy-

drates) have been investigated Weiss and Halliday (*Proc Soc Exper Biol & Med* 47 299, 1944) showed that a polysaccharide prepared by S Raffel by the method of Heidelberger and Menzel from human tubercle bacilli inhibits BA-amidase (Cathepsin II) We now wish to record that a carbohydrate prepared by R J Anderson from human tubercle bacilli as well as a purified tuberculo carbohydrate, freed from lipids (N Choucrour, *C R Acad Sci* 226 1477, 1948) inhibits leucine amino-peptidase from both normal and tuberculous rabbit organ homogenates Other polysaccharides, such as one extracted from *S marcescens* by M J Shear and non-bacterial carbohydrates such as dextrose, sucrose and glycogen are inert in this respect LA-peptidase has its origin in the lymphocytes and is widely distributed in the body (J Fruton) This enzyme functions in the processes of cellular growth and autolysis Since its activity is inhibited by tuberculo-polysaccharides, we have a possible factor in the mechanism of the cytotoxic action of tuberculin

Newcastle disease virus encephalitis in rhesus monkeys H A WENNER, A MONLEY, AND R N TODD (introduced by JULES FREUND) *Hixon Memorial Laboratory, Univ of Kansas School of Medicine, Kansas City*

Newcastle disease virus (NDV) caused encephalitis in *rhesus* monkeys The *Manhattan* and *California* strains of NDV (infected allantoic fluid) were inoculated in *rhesus* monkeys through intracerebral, intranasal, intradermal, and intraneural portals Monkeys inoculated in the brain developed fever, gross tremors, ataxia, and occasionally weakness of extremities The *California* strain caused a more severe and prostrating illness than did the *Manhattan* strain Clinical evidence of illness was not observed following other routes of inoculation The pathologic changes consisted of encephalitis, chorioiditis, meningitis and occasionally, myelitis NDV was detected in CNS, but not in blood or cerebrospinal fluid obtained prior to or during the acute period of illness The *California* strain caused illness by brain-to-brain passage in monkeys Specific antibodies appeared in the serum of monkeys during convalescence NDV infected allantoic fluid, in addition to hemagglutinins, contained a distinct complement-fixation antigen which reacted with an antibody in the serum of monkeys convalescent of NDV Serums obtained at various levels of convalescence following NDV encephalitis were studied with the use of complement-fixation and hemagglutinin-inhibition tests These tests complemented each other Complement-fixing antibodies appeared about a week earlier (14th day) than hemagglutinin-inhibiting

antibodies (21st day) Maximal antibody levels were reached usually by the 4th week

Lethal effect in hypersensitive guinea pigs of non-precipitinogenic substance in tuberculin
JANET McCARTER WOOLLEY (introduced by R. J. DUBOS) *Rockefeller Institute for Medical Research, New York City*

Fractions separated by ammonium sulfate precipitation from unheated culture filtrates of human tubercle bacilli have been investigated for biological activity. All fractions have skin potency for tuberculous animals in proportion to their protein content. One fraction (*J_r*, *J. Exper. Med.* 87:229, 1948) has a peculiar specific effect on guinea pigs sensitized with heat-killed tubercle bacilli in paraffin oil: animals given intracutaneously doses equivalent to 0.5 mg of protein develop symptoms of a generalized toxemia and die within 8 to 48 hours. Ten times the amount which would kill sensitized animals did not affect

a normal guinea pig. The substance responsible for this delayed toxic death is neither of the 2 precipitinogenic proteins of the culture filtrate since fractions containing one or both antigens are inactive, nor is it the common polysaccharide of culture filtrates. It is either identical or closely associated with the non-precipitinogenic protein of low sedimentation constant, about 1.0 S, previously found in an Old Tuberculin (67-2, *J. Immunology* 43:85, 1942). Of 14 animals tested with active preparations in doses equivalent to about 0.5 mg of protein, all died or became very weak, but fractions not having the lethal substance even in 3.0 mg doses of protein affected none of 10 animals tested. Thus we have a new phenomenon of hypersensitivity in this type of animal, specifically elicited by a substance which is not a precipitinogenic protein and is actually concentrated during the preparation of an Old Tuberculin. The substance must be part of a very active antigen in the bacterium.

ABSTRACTS RECEIVED TOO LATE FOR ALPHABETICAL INCLUSION IN SOCIETIES

AMERICAN PHYSIOLOGICAL SOCIETY

Ineffectiveness of estrogens in inducing steroid depression in WILLIAM B. LANGAN (introduced by ISRAEL S. KLEINER) *Dept. of Physiology and Pharmacology, New York Medical College, Flower and Fifth Avenue Hospitals, New York City*

It has been reported that estradiol does not induce any signs of a depressed state in *Rana pipiens* (LANGAN, 1947). In order to determine whether or not the ineffectiveness of estradiol is characteristic of estrogens, a series of estrogens and non-estrogens was tested. The substances to be tested were dissolved in sesame oil (except where otherwise noted) and introduced with a single intrapleuropertoneal injection in doses ranging from 1-10 mg. Frogs were kept at temperatures ranging from 16 to 21° C. The inhibition of the righting reflex was selected as the criterion of profound depression. The following substances did not induce any signs of depression: hexoestrol, benzoestrol, diethylstilbestrol, estradiol (aqueous), estradiol benzoate, estradiol dipropionate, estrone, ethinyl estradiol, androstendione, dehydroandrosterone acetate, pregnenolone (aqueous). The following steroids were very effective: desoxycorticosterone acetate and progesterone. A slight effect was observed for methyl testosterone, testosterone (aqueous), testosterone propionate and ethinyl testosterone. The estrogens tested were not effective in producing a state of depression. Also several non-estrogenic steroids were

not effective, whereas progesterone and desoxycorticosterone were most effective.

Localization of frontal oculomotor cortex in the cat ROBERT B. LIVINGSTON *Institut de Physiologie, Ecole de Médecine, Geneva, Switzerland*

Electrical excitation of the sensori-motor cortex of 18 adult cats lightly anesthetized with sodium Amytal gave rise to responses which included pupillary dilatation, retraction of the nictitating membrane, and conjugate or, more often, non-associated movements of the eyes. However, the eye movement responses evoked from these superior and lateral surfaces of the frontal lobes were not sufficiently consistent in appearance or contiguous in topographical representation to suggest an oculomotor representation analogous to the frontal eye field of the primate. On the medial surface of the frontal lobe, on the other hand, was defined an oval area, approximately 2.5 to 3 cm in diameter, centered in front of and slightly dorsal to the genu of the corpus callosum, from which could be evoked responses strikingly similar to those obtained by excitation of the frontal eye field of the monkey. Appropriate electrical stimulation applied to this medial cortex in a series of more than 40 cats permitted the elicitation of the following responses: pupillary dilatation with retraction of the nictitating membrane, responses previously localized to this general region of the cat's cortex by C. N. Woolsey (personal communication), conjugate deviation of the eyes in the horizontal plane, generally to the

side opposite the hemisphere stimulated, and movements of the eye lids Hess has recently elicited head turning as well as eye deviation by excitation of a posterior and inferior zone of this oculomotor area in the unanesthetized, freely moving cat (*Helv Physiol & Pharmacol Acta* 6 731, 1948) Certain other effects, not directly related to the ocular responses, have cortical representation which overlaps this region These include grunting vocalization, coughing, gagging, increased gastro-intestinal activity and respiratory responses

Mode of action of dinitrophenol on O₂ uptake of excised rat brain DAVID B TYLER *Dept of Embryology, Carnegie Inst of Washington, Baltimore, Md*

If the O₂ uptake of the brain in the presence of graded concentrations of dinitrophenol (DNP) is followed at half hour intervals for a period of 2 hours (instead of the usual one hour), 3 effects are seen 1) Low concentrations, up to 2.5×10^{-5} M, that produce only augmentation of O₂ uptake, 2) Intermediate concentrations, 5.0×10^{-5} M to 1.0×10^{-4} M, that first augment and then depress, and 3) High concentrations that produce only inhibition of O₂ uptake With 5.0×10^{-5} M the percentage augmentation is only slightly higher than that obtained with 2.5×10^{-5} M However, with the former concentration inhibition sets in at the end of the first hour, while with 2.5×10^{-5} M no inhibition occurs These results suggest the possibility that DNP is not limited to only one site or mode of action and that the accelerating and inhibitory actions of DNP are due to different mechanisms each requiring distinctly different threshold concentrations Under the conditions of these experiments (minced rat brain respiring in Ringer glucose phosphate media), the threshold concentration for the augmentation phase of action is about 1×10^{-6} M, for the inhibitory phase it is 50-fold higher

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α -Acetolactic acid, an intermediate in acetylmethylcarbinol formation ELLIOT JUNI (introduced by L O KRAMPITZ) *Dept of Microbiology, School of Medicine, Western Reserve Univ, Cleveland, Ohio*

It has been proposed (*Federation Proc* 6 301, 1947) that the formation of acetylmethylcarbinol occurs according to the following scheme $2\text{CH}_3\text{COCOOH} \rightarrow \text{CO}_2 + \text{CH}_3\text{COH}(\text{COCH}_3)\text{COOH} \rightarrow \text{CH}_3\text{CHOHCOCH}_3 + \text{CO}_2$ Cell-free extracts of *Aerobacter aerogenes*, *Bacillus subtilis*, *Staphylococcus aureus* and *Serratia marcescens* have the ability to form acetylmethylcarbinol and carbon dioxide from pyruvic acid and can also decarboxylate α -acetolactic acid to acetyl-

methylcarbinol A crude extract from *Aerobacter aerogenes* has been resolved into 2 components, 1) acts on pyruvic acid to produce α -acetolactic acid and carbon dioxide, 2) decarboxylates α -acetolactic acid but is without effect on pyruvic acid The former is dependent on cocarboxylase Both enzymatically formed and synthetic α -acetolactic acid form the osazone of diacetyl with phenylhydrazine with the formation of one mole of CO₂ per mole of α -acetolactic acid employed Analyses of both derivatives agree with theory Melting points and mixed melting points are identical Under the conditions which the osazone from α -acetolactic acid is prepared, no osazone is obtainable from acetylmethylcarbinol The enzymatically synthesized α -acetolactic acid is decarboxylated with aniline-citrate, and is also decarboxylated by the resolved enzyme component that decarboxylates synthetic α -acetolactic acid This latter component is specific for α -acetolactic acid and will not act upon acetoacetic acid or oxalacetic acid It also shows a specificity for one of the isomers of the racemic mixture of the synthetic α -acetolactic acid Preparations from microorganisms which do not ordinarily produce acetylmethylcarbinol were found to be incapable of decarboxylating α -acetolactic acid An enzyme preparation from pig heart capable of forming acetylmethylcarbinol from pyruvic acid has been obtained which can also decarboxylate α -acetolactic acid to acetylmethylcarbinol

Maximum rate of urea production from various amino acids in the dog HENRY KAMIN* AND PHILIP HANDLER *Dept of Biochemistry and Nutrition, Duke Univ School of Medicine, Durham, N C*

Dogs were given constant rate i v infusions of various amino acids, and blood and urine urea N measured Total urea N formation was calculated as the sum of urea N excreted and urea N accumulated in body water The maximum rates obtained, corrected for urea N production of control dogs subjected to diuresis, are indicated in the table

Maximum Urea Production, as mg N/kg/min

AMINO ACID	UREA PRODUCTION	AMINO ACID	UREA PRODUCTION
L-cysteine	0 05	DL-leucine	0 39
L-methionine	0 05	L-histidine	0 65
L-glutamic acid	0 11	glycine	0 80
L-aspartic acid	0 18	L-asparagine	0 80
L-alanine	0 20	'Amigen'	1 8
L-lysine	0 20	L-arginine	1 9
L-leucine	0 24	L-glutamine	2 8
L-tyrosine	0 35	L-arginine plus 'Amigen'	3 5

Direct analysis of tissue, as well as balance studies, indicated limited permeability of cells to the dicarboxylic amino acids. Liver samples from dogs infused with arginine showed only traces of that amino acid, but large quantities of α -amino N. Liver cells appeared to be freely permeable to the other amino acids, and the data are therefore a measure of the *in vivo* activity of the respective 'deaminases'. No correlation was observed between the *in vivo* rate of urea formation, and the *in vitro* rate of deamination in the isolated systems which have been studied by other workers. The high rate of urea production from glutamine suggests the possibility of a special role for that amino acid.

Synthesis of unusual quantities of pyridine nucleotides by human erythrocytes, *in vitro*
IRWIN G. LEDER,* W. A. PERLZWEIG AND PHILIP HANDLER, *Dept. of Biochemistry and Nutrition, Duke Univ. School of Medicine, Durham, N. C.*

Incubation of washed human erythrocytes suspended in a Ringer-phosphate medium containing glucose and 2% nicotinamide results in an 8- to 10-fold increase in their content of material which behaves like pyridine nucleotides in the fluorometric assay. The material is definitely not N-methylnicotinamide or nicotinamide nucleoside. It cannot be removed from the cells by washing, serves as a growth factor for *H. parainfluenzae* and is destroyed on incubation with a rabbit brain DPNase preparation for 90 minutes. It has not yet been established whether the material is mononucleotide, DPN, TPN or a mixture thereof. Liver slices and yeast suspensions behave similarly although the extent of synthesis is not as great as that observed in red cells. Both inorganic phosphate and glucose are essential in the medium and synthesis is completely inhibited by the usual glycolytic inhibitors, e.g. fluoride and iodoacetate. The synthetic velocity is roughly constant for about 16 hours and maximum synthesis is achieved within 20 hours. The addition of ATP is without effect on either the velocity or the total amount of material formed. Synthesis is dependent on the concentration of nicotinamide and ceases below 0.1% where the rate is only 8% of that achieved in a medium containing 2% nicotinamide. The nicotinamide, therefore, serves both as precursor and as inhibitor of DPNase.

Metabolism of Desoxyribosides in *Escherichia coli*
L. A. MANSON* AND J. O. LAMPEN, *Dept. of Biological Chemistry, Washington Univ. School of Medicine, St. Louis, Mo.*

Previous work with extracts of animal tissues has shown that purine and pyrimidine desoxyribosides undergo a phosphorolytic cleavage identical with that demonstrated for the corresponding

ribosides. In these systems, however, the resulting desoxyribose phosphate esters are not degraded. Washed, resting cells of *E. coli* (A T C 9723) were incubated with hypoxanthine desoxyriboside or thymidine and acid-soluble desoxypentose determined at intervals. In a phosphate or bicarbonate buffer (pH 7.0) the total desoxypentose disappears rapidly and the free nitrogenous base can be recovered in the medium in about 80% of the theoretical quantity. Hypoxanthine, thymine, and D-2-desoxyribose are not metabolized under these conditions. In the presence of arsenate the disappearance of total desoxypentose is slower and free desoxypentose appears in the medium. It is concluded that, in the absence of arsenate, the nucleosides undergo phosphorolysis, yielding the free base and a desoxypentose phosphate. The latter is then degraded by the cells. In the presence of arsenate, both phosphorolysis and arsenolysis occur. That portion of the nucleoside which undergoes arsenolysis yields free desoxyribose. This cannot be degraded and is therefore recovered in the medium. Cell-free extracts of the organism were prepared by grinding the cells with alumina by McIlwain's procedure. These extracts contain potent purine and pyrimidine nucleoside phosphorylases. The 2 phosphorylases appear to catalyze reversible reactions. The extracts do not degrade the desoxypentose phosphate formed from hypoxanthine desoxyriboside or thymidine. Evidently the enzymes involved are either inactivated or are not extracted.

Studies on adhesion and syneresis in fibrin clots

PETER R. MORRISON, ARTHUR L. SEILER AND JAMES M. HEAD (introduced by J. W. WILLIAMS), *Depts. of Physiology and Zoology, Univ. of Wisconsin, Madison*

Adhesion was measured as the force required to separate a disc from the attached substrate. A beam balance modified for automatic loading and kymographic recording of elongation and break with stress was used. This was suitable for different substrates and although technically difficult, interpretation was straightforward. Alternatively the pressure required to separate clot from an attached screen was measured. This limits substrates but is easier and somewhat more precise (average deviation = 5%) although conversion to absolute units is difficult. Adhesion values were surprisingly constant with changes in physical chemical variables. A 100-fold increase in either fibrinogen or thrombin increased adhesion by only 2-4-fold. Broad optima near physiological values were found for both pH and salt concentration with only a 2-fold range for both pH (6.0-8.5) and ionic strength (0.10-0.90). Substrates tested in order of increasing adhesion were silicone, petrolatum, octoil-S, glass, bakelite, collodion, cellophane, protein (heat-denatured fibrinogen),

koroseal, lucite, bakelite (sanded), aluminum, stainless steel, and 'tissue' Syneresis, as compaction under pressure, was followed by measuring expressed fluid at short intervals using a micrometer burette. In contrast to adhesion, values were strikingly influenced by the conditions under which the clot was formed.

Migration of lysine across the cell wall of *Streptococcus faecalis* VICTOR A. NAJJAR AND ERNEST F. GALE * *Unit for Chemical Microbiology, School of Biochemistry, Cambridge, England*

Two factors influence the passage of lysine across the cell wall,—the phosphate ion and the amino acids in the medium. Lysine migration across the cell wall in either direction is markedly suppressed by the presence of phosphate ions in the outside medium. No effect is exerted by chloride, sulfate and pyrophosphate ions. The uptake of lysine is inhibited about 30% both at 4° C and 37° C. However, outward migration is inhibited 100% at 4° C and no inhibition occurs at 37° C. Lysine migration into the cell is inhibited by the presence of various amino acids in the outside medium. The acidic amino acids show 50–75% inhibition and the basic 30–60% inhibition. No inhibition occurs in the presence of neutral amino acids. When lysine is taken up by the streptococcus by incubation in a medium also containing the acidic or basic amino acids, lysine does not migrate to the outside upon reincubation in phosphate but migrates freely in saline. Alternatively, when lysine is taken up in the presence of the neutral amino acids or from lysine solution alone, it migrates equally well in phosphate or saline upon reincubation in these salts. All migration experiments were performed on the resting cell in media of pH 7.2.

Cerebrospinal fluid findings in brain tumors

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Valuable information in various pathologic conditions has been obtained through ultraspectrophotometrical studies of the cerebrospinal fluid (CSF), especially in combination with chemical determination of the ascorbic acid and protein content (*Federation Proc.* 6–8, 1947–9, *Science* 109:335, 1949, *Am. J. Psych.* 109:697, 1948). Ultraspectrophotometric studies have been extended to a group of 40 CSF's in cases of tumor of the CNS. In 30 of these cases, the ascorbic acid (Robinson-Stotz's method) and the protein content were determined simultaneously. Twenty new controls of non-tumor cases have been added to the previously published 40 control cases. Besides, in 14 cases corresponding brain tumor extracts (*Arch. Path.* 44:307, 1947) could be prepared and

spectrographed. The extinction coefficients (E_{260}) of the tumor CSF's lie for the most cases between 2.0–4.6 while in the control cases the average values are $1.24 \pm .59$. After correction especially for ascorbic acid, 77% of the CSF's from tumor cases reach values above $E = 8$. Only 20% of the controls reach similar values after the same correction, while in about 50%, the E values are below 0.4. There is a certain parallelism between the E values of CSF's and those of the corresponding tumor extracts. A comparison of the CSF findings with the clinical data (size and location of the tumor, intracranial pressure) seems to indicate that the high E values are not only due to metabolic processes in the tumors but also to destruction and/or impairment of the brain tissue.

Ultraspectrophotometrical studies of irradiated mouse tumors M. SPIEGEL-ADOLF AND M. E. SANO * *Dept. of Colloid Chemistry and Tissue Culture, Temple Univ. School of Medicine, Philadelphia, Pa.*

The effects of x-ray radiation upon spontaneous tumors of hybrid mice were studied by physical, chemical and histological methods. In 40 tumor mice, the mammary carcinomas alone were irradiated with dosages varying between 300–4800 R. The rest of the animal was shielded. Control experiments showed that mice in which a benign tumor was irradiated as above, gained weight and apparently stayed in good health. The irradiated tumors were extracted as described before (*Arch. Path.* 41:307, 47) and the extracts ultraspectrographed. Ten non-irradiated mouse tumor extracts show average E_{260} values $= 2.1128 \pm .27$. If the extinction values at E_{260} of the irradiated tumors are plotted against the dosage, a graph is obtained that reaches the minimum of the E values at 900 R and returns after 1800 R to non-irradiated values. Further irradiation seems to be without effect. Gross pathology and histologic controls show comparable results. The tumors become smaller, hemorrhagic, picnotic, necrotic. After higher dosages new growth develops. Only the tumors under direct irradiation show the above mentioned changes of extinction coefficient and histology. Non-irradiated secondary tumors apparently remain unchanged. In 3 cases the irradiated tumors practically disappeared after dosages of 900–1500 R. New growths developing after 2 months did not respond to further irradiation of 1500 R. Contrary to references in literature protracted irradiation did not increase the incidence of lung metastasis. In an attempt to identify the substances causing the absorption at 260 m μ with nucleic acids, the optical studies have been supplemented by microchemical N and P determinations.

New nucleosidases in *Lactobacillus pentosus*

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New types of purine and pyrimidine nucleosidases have been demonstrated in *Lactobacillus pentosus* 124-2. Cell-free extracts prepared by grinding cells of *L. pentosus* with glass show the following new reactions: Adenosine + phosphate → adenine + ribose-phosphate, cytidine + phosphate → cytosine + ribose-phosphate, and xanthosine + phosphate → xanthine + ribose-phosphate. The evidence for the direct phosphorolysis (or arsenolysis) of adenosine and cytidine is: 1) one mole of reducing sugar was obtained per mole of nucleoside disappearing, 2) the spectral changes during the process corresponded to the respective listed, 3) no adenosine or cytidine deaminases could be demonstrated in this extract, (This excludes the possibility that these nucleosides are first deaminated and then undergo phosphorolysis) 4) the free bases were identified as follows: adenine as adenine picrate and cytosine by paper chromatography. The phosphorolysis of xanthosine was shown by the formation of reducing sugar and by the spectral changes which corresponded to the conversion to xanthosine to xanthine. Under the same conditions no reducing sugar was obtained with either xanthosine alone or xanthosine and heated extract. Uridine, guanosine and inosine also undergo phosphorolysis or arsenolysis with this extract. The relative rates are: uridine, cytidine > inosine > adenosine > guanosine > xanthosine. Thus, phosphorolysis is faster with the pyrimidine than with the purine nucleosides. A comparison of the rate of cleavage of uridine and cytosine in phosphate, arsenate and tris - (hydroxymethyl - aminomethane buffers, pH 7.5) indicates that these nucleosides are split by

phosphorolysis or arsenolysis rather than by simple hydrolysis.

AMERICAN INSTITUTE OF NUTRITION

Diminished ACTH production in rats on low protein diets, explanation of relationship between dietary protein and renal hypertension PHILIP HANDLER AND FREDERICK BERNHEIM *Depts of Biochemistry and Nutrition and of Physiology and Pharmacology, Duke Univ School of Medicine, Durham, N C*

The systolic blood pressure of subtotally nephrectomized rats falls to normal levels after a few weeks on an 8% casein diet. No single amino acid deficiency appears to be specifically responsible. The administration of 1 mg of ACTH promptly raises the blood pressure to the level maintained by rats receiving a 35% casein diet while ACTH is without effect on the pressure of the rats eating the high protein diet. ACTH also raises the pressure of rats eating a low protein, salt free diet to the levels found in rats eating a high protein, salt free diet, but is without influence in the latter group. Further, ACTH is without effect on the blood pressure of unoperated rats, regardless of diet. To determine whether 705-2-15-50 FP 5541 p 587 Take 2-16-3C Gal 21 rats on low protein diets are actually 'hypopituitary' with respect to ACTH production, 0.2 mg of epinephrine was given intraperitoneally to both operated and unoperated rats on high and low protein diets. The percentage fall in the circulating eosinophile count, 4 hours later, was measured as an index of ACTH activity. The fall in the low protein rats was less than half that observed in the high protein rats while both groups responded to ACTH itself. It is concluded that the normotension of partially nephrectomized rats on low protein diets is, in large measure, the result of a diminished ACTH production.

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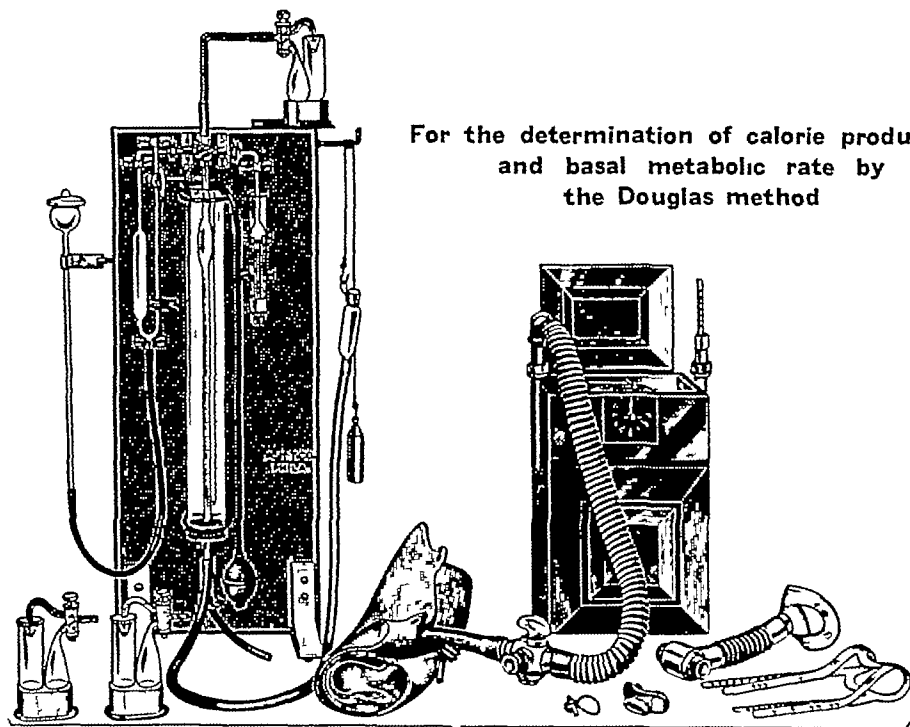
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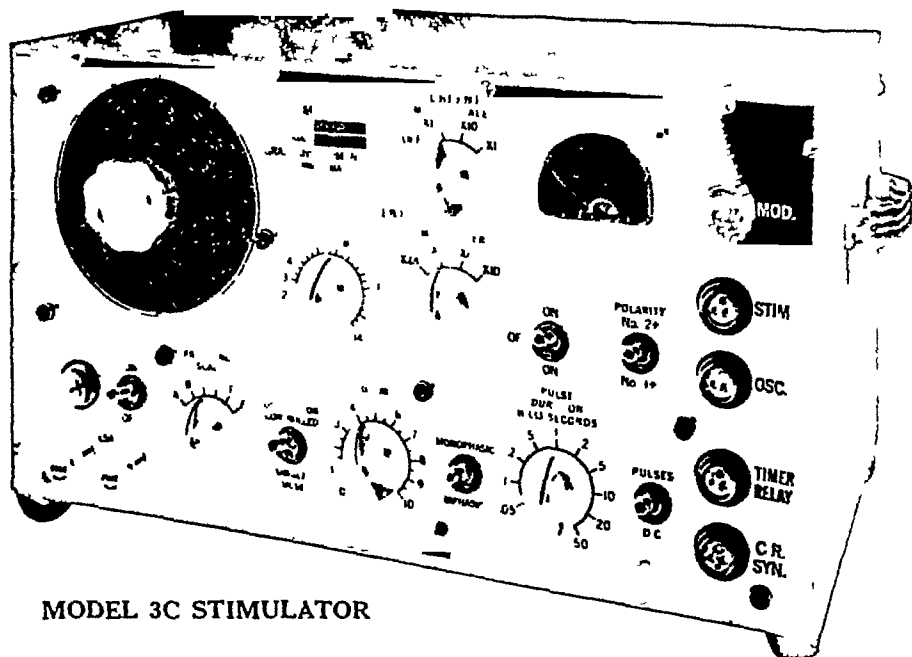
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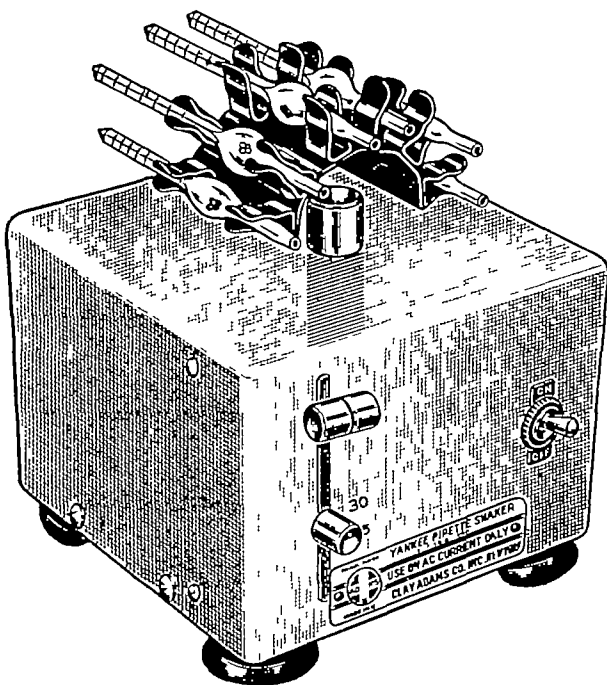
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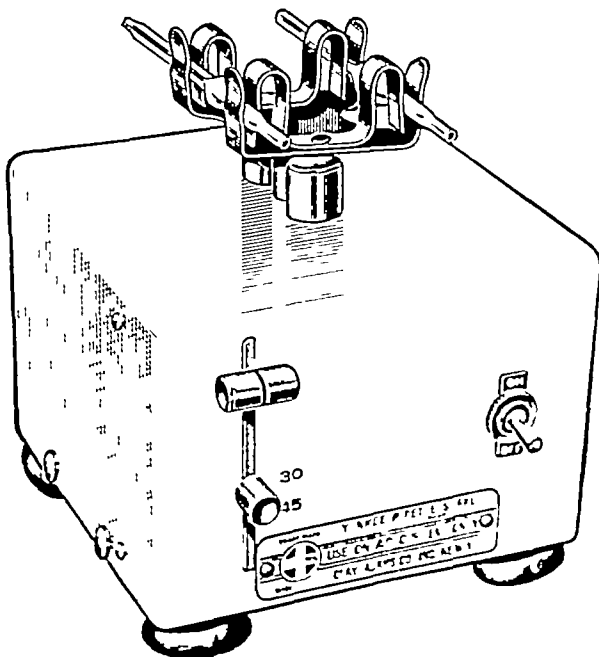
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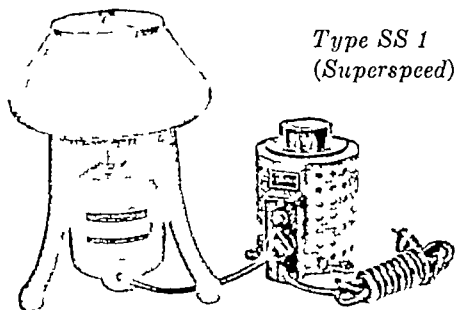
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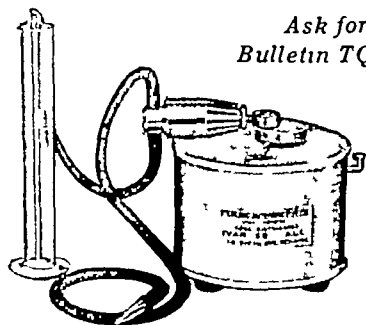


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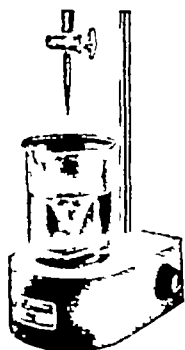
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Sections B, F—Physics, Zoology Scientific Implications of Nuclear Phenomena in the Field of Biophysics, part of a three-session symposium, *afternoon, December 28*

Section C—Chemistry Two (of the 12) sessions on steroid hormones, program arranged by Hal G. Johnson, Monsanto Chemical Co., *morning and afternoon, December 28*

A third session on medicinal chemistry, arranged by Herbert Carter, University of Illinois, *evening, December 29*, Chemists' luncheon, *December 30*

Section F—Zoology, jointly with American Society of Zoologists Symposium on Genetics and Behavior, *afternoon, December 29*

Subsection Nm—Medicine Four-session symposium on Biological Effects of Radiation, *mornings and afternoons, December 29, 30*

Subsection Nd—Dentistry Three sessions on the dental research of all branches of the Federal government, *evening, December 29, morning and afternoon, December 30*

Subsection Np—Pharmacy, jointly with the American Pharmaceutical Association, Scientific Section Four sessions of papers, *mornings and afternoons, December 28, 29*

Oak Ridge Institute of Nuclear Studies, jointly with Oak Ridge National Laboratory Two sessions, *morning, afternoon, December 28*

American Society of Parasitologists Paper-reading sessions, *December 27-29, inclusive*, luncheon, demonstrations, *afternoon, December 28*

American Society of Protozoologists Paper-reading sessions, *December 27, 28, inclusive*, luncheon, *December 29*

American Society of Zoologists Symposium on Transition from Aquatic to Land Life, *afternoon, December 27*, demonstrations, *afternoon, December 28*, paper-reading sessions, *December 28-30, inclusive*, Zoologists' dinner, Biologists' smoker, *evening, December 29*

Society of Systematic Zoology Symposium, *evening, December 27*, breakfast meeting, *afternoon business meeting, December 29*

American Microscopical Society Two sessions, *morning and afternoon, December 29*

Biometric Society, Eastern North American Region Six paper-reading sessions, *December 27-29, inclusive*

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American Dietetic Association Session, *evening, December 29*

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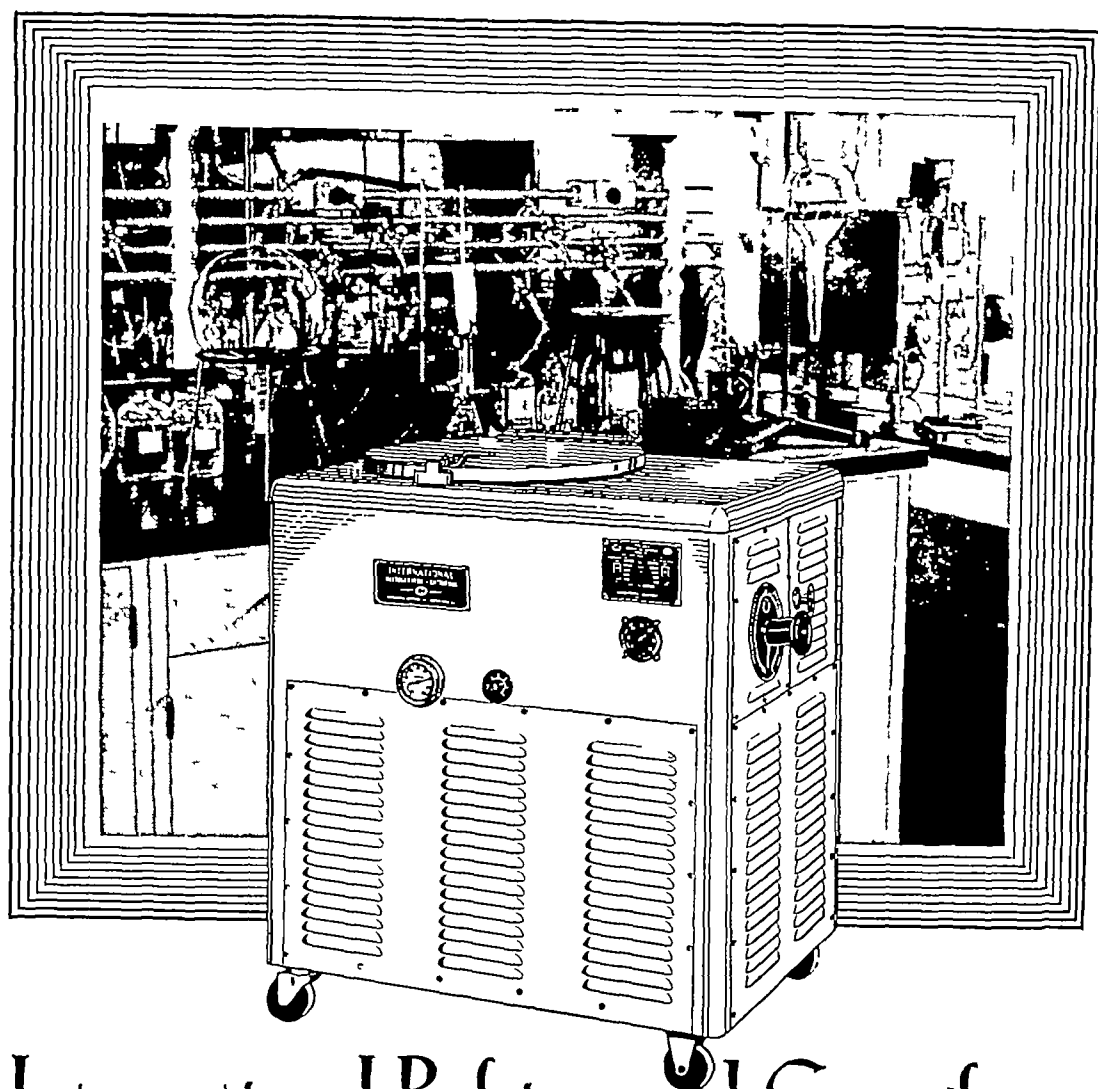
Society of the Sigma Xi Annual Lecture, Ralph W. Wyckoff, The Macromolecular Texture of Biological Materials, *evening, December 27*

United Chapters, Phi Beta Kappa Annual Address, Speaker, Detlev W. Bronk, *evening, December 29*

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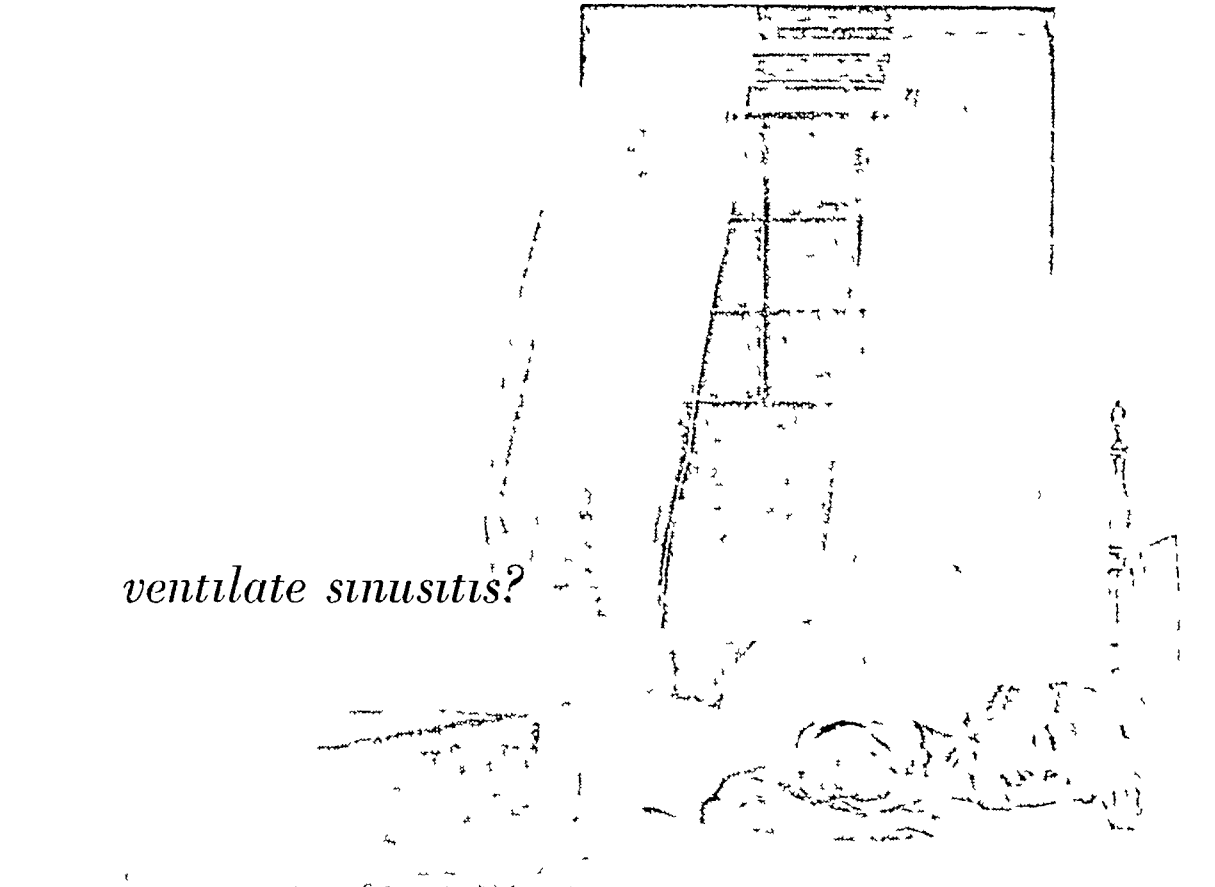
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Federation Proceedings

VOLUME 9

September 1950

NUMBER 3

AMERICAN ASSOCIATION OF IMMUNOLOGISTS

Symposium on Coxsackie Group of Viruses¹

Chairman GILBERT DALLDORF

THE COXSACKIE VIRUSES

GILBERT DALLDORF

From the Division of Laboratories and Research, New York State Department of Health

ALBANY, NEW YORK

IT SEEMS TO BE SOUNDLY ESTABLISHED that what we have called the Coxsackie viruses are common infections of man. The speakers that follow me will discuss the typing of various strains of Coxsackie viruses, their behavior in complement-fixation tests, their isolation under various circumstances and something of the nature of the human disease with which they have been associated (1).

It will be well to bear in mind that we are sadly deficient in our understanding of the human disease and know nothing regarding the lesions, if any, in man. That is why it seemed unwise to give the virus a descriptive name.

It may prove to be significant that the strains we have studied so far can be readily classified in two groups on the basis of the experimental disease (2). This may easily and definitely be done by histologic examination. *Group A* includes strains that induce lesions only of the skeletal muscles. *Group B* strains also cause lesions in the brain, sometimes in the fat pads, heart and other organs. We find the two kinds of Coxsackie virus differ in size and other respects. In our work it has been an advantage to be able to distinguish between the two and I suspect others will find it equally helpful.

So far these agents have been found associated only with man, in human feces and throat washings, in sewage and in flies trapped near human habitations. They have not been found as natural

infections in other animals. They have commonly been associated with epidemics of poliomyelitis or poliomyelitis-like disease. The association of Coxsackie and poliomyelitis viruses deserves attention and poses several interesting questions.

Our experience in New York during the past 3 years has been that Coxsackie virus may sometimes be found in the feces of poliomyelitis patients, sometimes alone, sometimes in combination with poliomyelitis virus. The data we have indicate that infection has distinct epidemiologic patterns, is largely limited to the young (the age distribution is similar to that of poliomyelitis some years ago), and is associated with symptoms indistinguishable from those of poliomyelitis. At any rate, we have not been able to distinguish between the symptoms and signs in the patients from whom we can and cannot isolate Coxsackie or poliomyelitis virus. This may simply mean that the Coxsackie viruses flourish during the summer months and have little clinical effect, that the disease we see is due in large measure or entirely to poliomyelitis virus.

Others have found Coxsackie virus associated with outbreaks of diseases of a different kind. It may be noted that so far these reports have been of *group B* virus infections. Dr. Kilbourne will describe such an outbreak. The observations of Curnen, Shaw and Melnick have much in common. We should look for Coxsackie viruses in several diseases for they seem to be associated with diverse clinical conditions.

COMPLEMENT-FIXATION TESTS WITH SOME OF THE VIRUSES IN THE COXSACKIE GROUP¹

JORDI CASALS AND PETER K. OLITSKY

From the Laboratories of the Rockefeller Institute for Medical Research

NEW YORK CITY

AN EARLY INTEREST of the writers concerned possibilities of laboratory diagnosis of the viruses comprised in the group designated by Dalldorf (1) as Coxsackie. While other members of the Symposium will discuss various methods of detection and identification of the viruses, this presentation is limited to complement-fixation tests. For the purposes of the study 4 laboratory strains of viruses of this group have been used: *type 1* (Dalldorf) (2, 3), *type 2* (Dalldorf) (2, 3), Conn 5 (4) and 1135K (5). Other colleagues of the Symposium will hold forth on the problem of the taxonomy of these agents: whether they are distinct, independent viruses or whether they are strains of the same virus. It is, however, of considerable interest that the viruses here employed have dissimilar properties in the experimental animal and that *type 1* was isolated from the feces of a child with a diagnosis of paralytic poliomyelitis, *type 2* and Conn 5, from stools of patients considered as cases of non-paralytic poliomyelitis, and 1135K, from the throat washings of a patient having an acute, febrile, influenza-like illness and whose chief complaint was abdominal pain (6). Only the future will tell whether one is dealing with a disease of protean manifestations, assuming a wide variety of clinical syndromes or whether the illnesses encountered, from which the Coxsackie viruses have been recovered, are different nosological entities caused by different viruses. From the results of the present investigations it would appear that, conforming with the designations commonly made by Dalldorf (1, 2), Curnen (6), Melnick (4) and others, the use of the term 'group of viruses' would at this time be preferable.

These facts are mentioned because they have bearing on the subject of complement fixation as

a test for detection of specific antibody in the serum of individuals or for identification of a particular virus. An antigen used in such tests primarily a) should be free from any nonspecific reactions since so many agents, be they strains or different viruses, have already been recovered, b) should have a maximal titer, i.e. should be powerful enough to detect the smallest quantity of specific antibody, and c) should be practical and simple, easy to adapt to ordinary laboratory equipment or field conditions and to available technical assistance. It is believed that such an antigen can readily be prepared, its preparation and the details of a complement-fixation test with it has already been described (7).

Several reports have appeared since then by others describing a diversity of methods for performing this test with Coxsackie viruses. Thus Howitt and Benefield (8) have used unfractionated suspensions of infected, isolated muscle repeatedly frozen and thawed. Melnick and co-workers (4, 9) have devised antigens prepared by high-speed centrifugation and precipitation by protamine combined with a microtest performed on lucite plates, the readings being plotted and the area under each curve determined by means of a planimeter. Recently Manire, Sulkin and Farmer (10) have described antigens made from suspensions of infected mouse muscle either uncentrifuged or as supernate fluid after centrifugation at 2500 rpm for 30 minutes.

The method adopted for the preparation of antigens now used in this laboratory for the Coxsackie viruses, and the details of the procedure of the test are those that have already been applied to neurotropic viruses in general (11). The preparation of an antigen is based on successive extractions of infected tissue with acetone and ethyl ether, after drying, the final residue is suspended in saline solution to a concentration of 3:1 with respect to the original wet tissue weight. The use of acetone-ether extraction is considered necessary since known Wassermann positive sera react with normal, nonextracted

¹ We are grateful to Major L. C. Murphy, V. C., U. S. Army and Professor Italo Archetti of our laboratory and to Drs. G. Dalldorf, E. D. Kilbourne and R. M. Taylor for their valuable cooperation.

tissue, thereby giving rise to false positives. Following extraction, however, nonspecific reactions are eliminated in most instances or they are reduced to such a degree that a practical specific test results (table 1). The tissue from which the antigen is made is obtained from Swiss-W, infant mice which are etherized to death. Either the muscle can be stripped off the bones, which is difficult, or the entire limb can be used which is a simpler method and yields as good

readily prepared so that a one-way test can be performed, i.e. a test to reveal antibody in 1135K antiserum against antigens other than 1135K. The antiserum is then found to contain antibody reactive against Conn 5 virus but not against *types 1* and *2*. In addition, *types 1* and *2*, and Conn 5 each appears to be antigenically specific (table 2, which shows a test with mouse antiserum). The relationship of 1135K virus to that of Conn 5 is further discussed by Dr

TABLE 1 REACTION OF HUMAN WASSERMANN-POSITIVE SERA WITH ACETONE-ETHER EXTRACTED AND NON-EXTRACTED ANTIGENS

SERUM	ANTIGEN												SALINE CONTROL
	Acetone-ether extracted Albany <i>type 2</i>						Nonextracted normal tissue						
Serum dilution reciprocal	2	4	8	16	32	64	2	4	8	16	32	64	2
Serum no 1	2	2	0	0	0	0	4	4	4	3	2	0	0
2	±	0	0	0	0	0	4	4	4	±	0	0	0
3	1	±	0	0	0	0	4	4	4	4	4	1	0
4	2	1	0	0	0	0	4	4	3	2	0	0	0
5	1	0	0	0	0	0	4	2	2	2	0	0	0
6	0	0	0	0	0	0	2	2	±	0	0	0	0
7	0	0	0	0	0	0	4	4	2	0	0	0	0

results. Brain tissue, owing probably to a low content of virus, does not produce effective antigens.

By this method antigens can readily be obtained with *type 1* and *2* from infected mice up to 14 to 15 days of age at the time of infection, best results, however, follow the injection with virus of 6- to 7-day-old animals, for the tissue of such mice yields an antigen of higher titer which exhibits least anti-complementary effects. In addition, mice are injected intraperitoneally and infected tissue harvested on the third or fourth day.

On the other hand, mice are relatively less susceptible to the effects of either Conn 5 or 1135K viruses. In these instances animals 1, 2 or 3 days old are needed, for older ones either fail to be affected or develop mild illness from which they can recover. With the limb tissue of such mice an effective antigen can be prepared with Conn 5 virus. Up to the present time, and work is still in progress, no usable antigen has been obtained with the 1135K virus. The probability is that a sufficiently large amount of virus per unit weight of tissue has not yet been secured. Anti-serum against this virus can, however, be

TABLE 2 A COMPLEMENT-FIXATION TEST WITH COXSACKIE VIRUS

ANTIGEN AND TISSUE	SERUM			
	<i>type 1</i>	<i>type 2</i>	Conn 5	1135K
<i>Type 1</i> limb	1/64	0	0	0
<i>Type 2</i> limb	0	1/64	0	0
Conn 5 limb	0	0	1/64	1/64
Conn 5 brain	0	0	0	0
1135K limb	0	0	0	0

Kilbourne, a member of this Symposium who first isolated the former active agent.

Immune sera to be used as positive controls for testing antigens are prepared by the repeated intraperitoneal injections of 0.5 cc. of 10⁻⁴ dilution of suspensions of infected limb tissue into 50- to 60-day-old mice. The inoculations are repeated 4 times at 3-day intervals and animals are bled for serum collection 7 to 10 days after the last injection. The other half of the inoculation of material for the complement fixation tests have already been reported (12, 7) and offers an example of the method.

On the basis of the

as described here, 60 sera were studied which were obtained from 28 human beings, several of whom were bled more than once. The individuals from whom specimens were secured fell into 3 groups: 1) laboratory contacts, i.e. those exposed during the course of handling the viruses, 2) patients with various illnesses now suspected as being of the Coxsackie types and 3) persons having no recollection of such illness or of contact with the virus. Of the first group, viz. laboratory contacts, there were 7, the serum of all was positive: all reacted with *type 1*, 6 also against Conn 5, and 4 also against *type 2* antigens. As already stated, no satisfactory antigen of the 1135K agent is as yet available. The results are interesting in view of the fact that the personnel

persons who had no history of illness suspected as being the Coxsackie types nor known contact with the virus. Of these the serum of only 3 could be collected for study: none reacted with *type 2*, one with *type 1* only, one with *type 1* and Conn 5, and the third, with none of the 3 antigens. In table 3 is shown a series of selected 8 cases which are illustrative of the results obtained with the aforementioned 3 groups of tests.

From the results of the present studies it appears therefore that the complement-fixation test performed under controlled conditions offers a means for determining the presence of antibody in sera of man and experimental animals and for measuring its relative amount. The results were generally confirmed in later reports by others.

TABLE 3. COMPLEMENT FIXATION WITH HUMAN SERA COXSACKIE-VIRUS ANTIGENS

NO	SERUM History	ANTIGEN			
		Albany <i>type 1</i>	Albany <i>type 2</i>	Conn. 5	Normal tissue
1	Laboratory contact with 3 strains	1 4	0	1 32	0
2	Laboratory contact with 3 strains	1 16	1 8	1 16	0
3	Laboratory contact with 3 strains	1 16	1 8	1 16	0
4	Clinical infection				
	a) before onset	1 2	0	0	0
	b) month after	1 8	0	1 2	0
5	Clinical infection				
	a) day of onset	1 2	0	0	0
	b) 2 months after	1 4	0	1 8	0
	c) 1 year after	1 2	0	1 2	0
6	No contact or recognized infection	1 4	0	0	0
7	No contact or recognized infection	1 8	0	1 8	0
8	No contact or recognized infection	0	0	0	0

of the laboratory was exposed to all of the 4 viruses, and most active work being carried on with *type 1*. The second group comprised persons from a wide geographic distribution. Our thanks are due Dr. R. M. Taylor for the specimens from Iceland, to Miss B. F. Howitt for the ones from Texas and to Dr. F. P. Nagler for those from Prince Edward Island, Canada. Of the serum of 18 individuals, 11 were tested with *type 1*, 2 and Conn 5 antigens: one was positive with all of them, 4 with *type 1* and Conn 5 only, 5 with *type 1* only and the eleventh was negative with all 3. Seven were tested with *type 1* and 2 antigens: 5 were positive with the former and all negative with the latter. It is noteworthy that complement-fixation antibody against *type 1* virus is generally more commonly met with here than against the others. The third group consisted of

The pattern of antibody level in several neurotropic virus infections runs along the following lines: neutralizing antibody appears, as a rule, early after exposure to a virus, rapidly reaches a high level and then either remains on a higher or lower plateau for a considerable period of time, perhaps for the life of the individual. With the complement-fixing antibody, however, the course is often different. Here, the antibody arises somewhat later, reaches promptly its highest level and then, in a matter of months, slowly diminishes to complete extinction. Whether the same pattern of antibody production is followed by the Coxsackie viruses cannot be determined at the present time.

It will be observed that in certain instances in which paired sera were available, the complement-fixing antibody showed an increase cor-

related with the course of the infection, after the manner of a specific antibody response. At the same time several specimens exhibited positive reaction to more than one antigen. In view of the specificity of the antigens as revealed by control tests with normal sera and prepared, known antisera, such reactions are considered to be essentially not cross reactions, but rather specific responses to the antigens in question.

It is clear, as shown here and by the work of others, that the Cocksackie viruses are widely distributed and that infection with them is not uncommon. Many persons show positive reactions for serum antibody among whom are those who have no recollection of contact with the virus or of suspected illness. Infection is, moreover, easily contracted as noted by the large incidence of positive reactions in workers handling the virus, several of whom have been unaffected by illness during this period of contact, although it is difficult to state this as cause and effect since

these persons may have been positive before they came in contact with the viruses (viz. Curnen (6) for several clinically recognizable laboratory infections). The situation in the case of Cocksackie viruses is one that has similarities with that of poliomyelitis virus in which a large percentage of apparently normal human beings sooner or later develop antibody.

Since subclinical infections with the Cocksackie viruses may be a common occurrence (2, 4, 8) and in view of the fact that such infection leaves its signature in antibody production, it is clear that an antibody test per se on a single sample of serum or even on repeated samples is not a means for diagnosis, unless it can be shown by examination of sera deriving from early stages of a suspected illness and from later convalescent periods that there has been a distinct rise in antibody which is correlated with the course of the disease.

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ISOLATION AND DIFFERENTIATION OF THE COXSACKIE GROUP OF VIRUSES

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DURING THE PAST YEAR AND A HALF, material has been received either from sporadic cases or from groups of patients showing ill-defined clinical pictures such as mild fevers, poliomyelitis-like, encephalitis-like, or influenza-like syndromes. At first it was thought (1) that these cases might have some relationship to Newcastle Disease of chickens but since this virus was never isolated and as it was shown that the positive neutralization tests were due to a non-specific heat labile factor in the human sera, (2) this idea was abandoned. Following the report by Dalldorf and Sickles, (3) on recovery of the Coxsackie group of viruses from human feces, (4, 5) all the available material on hand was inoculated into suckling mice. Coxsackie viruses were recovered from the feces and nasopharyngeal washings of many of these patients. Subsequently more specimens were examined from various sources, and strains have been recovered from 105 individuals in 10 different states, Alabama, Colorado, Delaware, Florida, Georgia, Louisiana, Oklahoma, South Dakota, Tennessee and Texas.

METHODS

1) *For Isolation of Viruses* The fecal and oral specimens were prepared according to a previously described method (6). Twenty per cent suspensions of feces were made in distilled water and run in the angle centrifuge $\frac{1}{2}$ hour at 13,000 rpm in the cold room. One thousand units of penicillin and 10 mg of streptomycin were added for each ml of the supernatant fluid and the mixtures were left at room temperature for $\frac{1}{2}$ hour before inoculation of 3- to 4-day-old mice. Each animal received 0.01 ml intracerebrally, 0.03 ml intramuscularly and 0.03 ml intraperitoneally of the treated feces. The oral washings were treated in the same manner, except that the antibiotics were reduced to $\frac{1}{2}$ the quantity.

If the mice showed muscle paralysis or were

prostrate after 3 or 4 days' incubation, they were killed and the skeletal muscles removed. Because a higher concentration of virus was found in the muscles, Seitz filtrates of the latter tissues were used for all future work.

2) *Immune sera* were produced by inoculation of mice, guinea pigs, hamsters, rabbits or monkeys with either brain or the filtered muscle suspensions. An effective serum was obtained in about 3 weeks by bi-weekly intravenous injections of rabbits with filtered infected mice muscles.

3) *Neutralization tests* were performed according to the method in the U. S. Army Laboratory Manual (7), except that the serum-virus mixtures were incubated for 20 minutes at 37° C and held for 20 minutes in the cold room before inoculating 0.03 ml intramuscularly into 3- to 4-day-old mice. All sera were inactivated at 56° C for 30 minutes before use. Although the virus strains had LD₅₀ titers of between 10⁻⁵ and 10⁻⁸, many undiluted sera gave such high protection that a neutralization index often was not obtained when only 2 or 3 dilutions of virus were used. In tests reported as positive, however, the serum neutralized at least 1000 LD₅₀ doses of virus. Occasionally, the tests were performed on different dilutions of sera against a constant amount of virus, usually 100 LD₅₀ doses.

4) The methods used for the complement-fixation tests have been recently described (8). Filtered mice muscles were used for antigens and the tests were performed according to a modified Kolmer technique with over-night fixation in the cold.

VIRUS ISOLATIONS

The first 5 viruses that gave symptoms of muscle paralysis in suckling mice were isolated from human feces at monthly intervals from October 1948, to January 1949. After these original isolations of the Coxsackie virus, a systematic study was made of all material remaining from the earlier collections beginning with 1947 and continuing through 1949. This material had been kept frozen at either -10° C or -60° C and in

¹ From the Virus and Rickettsia Section, Montgomery, Ala.

many instances the virus remained viable after storage for over 2 years at the latter temperature

ings, mouth vesicles, nasal swabs, saliva, sputum, serum, whole blood, lung, cord and brain, but not spinal fluid that had been sent in at various

TABLE 1 SUMMARY OF ISOLATIONS OF COXSACKIE VIRUSES FROM HUMAN SOURCES¹

STATE	ORAL WASHINGS		FECES		BLOOD		BRAIN OR CORD		SPINAL FLUID	
	No tested	No positive	No tested	No positive	No tested	No positive	No tested	No positive	No tested	No positive
Ala	34	24	16	5	21	11	5	3		
Colo			2	2			1	0		
Del	48	7	31	9					20	0
Fla	4	4	6	5	4	3				
Ga	11	7	13	7	2	2				
La	6	2	5	1	1	1	4	3		
Okla	1	1	4	1	1	1				
S D							1	1		
Tenn	16	5	6	1	10	8	4	2		
Tex							1	1		
Totals	120	50 (41.6%)	83	31 (37.3%)	39	26 (66.6%)	16	10 (62.5%)	20	0

¹ Includes 2 sputum specimens

TABLE 2 COXSACKIE VIRUS ISOLATIONS FROM FATAL CASES

CASE NO	AGE	C	SEX	LOCATION	DATES OF		MATERIAL TESTED	DIAGNOSIS	TYPE OF VIRUS ISOLATED
					Onset	Death			
1	56 yrs	C	M	Ala	1/31/49	2/14/49	Brain	TB of lungs, Encephalomyelitis	2
2	4	W	F	Ala	9/1/49	9/15/49	Spinal cord	Polio myelitis	4 ¹
3	2	W	M	Ala	9/3/49	9/27/49	Spinal cord	Polio isolated	
4								Autopsy findings negative	4
5	6	W	M	Conn	12/49	2/5/50	Lung	Pneumonitis	
6	2	C	M	Ga	5/30/49	6/2/49	Serum	Encephalitis	2
7	34	W	M	La	?	9/1/49	Serum	Polio myelitis	2 ¹
				La	8/3/49	8/11/49	Brain	Polio myelitis or Encephalomyelitis	4 ¹
8	12	W	M	La	10/1/49	10/18/49	Brain	Encephalitis ?	5
9	20	W	F	S D	12/10/49	12/17/49	Brain	Polio myelitis	4
10	3	W	M	Tenn	7/15/48	7/25/48	Nasal washing	Encephalomyelopathy	4 ¹
							Brain		4 ¹
							Serum		4 ¹
11	34	W	M	Tenn	11/16/49	11/23/49	Brain stem	Polio myelitis	2
12	?		F	Tex	No information		Brain stem	Polio myelitis	5

¹ Virus of the same type isolated a second time from the same material

Coxsackie viruses have been isolated from human feces, nasopharyngeal or mouth wash-

times from scattered localities in 10 different states Table 1 gives a summary of 117 isolations

and shows both the geographical distribution and the number obtained from the different kinds of specimens

While it has been believed that the Coxsackie viruses are associated with a benign type of infection, it is interesting to observe, as shown in table 2, that they have been recovered from the oral washings of one, the lung of one, the serum of 2 and the nervous tissues of 9 fatal cases

samples had been kept frozen at -10°C and some were held for over a year. While 14 of the specimens were obtained during the first week of illness, one was taken in 9 and another in 13 days after the onset. The dates of onset were unknown for the other patients.

Specimens were received from a number of outbreaks which, although characterized by diverse clinical pictures, were all suspected of hav-

TABLE 3 OUTBREAKS ASSOCIATED WITH ISOLATIONS OF COXSACKIE VIRUS

LOCATION	YEAR	GENERAL TYPE OF CASE	PREDOMINANT SYMPTOMS	TOTAL NO OF CASES TESTED	VIRUS ISOLATIONS FROM					
					Feces		Mouth Garglings		Blood	
					No tested	No positive	No tested	No positive	No tested	No positive
Wilmington, Del	1947	Polio-myelitis-like	Fever, headache, some muscle weakness	46	31	9	36	1		
							12 ¹	6		
Enterprise Ala	1948	Polio-myelitis-like	3-5 day fever, headache, nausea, vomiting	19	6	2	5	2	7	6
Shreveport La	1948	Mild fever	3-day fever, headache, no neurological symptoms	5	4	1	6	2		
Nashville Tenn	1948	Encephalitis-like or atypical syndromes	Varied symptoms, headache, fever	17	3	0	13	3	10	8
Montgomery Ala	1949	Influenza-like	Fever, aches, some mouth vesicles	21			21	12	9	3
Swainsboro, Ga	1949	Generalized Febrile	Fever, headache, vomiting, malaise	14	7	2	9	5	1	1

¹ Contact nurses

Six of these 12 fatal cases were diagnosed as having poliomyelitis. Poliomyelitis and Coxsackie viruses were recovered simultaneously from the spinal cord of one of these patients. They were obtained also from the feces of 2 other non-fatal cases. It may be seen likewise that the same type of virus was recovered twice from 3 different materials (brain, nasal washings and serum) from the same patient.

Virus was obtained from either the clotted blood or the serum of 26 patients. All of the blood

ing a viral origin. The symptoms were usually those of a non-paralytic poliomyelitis or a mild 3- to 5-day fever accompanied by headache and often muscle pains. In one group of adults influenza was suspected. Because no influenza virus was isolated, the throat washings were tested for the Coxsackie strains with the results as shown in table 3.

The largest number of isolations has been from material of patients becoming ill during August and September, although specimens have been

positive from cases occurring throughout the year, especially in the spring Curnen, Shaw and Melnick (9) have reported likewise that the virus has been more often recovered from the August and September specimens

Differentiation into Serological Types Early in these studies it was observed that the antiserum from one strain of virus did not afford protection against several others. When more viruses were isolated, it became apparent that they belonged to serologically distinct types. The presence of a multiplicity of strains has been reported by both Dalldorf *et al* (3-5) and Melnick *et al* (10). Through the courtesy of Dr G Dalldorf of the Division of Laboratories and Research of the

but had certain properties in common 1) They could be propagated only in suckling mice or hamsters, did not infect 14- to 21-day old mice, embryonated eggs, young chicks, baby guinea pigs, rabbits or monkeys, including one 4 days old, 2) they all passed through a Seitz filter and 3) produced myositis but no neurological lesions in suckling mice. Virus could be found in the blood stream, the brain, cord, lungs, liver, spleen, kidneys and intestinal contents of infected mice and was present in high concentration in the skeletal muscles. Likewise the baby mice could be infected by various routes, intracerebrally, intraperitoneally, intramuscularly, subcutaneously and intranasally.

TABLE 4 DISTRIBUTION OF VIRUS TYPES

STATE	TYPE 1 ¹	TYPE 2 ¹	TYPE 4	TYPE 5
Ala	1	5	18	9
Colo			2	
Del	1	6	3	
Fla				7
Ga		3	6	3
La		3	1	1
Okla		1	2	
S D			1	
Tenn	1	5	6	1
Tex				1
Total no types ²	3	23	39	21

¹ Types 1 and 2 Sickles and Dalldorf

² Each number represents an individual case

New York State Department of Health, antisera were received for the viruses which he has designated as *types 1, 2 and 3 of group A*.

By means of both the neutralization and the complement-fixation tests (8), it was found that certain of the strains isolated in this laboratory fell into *types 1 and 2* but none so far into *type 3*. In addition 2 other immunologically distinct viruses have been found that are tentatively called *types 4 and 5*. Table 4 shows the geographical distribution according to states of the 86 strains that have been typed. So far, *type 4* has been found most frequently while *types 2 and 5* are next in prevalence. Although there was a tendency for a certain type to predominate in one area or in a particular outbreak, yet often one or more other types might be present also. *Type 5* seems to be prevalent in Florida and in Alabama.

All 4 of the strains were serologically distinct

ISOLATION OF TYPE 4 VIRUS FROM NORMAL MICE

Early in this study a question arose as to whether or not one or more of the Coxsackie strains might not be latent in normal mice. In May 1949, the filtered muscles of 4 normal 3-day old mice were inoculated into a group of 5-day old mice. In 4 days the animals became paralyzed and a second passage of filtered muscles to normal suckling mice resulted in establishment of a virus that was serologically different from the other 2 strains on hand and was later called *type 4*.

On reviewing the records, it was noticed that 2 Coxsackie strains that were later proven to be *type 4* were inoculated on the same day as the no. 1 normal mouse strain and it also happened that at least 7 strains of virus that later proved to be *type 4* had been isolated in the laboratory from human material during the preceding 2 months. This was unknown at that time.

The question now arose as to the origin of this *type 4* strain. Was it a latent mouse virus inactivated by inoculation or was it originally of human origin but so easily passed in the laboratory that it could be picked up in normal mice on serial passage?

From the facts that antibodies for *type 4* strain were found in human sera, that this virus could be obtained more than once from the same human tissues and could be found in mouth washings at weekly intervals, it seemed probable that the *type 4* strain might have a human origin but could be acquired by mice in the laboratory. Although no virus was recovered from the tissues of 3 pools of suckling mice tested at that time, yet from later experiments it seems highly prob-

able that accidental infection can occur in the laboratory. The same *type 4* virus has been isolated on 2 other occasions from the filtered muscles of normal mice that had been inoculated with filtered muscles of other normal mice.

ANALYSIS OF VIRUS ISOLATIONS

Because of the above findings and because so many viruses were being obtained, the isolations were analyzed according to the number of trans-

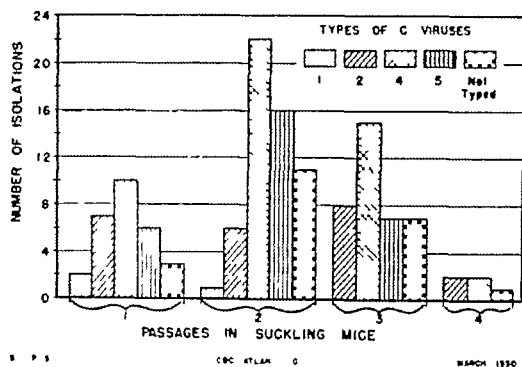


FIG 1 PASSAGES IN MICE before muscle paralysis occurred

fers in mice to recover 5 (3.9%) of the strains. In each instance such symptoms as lack of growth, slight muscle weakness and ataxia were observed in the mice that warranted the transfer of the muscle filtrates to more animals.

Besides these 126 isolations of the Coxsackie viruses, 200 other human specimens were inoculated into baby mice without recovery of a virus. One hundred and forty-six of the specimens were passed only once, while 44 were passed twice in mice, and 10 were given 3 passages without virus isolation. Therefore, out of 326 attempted isolations, 126 (38.6%) were positive for the Coxsackie viruses. It seems probable that some of these recoveries were not obtained from the human cases, but resulted from accidental transfer in the laboratory. The authentic strains are most likely those that show definite muscle paralysis on the first passage, although many obtained after 2 mouse transfers may also be considered.

SEROLOGICAL TESTS

Both neutralization and complement-fixation tests have been done on groups of human sera,

TABLE 5 RESULTS OF NEUTRALIZATION TESTS AGAINST COXSACKIE VIRUSES

Sera	TESTS AGAINST VIRUS TYPES							
	1		2		4		5	
	No	Positive	No	Positive	No	Positive	No	Positive
Patients	43	19 (44.1%)	59	53 (89.8%)	29	19 (65.4%)	19	14 (73.6%)
Normal			4	4 (100%)	14	10 (71.4%)		

Total number of paired sera tested	55
Cases with negative acute phase sera but positive convalescent	15
Sera positive to 2 viruses	26
Sera positive to the patient's virus	26
Sera negative to the patient's virus	6
Sera positive to a heterologous virus	4

fers made in suckling mice before typical muscle paralysis was observed. Figure 1 shows the number of isolations of each virus type obtained after 1, 2, 3 or 4 passages, respectively, in suckling mice. Of 126 isolations, only 28 (22.2%) viruses gave typical symptoms in mice after the first inoculations, 56 (44.4%) were definitely positive after a second transfer, 37 (29.2%) strains were obtained after a third passage, while it took 4

many of them taken during the acute and convalescent stages (8). As shown in table 5, of 55 paired sera only 15 (27.2%) showed evidence of a rise in neutralizing antibodies between the early and late specimens. Twenty-six (27.1%) of 95 sera tested neutralized more than one virus, although not all the specimens were tried against each of the 5 strains. The sera of 26 (72.2%) out of 36 patients from whom a virus was iso-

lated contained antibodies for the homologous type of Coxsackie virus, while 6 sera failed to be neutralized by the homologous strain and 4 were positive to a different type

The results of the neutralization tests against each of the 4 strains of virus are given also in table 5. Of 17 normal sera tested against types 2 and 4, only 4 were negative against the viruses employed

DISCUSSION

Although 126 isolations of the Coxsackie viruses have been made from tissues or secretions of 105 individuals, yet from the fact that the most prevalent type (type 4) has been recovered from filtered muscles of normal suckling mice, it seems quite likely that many of these isolations were due to dissemination of the virus in the laboratory. This is to be considered particularly probable, because on several occasions 2 different types of Coxsackie virus were obtained either from the same or from different specimens from one individual. These irregular findings have occurred at least 4 times.

In this study it has been of special interest to have recovered the Coxsackie viruses (types 2, 4 and 5) from the tissues or secretions of 12 different fatal cases, 6 of which were diagnosed as poliomyelitis. Poliomyelitis virus was isolated from the spinal cord of one of these patients and also from the feces of 2 recovered cases. Because of the lack of uniform symptoms, however, it is still a matter of conjecture whether or not the Coxsackie viruses were responsible for the fatalities.

The isolation of the Coxsackie viruses from the blood stream of human cases is likewise of significance, not only because it suggests a simple method of recovering virus, but also because the blood is thus a potential source of virus for blood sucking vectors. Melnick *et al* (10) have reported previously on the recovery of the Coxsackie virus from flies taken in nature. However, because of the frequency of isolating the virus from the oral passages, the evidence at present seems to indicate a person-to-person transmission.

Certain strains of the Coxsackie viruses seem to predominate in particular outbreaks and in certain areas. Where the infection seems to be mixed, the possibility of accidental contamination is to be considered. If one adheres to the following criteria, however, one should be reasonably

certain that the virus has come from the patient, 1) typical muscle paralysis in the first mice inoculated with the original specimen, 2) ability to recover the same strain of virus more than once from the same material and 3) presence of antibodies in the patient's serum to the same strain of virus isolated from the patient, preferably with evidence of a rise in titer.

SUMMARY

Strains of the Coxsackie group of viruses have been isolated from the tissues or secretions of 105 patients from 10 different states with multiple isolations from different kinds of specimens from 6 separate individuals. The widespread prevalence of this group of viruses is thus made apparent. Four distinct serological types have been obtained. Two of them correspond to types 1 and 2 of Dalldorf, the other 2, tentatively called types 4 and 5, are antigenically different, although giving the same clinical and pathological pictures in mice. These viruses have been recovered from human blood, serum, brain, cord, mouth or nasopharyngeal washings, saliva, sputum, nasal swabs, mouth vesicles, and feces, with a predominance of types 4, 2 and 5, in order of frequency. The viruses have been found in material from 12 fatal cases from 7 different states and have been recovered many times from the blood stream.

Neutralizing antibodies for the Coxsackie viruses have been found in the sera of both patients and normal individuals. Of 55 paired sera 15 (27.2%) showed an increase in antibody content between the acute and convalescent specimens, while 26 sera neutralized more than one type of virus. Antibodies for the homologous strain of virus were found in 26 (72.2%) of 36 sera of patients from whom a virus had been isolated. One of the strains of Coxsackie virus has been recovered 3 times from muscle filtrates of normal suckling mice inoculated with other normal mouse muscles and there is strong evidence to show that these viruses may spread accidentally in the laboratory, thus accounting for some of the isolations. To be assured that a particular virus has etiological significance, it should 1) produce typical muscle paralysis in suckling mice on the first inoculation, 2) should be recovered a second time from the same material and 3) should be neutralized by the patient's serum.

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DIVERSE MANIFESTATIONS OF INFECTION WITH A STRAIN OF COXSACKIE VIRUS

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SINCE THE ORIGINAL ISOLATION by Dalldorf and Sickles (1) of new viruses pathogenic for suckling mice, the properties of the Coxsackie viruses have been extensively investigated. However, studies of the disease produced with these viruses in man have been few, and largely limited to consideration of patients suspected of poliomyelitis. It is already evident that infection of the human with Coxsackie viruses may result in paralytic disease (2), aseptic meningitis (3), epidemic pleurodynia (4) or undifferentiated fever (4). The present report is concerned with a comprehensive study of the varied manifestations of infection with a strain of Coxsackie virus in a small, circumscribed outbreak unassociated with paralysis or meningitis.

In early August 1948, 5 of 6 physicians resident at the Hospital of The Rockefeller Institute and 2 non-resident physicians closely associated with the Hospital fell victim within a 2-day period to an acute febrile illness. Sporadic instances of illness also occurred concomitantly in other members of The Rockefeller Institute staff unassociated with the Hospital. Efforts to demonstrate serologic evidence of infection with the viruses of influenza A, influenza B, and Newcastle disease were unsuccessful in the early study of this influenza-like epidemic. Infectious mononucleosis, suspected because of the presence of atypical lymphocytes in blood smears, was excluded by the absence of significant concentrations of sheep red blood cell agglutinins in convalescent sera of patients. Attempts to recover a virus from blood and throat washings by inoculation of chick embryos and 3-week old Swiss mice were fruitless.

The report, in 1949, by Melnick, Shaw and Curnen (3) of the isolation in suckling mice of a virus from aseptic meningitis patients in New England in the summer of 1948, prompted re-investigation of The Rockefeller Institute outbreak because of its temporal coincidence with the New England epidemic. Neutralization tests em-

ploying the Connecticut strain (Conn 5) of virus (kindly supplied by Dr J L Melnick) against the paired sera of 7 patients promptly defined the etiology of The Rockefeller Institute illnesses. Because these patients had presented no clinical evidences of paralysis or meningitis, as described in earlier reports of Coxsackie virus infection, it seemed of importance to review the comprehensive clinical and laboratory data assembled on the 5 hospitalized patients in order to aid in the delineation of this newly recognized disease.

CLINICAL MANIFESTATIONS

Within the same 12-hour period, 6 of the 7 patients studied experienced the onset of fever. In all but one patient insidious prodromata of insomnia, malaise and migratory aches of low intensity had occurred during the preceding 24 to 48 hours.

Certain features of the disease encountered in 3 or more of the 5 hospitalized patients are listed in table 1. The major complaint in all patients was pain of aching character, usually described as 'muscular'. Pain involved the neck, eyes, back, head, legs and shoulders with greatest frequency. Restlessness was also described by all patients, and occurred chiefly in the afebrile, prodromal period. It is notable that 4 patients complained of mild pharyngeal soreness, in 3 this symptom appeared 48 hours after onset of fever. Fever and muscle tenderness were physical signs common to all patients. In 4 patients fever lasted less than 24 hours. The fifth patient suffered a febrile relapse on the sixth day of illness unassociated with the presence of bacterial pathogens in blood or throat cultures.

Except for the non-specific common complaints of pain and fever, there was little clinical indication that the patients studied were victims of the same disease. The diverse clinical manifestations of infection are contrasted in table 2. It is apparent that such widely divergent clinical entities as infectious mononucleosis, acute appendicitis, sinusitis, pleurodynia and influenza

were simulated by Coxsackie virus infection. Despite the brevity and benignity of the symptoms, the intensity of the abdominal and 'sinus' pain encountered was sufficient to instigate surgical consultation, while the severity of the chest pain in *patient H A* suggested acute pleuritic or

Despite the promptness of defervescence, convalescence in all patients was protracted, symptoms of transient aches, lassitude, generalized weakness and easy fatigability persisting for 1 to 5 weeks. During the ensuing 22 months no sequelae of infection have become apparent.

TABLE 1 COMMON CHARACTERISTICS OF DISEASE

ONSET	GRADUAL
Common complaints	Pain (5) ¹ neck, eyes, back, head, legs, shoulders Restlessness (5) Mild sore throat (4) Nasal congestion (3)
Common signs	Fever (5) $av = 102.4$ (R) Muscle tenderness (5)
Average fever duration	30 hours
Complications	None
Convalescence	Protracted asthenia

¹ Number of patients

LABORATORY DATA

Routine urine analyses were negative, and none of the common pathogens was isolated from throat or blood cultures. Hematologic findings of interest are summarized in table 3. Unequivocal leukopenia was evident in 2, and low normal total leukocyte counts were observed in 2 other patients. Relative lymphocytosis was a feature of differential leukocyte counts made during the acute illness and also one month later. Large, atypical, young lymphocytes with deeply basophilic cytoplasm and lobulated nuclei were noted in 2 acute phase smears, and in the 4 convalescent phase smears studied. Eosinophilia occurred in 2 patients in early illness.

TABLE 2 DIVERSE CLINICAL MANIFESTATIONS OF INFECTION

PATIENT	CHIEF SYMPTOMS	CHIEF PHYSICAL SIGNS	ILLNESS SIMULATED	NET ANTIBODY ACUTE/CONV	VIRUS ISOLATION
<i>E K</i>	Headache	Splenomegaly	Infectious mononucleosis	<4/600	0
<i>H K</i>	Abdominal pain	Abdominal tenderness	Appendicitis	<10/62	+
<i>H E</i>	Backache	Sinus tenderness	Sinusitis	<10/390	0
	Nasal obstruction	Nasal obstruction			
<i>H A</i>	Chest pain	Splinting of chest	Pleurodynia	<10/256	0
<i>C S</i>	Chest pain	Pain on eye movement	Influenza	<10/427	0
	Ocular myalgia				
	Cough				

pericardial disease, the latter belied by a normal electrocardiogram.

Physical signs of disease were few. Slight pharyngeal injection and posterior pharyngeal lymphoid hyperplasia were noted in 3 patients. Generalized lymphadenopathy was not detected, but tender submaxillary lymphadenopathy was found in patients with pharyngeal soreness, and in one patient a small, tender axillary node was discovered. Splenomegaly of slight degree was discerned in 2 patients, one of whom complained of tenderness of palpation in the right hypochondrium and suprapubic region. The latter complaint was not associated with urinary tract signs or symptoms. Neurological examination in all patients was negative although *patient H E* remarked transient paresthesia in the right axilla.

DIAGNOSTIC AND IMMUNOLOGIC STUDIES

Confronted with the problem of re-investigating, 14 months after its occurrence, an epidemic from which no etiologic agent had been recovered, the factor of geographical propinquity dictated the choice of the Conn 5 strain of Coxsackie virus for trial neutralization tests with patients' sera. Tests were performed in 1- to 2-day-old Rockefeller Institute white mice by the intraperitoneal injection of 0.03 cc of virus-serum mixtures containing 50 median effective doses (using death and paralysis as the end point) of virus in the form of saline suspensions of eviscerated suckling mouse carcasses. Preliminary tests in which 1:10 (final) dilutions of sera were used demonstrated no neutralizing antibody in any of

the 7 acute phase specimens, and complete protection by all convalescent sera

Attempts to recover virus from the throat washings and blood of patients resulted in the isolation, on 2 occasions, of virus from the throat washing of patient *H K*. This throat washing, taken in phosphate buffered saline (pH 7.2) had

TABLE 3 COMPARATIVE LEUKOCYTE COUNTS

PATIENT	TOTAL LEUKOCYTE COUNTS (ACUTE)	DIFFERENTIAL LEUKOCYTE COUNTS ¹									
		Acute					Convalescent				
		P	L	AL	E	M	P	L	AL	E	M
<i>E K</i>	2400	14	58	16	2	10	32	34	32		2
<i>H K</i>	11,200	52	12	22	8	3					
<i>H E</i>	3400	51	38		3	8	32	47	18	3	1
<i>H A</i>	6600	55	38			7	50	20	20	4	6
<i>C S</i>	5400	34	32		30	4	35	21	37	6	1

¹ P = polymorphonuclear leukocyte L = lymphocyte AL = atypical lymphocyte E = eosinophile M = monocyte

5 strain, while complement fixation occurred in the presence of HK antiserum and the Conn 5 virus (This and complement-fixation reactions subsequently mentioned were generously performed by Dr Jordi Casals)

Titration of neutralizing antibody in serum specimens was effected by mixing serial 4-fold dilutions of serum previously heated at 56°C for 30 minutes with a constant quantity of virus (i.e. 50 median effective doses). This method of titration was chosen because it should provide accurate measurement of antibody concentration in sera with high neutralizing capacity. Studies by Horsfall of the precision of neutralizing antibody measurements with influenza A virus in the mouse (5, 6), and by Horsfall and Curnen of PVM neutralizing antibodies in the same species (7) have demonstrated with both viruses an identical linear relationship between the serum dilution end points and the quantity of virus employed. Morgan (8) has shown the same relationship using still another virus (WEE) in the mouse inoculated by either the intraperitoneal or

TABLE 4 NEUTRALIZING ANTIBODY TITRATIONS

PATIENT	VIRUS	TITER OF ACUTE SERUM 1-3 days	TITER OF CONVALESCENT AND LATE SERA				
			½ mo	1-2 mo	8 mo	14 mo	20-21 mo
<i>E K</i>	Conn 5	0 ¹	>4096 ²	600	117	144	112
	HK		2560	410	115	64	131
<i>H K</i>	Conn 5	0		62			32
	HK	0		132			16
<i>H E</i>	Conn 5	0		390			32
<i>H A</i>	Conn 5	0	256	256			128
<i>C S</i>	Conn 5	0		427			64
Complement-Fixing Antibody Titers							
<i>E K</i>	Conn 5	0		8	4	2	2
<i>H A</i>	Conn 5	0		2			

¹ No antibodies demonstrable in 1:10 dilution of serum ² Reciprocal of serum dilution

been stored in a sealed glass ampoule for 14 months in a CO₂ cabinet prior to intraperitoneal injection into a litter of one-day-old mice. Five days later, tremors, ataxia and generalized weakness were noted, and all mice were killed for passage. On second passage, a 20 per cent broth suspension of extremities and brain tissue, injected intracerebrally and intraperitoneally, initiated paralysis as early as the third day. Third passage by the intraperitoneal route alone resulted in death or paralysis by the 5th day of all 16 mice inoculated. This virus (HK) was neutralized by specific immune serum against the Conn

intracerebral route. In these systems the quantity of virus may be varied as much as 7-fold without causing a significant (i.e. 4-fold) change in the neutralization titer of a serum.

In table 4 the neutralizing antibody titers of the 5 hospitalized patients may be compared. In every instance antibody was undetectable in acute phase serum, but manifest in high titer in convalescence. In 2 instances (patients *E K* and *H A*) when serum was obtained less than 2 weeks after onset of illness, very high antibody concentrations were already evident. High concentrations of antibody were still present in the sera of

all patients nearly 2 years after infection. Serial antibody titrations in *patient E K*, using both the Conn 5 virus and the HK virus homologous to the epidemic, reaffirmed the antigenic similarity of the 2 strains, and provide an indication of the slow rate of decline of neutralizing antibody levels. Concomitant serial determinations of complement-fixing antibody are also recorded in table 4.

Studies of the sera of 2 laboratory workers heavily exposed to infected litters and tissue suspensions were made 4 and 7 months, respectively, after initial exposure. No neutralizing antibodies were manifest in the serum of the worker exposed for the longer period. However, serum drawn from the other worker 4 months after his original exposure to Conn 5 virus was found to contain neutralizing antibody not ini-

tially present. During the period of exposure this person had experienced no clinical illness.

This study of an outbreak of Coxsackie virus infection has adduced certain facts worthy of emphasis. Coxsackie viruses, currently identified with poliomyelitis-like illness, may produce disease with widely divergent manifestations unsuggestive of poliomyelitis. Convalescence may be slow, and abnormal differential leukocyte patterns may persist for 4 to 5 weeks. Neutralizing antibody response is prompt, maximum serum titers appearing 2 weeks or less after the onset of illness, and high titers persist at least 21 months. Inapparent infection may occur and lead to a specific antibody response. The stability of the virus is indicated by its repeated recovery from a throat washing stored at -70°C for 14 months.

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DIFFERENTIATION OF IMMUNOLOGICAL TYPES AMONG THE COXSACKIE VIRUSES¹

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SOON AFTER THE DISCOVERY of the Coxsackie group of viruses, it was clear that they existed in multiple immunological types (1, 2). In fact, in view of the differences in tropisms for certain murine tissues which exist among the types (3, 4), it is not unlikely that different viruses may now be included in this group. For the present, however, viruses whose capacity to produce overt disease in experimental animals is limited to infant mice and hamsters are being placed in the Coxsackie family. It is recognized that in mice some types (*group A*) produce a disease which seems to involve only the skeletal muscles, whereas other types (*group B*) also involve additional tissues, notably the brain. In any case, we shall deal here with methods for classifying strains of what are now considered to be the Coxsackie viruses into distinct immunological types.

The classical methods of immunology have been employed with certain modifications which have had to be adapted to these viruses and their infant hosts. The methods include 1) cross protection tests in infant mice born of vaccinated mothers, 2) production of pharyngeal and intestinal carriers of virus in chimpanzees following its oral administration, and a study of homologous and heterologous immunity in these animals, 3) cross neutralization tests, and 4) cross complement-fixation tests.

CROSS PROTECTION TESTS IN INFANT MICE

The susceptibility of mice to Coxsackie viruses is short-lived, and in a matter of a few days after birth they become naturally resistant to the disease. Consequently, cross protection tests in which challenge virus is inoculated into vaccinated mice cannot be performed, because the time required for an expected immunity to develop would place the mice out of their susceptible period. But by challenging infant mice

within 48 hours of birth from mothers vaccinated with different strains of active virus, we have been able to use this procedure as a typing method. The vaccinated females which were bred had neutralizing antibody titers of about 1:1000 against 100 ID₅₀ of virus and complement-fixing titers of about 1:64 against 8 units of antigen. It was found that homologous immunity was present whether or not the baby mice were nursed by their own or by foster mothers following the

TABLE 1. MATERNAL TRANSFER OF TYPE SPECIFIC IMMUNITY

CHALLENGE VIRUS	BABIES BORN OF MOTHERS VACCINATED WITH			
	Conn-5	Ohio-1	Texas-1	High Point
Conn-5	+	—	—	—
JLM	+	—	—	—
NL		—	—	—
Ohio-1	—	+	—	—
Texas-1	—	—	+	+
High Point	—	—	+	+
NH Fly 1943				+
Easton-2	—	—	—	—
NY-5		—	—	

+ indicates mice resistant to challenge inoculation

— indicates mice susceptible to challenge inoculation

challenge inoculation of 100 ID₅₀ doses of virus. However, in practically all instances the infant mice received at least one feeding of colostrum from their own mother before the inoculation of virus. The results are shown in table 1.

The immunity was type specific and was completely absent when mice were challenged with heterotypic viruses. Strains which fall into the same antigenic type by other tests (neutralization and complement fixation) also fall into the same type in these cross protection tests. Thus mice

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vaccinated with the High Point or Texas-1 strain had babies immune to each of these strains and to the related NH Fly 1943 strain. Also when 2 strains (*JLM*, *NL*) related to Conn 5 were used for challenge, only mice from Conn-vaccinated mothers were protected. And when 2 strains (Easton-2 and NY-5) related to Dalldorf's type 1 were used, none of the offspring from mothers vaccinated with any of the 4 strains was protected.

HOMOLOGOUS AND HETEROLOGOUS IMMUNITY IN CHIMPANZEES

In following the immune response in chimpanzees which have no obvious signs of illness follow-

few days after the exposure and continuing for 3 to 6 days. Some, if not the bulk, of the virus fed is excreted within 3 to 5 days, and this is followed by new virus, 'manufactured' by the animal, for a period of 2 to 4 weeks. Antibodies to the virus appear within 2 weeks and maintain their titer for many months.

Several weeks later, if the same strain is re fed to such a chimpanzee, then the virus may be found in the stools for a period of about 5 days, having passively traveled through the alimentary tract. No true carrier state occurs, and it has not been possible to isolate virus from the blood or throat. However, if the Texas (heterotypic)

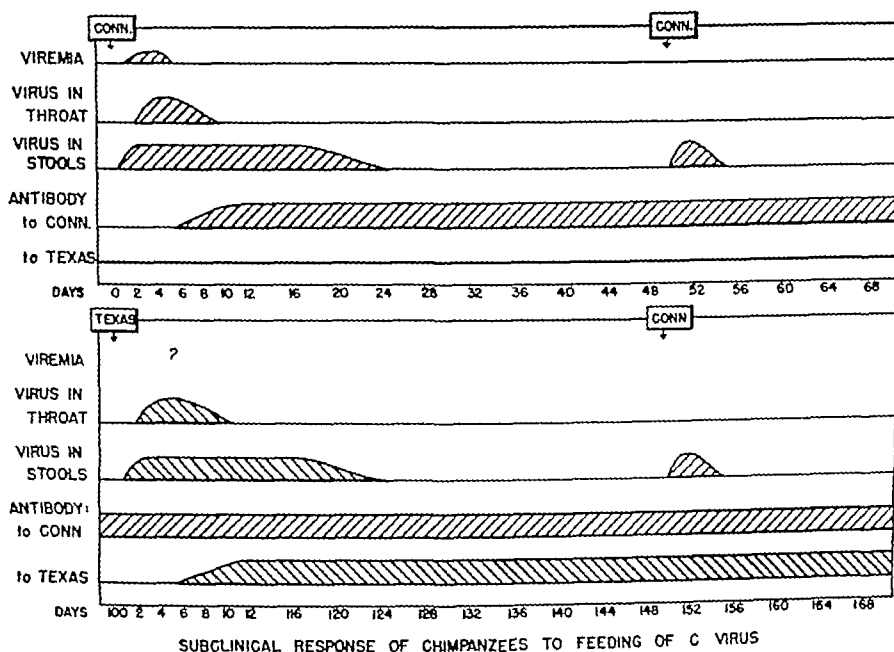


Fig 1 DEMONSTRATION OF HOMOTYPIC IMMUNITY and heterotypic susceptibility in the chimpanzee

ing oral infection with Coxsackie virus, criteria other than clinical illness must be used. The measure of infection is based on the development of a pharyngeal and intestinal carrier state (with viremia) and the accompanying appearance of neutralizing antibodies in the serum. The measure of immunity is based on whether or not the carrier state can be again produced by a challenge dose of virus. Similar studies in chimpanzees infected with poliomyelitis virus have recently been reported (5, 6).

The pattern of the results obtained (7) is shown in figure 1. If the Conn-5 strain is fed to a chimpanzee, the virus will appear in the blood for a few days following the feeding. Virus may be recovered from the throat generally starting a

strain of virus is now fed, then the chimpanzee appears to respond in the same manner as it did when the Conn virus was first fed, and a new antibody (Texas type) appears in the blood. Such a chimpanzee is now immune to both types, but may still respond like a new animal when fed a third type (Ohio).

With this method we have found that the Conn-5, Texas-1 and Ohio-1 strains each belong to a distinct immunological type and that the High Point strain is related to the Texas type.

CROSS NEUTRALIZATION TESTS

Type-specific neutralizing antibody is one of the most widely used reagents for identifying a virus, and for the Coxsackie group of viruses the

neutralization test works admirably if adequate numbers of infant mice are available for the performance of the tests. For diagnostic purposes, using paired sera, or for other quantitative antibody measurements, results are most satisfactory when a fixed quantity of virus is used with varying dilutions of serum. For typing of strains, however, we prefer to use a constant amount of serum (usually undilute or 1:5 mouse or monkey immune serum) and determine the titer of the strain in the presence of this serum. In conformity with the procedure used for other viruses, the neutralization index is the difference between the titer of the virus in the presence of the immune serum and that in the presence of normal serum. Pre-heating of the serum at 56° C for 30 minutes

in New Haven, Connecticut and Winston-Salem, North Carolina, and in the same year from sewage collected in Hartford, Connecticut. Antibodies to this type are also widespread, occurring in human sera collected on both the east and west coasts of the United States as well as in Denmark and Sweden. That the detection of these antibodies reflect prior infection with specific types of virus is suggested by the presence of Texas type antibodies and the absence of Conn type antibodies in the sera of over 50 individuals, both young and old, living in northern Alaska (7).

Further information on the specificity of the neutralizing antibody response has been obtained from an extensive study of the occurrence and development of antibodies in 6 patients who

TABLE 2 CROSS NEUTRALIZATION OF STRAINS OF COXSACKIE VIRUS

VIRUS	CONTROL ID#	IMMUNE SERUM LOG OF NEUTRALIZATION INDEX							
		Conn	Ohio	Texas	Hi.Pt	Easton	D 1	D 2	D 3
Conn -5	10 ^{-5.7}	>4.7	0	0	0	0	0	0	0
W-S, NC	10 ^{-4.3}	>3.3	0	0	0	0	0	0	0
Ohio-1	10 ^{-5.2}	0	>4.2	0	0	0	0	0	0
Texas-1	10 ^{-7.0}	0	0	6.0	5.0	0	0	0	0
Hi Pt	10 ^{-6.5}	0	0	4.6	5.0	0	0	0	0
NH '43	10 ^{-7.5}	0	0	6.0	6.0	0	0	0	0
Easton 2	10 ^{-6.0}	0	0	0	0	5	5	0	0
D-1	10 ^{-6.0}	0	0	0	0	5	5	0	0
D-2	10 ⁻⁵	0	0	0	0	0	0	>4	0
D-3	10 ⁻⁵	0	0	0	0	0	0	0	>4
Nancy	10 ⁻⁶	0	0	0	0	0	0	0	0

has no measurable effect on the content of antibodies.

The results of table 2 indicate that we can now recognize at least 7 antigenic types (7). Of these, 2—Conn -5 and Ohio—belong to *group B* and 5, to *group A*. It is noteworthy that strains belonging to one type may be widespread in their occurrence. For example, the *group A* Texas type, named from the fact that it was first isolated from flies trapped in Texas in 1948, has been recovered a) from human stools collected that year and the next in Texas, b) from sewage collected in North Carolina in 1948, and c) from flies collected in New Haven in 1943 and stored on dry ice until 1950. Furthermore, antibodies to this type are widespread in the human population, occurring in sera collected in several areas in this country, in Europe and in northern Alaska. *Group B* types may also be widespread, as evidenced by the isolation of the Conn -5 type in 1948 from patients

contracted infections with one or another of the Coxsackie viruses while working with them in the laboratory (7-9). From each patient a virus was isolated during the acute phase of illness which was typed by following the reactions with specific hyperimmune animal sera (see bottom three rows of table 3). The data in table 3 also show that no patient before his illness had detectable antibodies to the strain subsequently isolated from him or to the prototype strain. But soon after illness, there developed simultaneously antibodies to the strain isolated from the patient and to the prototype strain to which it was related.

The reactions of the sera of the laboratory patients with strains not related to the one isolated from each patient are instructive. Only 2 of the 6 patients had an illness due to Texas type infection, yet the other 4 must have had a subclinical infection with this type. The results with the serial samples of blood taken from patient

JLM starting several months before his illness emphasize this point on November 29, 1948, antibodies were absent to both the Conn and Texas types By February 21, 1949, antibodies to the Texas type had appeared in titer of 1 100

prototype By May 26, such antibodies were present

Concerning antibodies to the Ohio type, 2 patients had such antibodies before they became ill (to a heterologous type) Three of the remain

TABLE 3 ANTIBODY RESPONSE IN SIX PATIENTS INFECTED WHILE WORKING WITH COXSACKIE VIRUSES

PATIENT AND DATE OF ONSET	DATE OF SERUM	SERUM TITER OBTAINED AGAINST 100 ID ₅₀ OF VIRUS								
		Virus stain used in neutralization test								
		<i>ESm</i> ₁ ¹	<i>JLMm</i> ₁	<i>NLM</i> ₁	<i>LMKm</i> ₁	<i>FLm</i> ₁	<i>GJm</i> ₁	Conn. 5 <i>m</i> ₅	Texas <i>m</i> ₄	Ohio <i>m</i> ₂
<i>EWS</i> 2/14/49	1/21/49	0						0	0	0
	2/21/49	50						100	100	0
	3/25/49	250						100		0
<i>JLM</i> 5/16/49	11/29/48		0					0	0	100
	2/21/49		0					0	100	10
	5/18/49		0					0	100	100
	5/26/49		250					100	100	1000
	6/12/49		1250					100	100	100
	10/20/49							100		100
<i>NL</i> 5/21/49	2/10/49			0				0	0	100
	5/26/49			1250				100	100	100
	11/2/49							1000	100	100
<i>LMK</i> 10/20/49	3/25/49				0			10	10	0
	5/24/49				0			10	1000	0
	10/28/49				100			1000	100	100
	12/27/49							100		1000
<i>FL</i> 7/16/49	6/15/49					0		0	0	0
	7/25/49					100		0	1000	0
<i>GJ</i> 7/6/49	6/15/49						0	0	0	0
	7/25/49						250	0	100	0
Animal Immune	Conn mouse 10/20/49	>100	>100	>100	0	0	0	1000	0	0
	Texas mouse 10/22/49	0	0	0	0	>100	>100	0	1000	0
	Ohio Rh 4332 8/11/49	0	0	0	>100	0	0	0	0	1000

¹ Subscripts after *m* indicate number of passages the virus had undergone in mice before it was used in the neutralization tests

and this serum level was maintained in the next 4 bleedings carried out in May, June and October of that year The patient became ill (diagnosis compatible with epidemic pleurodynia or myalgia) on May 16, 1949, at a time when he had no antibodies to his own strain or to the Conn

ing 4 persons did not develop Ohio-type antibodies during the period studied, whereas a fourth, *LMK*, did at the time of illness The *LMK* strain proved to belong to the Ohio type

It is noteworthy that this experience serves as an accidental but nevertheless clear example that

strains belonging to the Conn -5, Ohio, and Texas types are capable of producing disease in man

At the time of *JLM*'s infection with the Conn type, his family was infected with yet another type. His daughter, Nancy aged 8, was ill with 2 bouts of fever (maximum 103.5°), the first from May 14 to 16 and the second from May 27 to 28

TABLE 4 FAMILY INFECTION WITH TWO TYPES OF COXSACKIE VIRUS

SERUM	SERUM TITER OBTAINED AGAINST 100 ID ₅₀ OF VIRUS					
	<i>JLM</i> <i>m</i> ₁ ¹	<i>Nancy</i> <i>m</i> ₁	<i>SM</i> <i>m</i> ₁	Conn 5 <i>m</i> ₁	Texas <i>m</i> ₁	Ohio <i>m</i> ₂
<i>JLM</i> ²						
2/21/49	0	0	0	0	100	10
5/18/49	0	0	0	0	100	100
5/26/49	250	1250	250	100	100	1000
6/12/49	1250	1250	250	100	100	100
<i>Nancy</i> ³						
3/23/49	0	0	0	0	250	0
6/3/49	0	100	50	0	250	0
<i>SM</i>						
6/3/49	0	0	0	0	250	0
12/29/49	0	0	0	0	250	0
Conn	>100	0	0	1000	0	0
Texas	0	0	0	0	1000	0
Ohio	0	0	0	0	0	1000
D-1	0	0	0	0	0	0
D-2		0	0	0	0	0
D-3		0	0	0	0	0

¹ Subscripts after *m* indicate number of passages the virus had undergone in mice before it was used in the neutralization tests

² *JLM* had fever from 5/18 to 5/23

³ *Nancy* had fever from 5/14 to 5/16 and recurrence on 5/27 to 28

Virus was isolated from fecal samples collected May 21 and June 5. Her strain belonged to a hitherto unknown type (7), shown by the data in table 4. *Nancy*'s mother, *SM*, was a healthy carrier of this strain as evidenced by isolation of the virus from her stools collected on June 10. However, unlike *Nancy*, she did not develop neutralizing antibodies to her own virus, nor to the Conn type which was the causative agent of

her husband's illness. *Nancy* developed antibodies to her own virus, to her mother's virus, but not to her father's virus. Between May 18 and 26, her father developed antibodies not only to his virus and to the Conn type to which it was related, but also to the type which infected the other 2 members of the family. The only reason for interpreting his illness as due to the Conn type and not to *Nancy*'s type was the isolation of a Conn type virus during his illness.

COMPLEMENT-FIXATION TESTS

In determining the cross reactions between strains by the complement-fixation test, the plate method of Fulton and Dumbell (10) has been employed whereby complement and serum are varied with a constant amount of antigen. Table 5

TABLE 5 CROSS REACTIONS OF COXSACKIE VIRUSES BY COMPLEMENT FIXATION

SERA	ANTIGENS						
	Texas 1	Hi Pt.	Conn 5	Ohio-1	D-1	D-2	D-3
Texas-1	100	122	0	0	0	0	0
Hi Pt.	51	100	15	4	0	0	0
Conn -5	0	10	100	0	0	0	0
Ohio-1	0	0	0	100	0	0	0
Dalldorf 1	0	0	0	0	100	0	0
Dalldorf 2	0	0	0	0	0	100	0
Dalldorf 3	0	0	0	0	0	0	100

shows the extent of the cross reactions between 7 strains of Coxsackie virus. The means for arriving at these figures are similar to those described for influenza virus (10) and will be described in detail for Coxsackie virus, but for the present it will suffice to say that '100' indicates a complete cross and '0' no crossing whatever. It is evident that the only extensive cross reaction occurs between strains High Point and Texas which together form a type immunologically distinct from the other 5 strains listed which in turn fall into 5 distinct types.

In preparing complement-fixing antigens for use with sera from heterologous animal species, antigens have been rendered specific for Coxsackie virus by treatment with protamine sulfate according to the method of Warren (11, 12). Table 6 shows the results of protamine treatment of mouse muscle antigen tested against immune sera of monkeys inoculated with mouse tissue. It

is seen that the nonspecific reactions of immune as well as of normal sera obtained with crude antigens (untreated) are eliminated when protamine treatment is employed

We have occasionally failed to make satisfactory antigens for the low-titer types (Ohio, Conn, Dalldorf's type 1) and have resorted to concentration by ultracentrifugation (7, 12). Numerous tests have been performed supporting the logic of this procedure, and table 7 indicates

TABLE 6 SPECIFICITY OF PROTAMINE-TREATED ANTIGENS

MONKEY NO	IMMUNE TO	STATE OF IMMUNIZATION	TITERS WITH ANTIGENS			
			Normal crude	Normal + protamine	Texas crude	Texas + protamine
4198	Texas	Pre	0 ¹	0	0	0
		Post	4	0	64	16
3955	Texas	Pre	0	0	0	0
		Post	16	0	64	16
4151	Conn	Pre	16	0	16	0
		Post	16	0	16	0

¹0 = <2

TABLE 7 TITERS OF A SINGLE SERUM WITH ANTIGEN (EASTON-2) CONCENTRATED IN THE ULTRACENTRIFUGE

NO OF TIMES ANTIGEN WAS CONCENTRATED	TITER OF SERUM
20	40
16	24
8	3
4	<2
With unconcentrated antigen	<2

typical results when the titer of a single serum is determined with various concentrations of a single batch of antigen

The development of humoral complement-fixing antibodies in human infections with the Ohio type of Coxsackie virus has been studied (7, 12). Table 8 indicates the serum titers obtained at various times after onset of illness in 6 patients. Concentrated antigen was employed in the tests, which were carried out in tubes using the 50 per

* All but one of these sera were obtained through the courtesy of Drs Albert B Sabin and Alex J Steigman who made their collections in Ohio in 1947 (13). Isolations of Coxsackie virus from the stools of 3 of their patients were made (12).

cent hemolytic endpoint. Patient LMK had a proved laboratory infection with the Ohio type, and the same type had been isolated from Red, Lan and Hux. In the case of Ric and Ris no isolations were attempted, but it would appear from the complement-fixation results that Ric was infected whereas Ris was not.

TABLE 8 COMPLEMENT FIXATION IN HUMAN DISEASE USING CONVALESCENT SERA WITH OHIO TYPE OF COXSACKIE VIRUS

	TITER OF SERUM							
	Onset	2 wk	1 mo	3 mo	4 mo	7 mo	1 yr	2 yr
Red	0 ¹	7	32	112				8
Lan	0	3	4	56				6
Hux	0		4					2
Ric	0	0	0	192			96	
Ris	0		0	0				
LMK	0		0	24	30	12		

¹0 = <2

TABLE 9 DIFFERENTIATION OF COXSACKIE VIRUSES BY FOUR METHODS

VIRUS TYPE	VIRUS NEUTRALIZATION	COMPLEMENT FIXATION	PROTECTION OF INFANT MICE BORN OF VACCINATED MOTHERS	IMMUNITY IN CHIMPANZES TO RE INFECTION
Conn-5	X	X	X	X
Ohio-1	X	X	X	X
Texas-1	X	X	X	X
D-1	X	X		
D-2	X	X		
D-3	X	X		
Nancy	X			

X indicates that by the method employed, the type indicated has been proven different from each of the other types tested by that method. A blank space indicates not done.

The temporal pattern of complement-fixing antibody obtained with the Ohio type, however, is not necessarily the same as that experienced in infections with other types. Patients infected with the Easton, 1949, strain (belonging to Dalldorf type 1) seemed to develop such antibodies much earlier than those infected with the Ohio type.

SUMMARY

Immunological evidence has been presented that the Coxsackie group of viruses exists in multiple antigenic types. At least 7 types are

now recognized. The methods for identifying these types have been reviewed in this paper. They are those of classical immunology with some modification to fit the virus-host systems involved and include 1) cross neutralization tests, 2) cross complement-fixation tests, 3) cross pro-

tection tests in infant mice born of vaccinated mothers and 4) cross protection tests in chimpanzees with subclinical infections. It can be seen from table 9 that each method yields results in agreement with those obtained by each of the other methods employed.

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EVALUATION OF THE SYMPTOMS AND SIGNS OF
DEFICIENCY DISEASES¹

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NUTRITION WORKERS oftentimes, and with some justification, accuse physicians generally of disregarding nutritional factors in the development of disease and of failing to accept some nutrition investigators' conclusions of widespread deficiency diseases within the population. Clinicians might reverse this indictment and accuse nutrition workers of oftentimes ignoring well-established clinical facts and of sometimes exhibiting a surprising ignorance of some fundamental medical concepts.

It is the writer's belief that the physician's sometime lack of enthusiasm for properly established nutritional claims has been attributable in part to the disappointment experienced when at other times nutritional claims were enthusiastically adopted, tested and found unsound. Accordingly, physicians have become cautious and even skeptical of all claims made by nutritionists. Because of such experiences, some clinicians are likely to regard nutrition workers as enthusiastic propagandists lacking in a knowledge of clinical medicine. It well behooves us as nutrition workers, therefore, to appraise critically the bases upon which we make claims, statements, policy recommendations and so forth. Otherwise we lose the confidence of other physicians and become ineffective.

Several examples could be cited to illustrate the above type of error. One such was the confusion of ordinary conjunctival thickening and pingueculae with xerosis conjunctivae or Bitot's

spots. The claims were made (1) that these thickened areas in the conjunctivae were due to chronic vitamin A deficiency, that 99 per cent of a low income population exhibited this condition and therefore "had microscopic ocular lesions characteristic of avitaminosis A." In another study (2) it was stated that "according to the above criterion every subject carried unresolved degenerative changes resulting from vitamin A deficiency, most of them of a moderate to severe character."

The year following the appearance of these claims of a startlingly high incidence of avitaminosis A, an experienced ophthalmologist (3) pointed out that "The 'spots', or pingueculae, are subepithelial infiltrations, the epithelium over them being unaffected, a condition different from xerosis in which the changes are in the epithelium, and that such spots are not associated with low states of vitamin A, as proved by blood tests." Furthermore, this ophthalmologist pointed out that these states represent common presenile or senile alterations.

Grossly the Bitot spot is characteristically laterally placed, although they may be both laterally and medially. The lesion is greyish, dull, and has somewhat the appearance of meringue. This is in contrast to the more elevated, gelatinous appearance of pingueculae. Illustrations of a true Bitot's spot may be found in Berliner's monograph (4).

Epidemiologic investigations consisting of simultaneous dietary studies, measurement of vitamin A concentration in the blood plasma, and observations of the conjunctivae with the slit lamp were made in two separate areas in North

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Carolina (5, 6) The samples comprised approximately 1000 persons each. In neither study was a correlation found between the severity or incidence of the conjunctival changes and the level of vitamin A in either the diet or plasma. In a well-designed therapeutic study which tested the effect of the daily administration to nurses of 50,000 IU of vitamin A over a period of up to 2 years, Robertson and Morgan (7) found that upon examination with the slit lamp "it was not possible to determine which nurses were receiving vitamin A and which were not and that in none of the subjects with conjunctival elevations at the beginning did therapy cause the elevations to disappear. Therefore one would conclude that the conjunctival thickening is not caused by a deficiency of vitamin A." A similar lack of influence of vitamin A on the appearance of the thickenings was noted by Borsook and Wiehl (8).

In studies designed to produce avitaminosis A in human subjects, neither of these conjunctival lesions has been observed. The most extensive experimental study of vitamin A deprivation in man is that of the Vitamin A Sub-Committee of the Medical Research Council (9). Despite the production by dietary restriction of lowered carotene and vitamin A levels in the plasma and of abnormalities of dark adaptation, it was found that the various biomicroscopic changes observable initially in both the deficient and control groups did not vary with vitamin A intake. No Bitot spots developed.

The abundant evidence which has accumulated justifies the conclusion that the conjunctival changes which are commonly observed in our population are not related to avitaminosis A nor do they serve as useful measures of nutriture. Bitot spots are very infrequently seen in the population of the United States. When present they may at times indicate avitaminosis A but frequently are not evidence of vitamin A deficiency unless associated with other significant findings (10). Extravagant claims (11) concerning widespread avitaminosis A based upon the prevalence of these lesions in the population should be discarded.

What is the status of the skin lesions which have been said to signify avitaminosis A? First, one must be certain of the definition of these lesions, for confusion exists as to their characteristics. The workers who are most familiar with these lesions are investigators with experience in the Orient, India or Africa. The descriptions of the lesions indicate that they are hyperkeratotic

papules occurring at the site of the hair follicles and initially, at least, over the extensor surfaces of the body. Because of the plugging of the sweat glands, the skin is excessively dry. The lesions are not to be confused with the congenital condition known as ichthyosis—a confusion which I have observed repeatedly to occur when enthusiastic but inexperienced persons undertake their first nutrition surveys. Sometimes pityriasis rubra pilaris offers a differential problem (12). Furthermore, these lesions have been confused with a less common condition known as Darier's disease.

A more frequent and serious confusion seems to be that of hyperkeratosis follicularis with the common abnormality of skin texture known to dermatologists as keratosis pilaris. This condition would seem to be that which is sometimes referred to in the literature as 'permanent goose flesh'. It is not infrequent in children under 12 to 13 years of age regardless of their nutriture. These lesions tend to be less noticeable during the summer months when there is increased moisture of the skin and most prominent during the cold winter months when the moisture of the skin is decreased. I have observed these lesions in children who have had well-rounded diets plus supplementary vitamin A for the first 8 years of their lives and who exhibited satisfactory levels of vitamin A in the blood. Krause and Pierce (13) found no significant difference in the blood levels of children with and without the condition. These lesions are commonly seen in the United States. They cannot be taken as evidence of avitaminosis A. As a matter of fact, in a too-little-known paper which reports on 207 cases of avitaminosis A observed at Peiping Union Medical College, Frazier and Hu (14) pointed out that "The cutaneous eruption was characteristic of vitamin A deficiency only after the patient had attained sexual maturity. Before the age of puberty the only common abnormality of the skin (due to avitaminosis A-WJD) was xerosis." These workers noted that of the 91 children under 15 years of age and diagnosed as having avitaminosis A, only 4 exhibited the keratotic lesions, and these 4 were all in the 14- to 15-year age group.

It appears, then, that lack of familiarity with the skin lesions of avitaminosis A has most likely led many workers in America erroneously to assign to this deficiency all of the 'nutmeg grater-like' skin lesions which they have observed. Most certainly, the frequency of occurrence of rough, dry skins with or without 'permanent goose flesh' cannot be used as the chief index of vitamin A nutriture. As a matter of fact, there is appearing

an increasing body of evidence relating skin lesions seemingly indistinguishable from the hyperkeratosis of which we have been speaking to a lack of other dietary essentials, especially the B vitamins, and even unsaturated fatty acids (15)

Recent studies show that clinically manifest avitaminosis A will be accompanied by serum levels of vitamin A of less than 50 IU per 100 cc and that the earliest physiologic manifestation of this deficiency is the change in dark adaptation (9, 16). In my opinion, the only manner in which one may arrive at reasonable estimates of the incidence of vitamin A deficiency within a population is by the combined application of the clinical, biochemical and physiologic study coupled with observations on the therapeutic response of subjects judged deficient by these tests. Dietary studies may give confirmatory information, but they will chiefly be of value in the description of the food habits.

The question of avitaminosis A has been considered in some detail to serve as an example of the sort of critical appraisal of clinical methods which is desirable at this time.

Similarly, experience with the sign of so-called 'corneal vascularization' observable with the slit lamp as evidence for riboflavin deficiency has proved valueless for assessing the nutritional level population groups in this country. Although corneal vascularization does occur in ariboflavinosis (17), the occurrence of prominent vessels at the limbus, or indeed even of actual corneal invasion, is not correlated with the estimated riboflavin intake in the diet nor with other signs suggestive of riboflavin deficiency (5, 6, 18). Further, these conditions are not amenable to long-continued therapy with large quantities of riboflavin (8). Lastly, it may be added that vascularization of the cornea occurs in all types of corneal injury, and, at least in experimental animals, it may result from a great variety of dietary deficiencies (19-22).

It is to be concluded that this sign alone gives us no information as to the prevalence of ariboflavinosis within a population. In individual cases, however, it is reasonable to employ this sign as one of the several manifestations included in the syndrome of ariboflavinosis. This is to say that, as in all clinical diagnosis, we must not base our judgment upon the presence or absence of a single isolated non-specific sign.

Sebrell and Butler (23) demonstrated that fissuring at the angles of the mouth occurs in ariboflavinosis. This sign also occurs in deficiencies

of iron (24, 25), niacin (26, 27) and vitamin A. It is often produced by illfitting dentures (28), in which instance it is termed 'pseudoariboflavinosis'. It may occur in chronic illnesses, especially arthritis, and not respond to vigorous nutritional therapy. Angular fissures are frequently observed to appear during periods of treatment with certain antibiotics. At times we have observed cases in which the lesions seem to be allergic in origin. Responses of angular fissures to pantothenic acid (29) as well as pyridoxin (30, 31) have been reported. It is apparent that interpretation of the significance of this sign must be made with considerable knowledge of the individual patient, and that a mere tabulation of the frequency with which it occurs in a population does not provide a reliable index of ariboflavinosis within that population.

The biomicroscopic appearance of the lingual papillae has been recommended (32) and used in some studies as an indicator of the niacin nutriture. The non-specificity of lingual changes is apparent from the variety of conditions which may result in glossitis. For example, Glossitis may be associated with iron deficiency anemia and respond to therapy with iron alone (24, 25). It is a frequently observed sign in sprue and responds to folic acid (33). The glossitis of pernicious anemia, a disease characteristic of the older age group, responds more satisfactorily or permanently to vitamin B₁₂ than to folic acid (34). The deficiency glossitis of pellagra responds to niacin, but similar lesions may be amenable to therapy with riboflavin (17). Pantothenic acid (29) has been reported as efficacious in some instances.

Perhaps no other area of the body is subject to so great trauma as the mouth and tongue. The wearing of plates and the chewing of tobacco are frequent causes of glossitis and cheilosis (35). Post-menopausal atrophy of mucous membranes may be resistant to all forms of nutritional therapy. A legion of other non-nutritional lingual changes can be found in any good textbook of dermatology or of oral diagnosis. Among those most frequently referred to us for evaluation have been Moeller's glossitis, leucoplakia and lichen planus.

From the great variety of conditions which affect the lingual mucosa, only a few of which I have indicated here, it is apparent that biomicroscopic study of the condition of the tongue cannot serve to assess the niacin nutriture of a patient or a population.

It is well known that swollen, red, friable,

bleeding gums are characteristic of scurvy. It has been proposed (36) that changes in the gingivae serve as an index of vitamin C deficiency. Such an interpretation of these lesions would seem to receive support from studies on chronic ascorbic acid deficiency in the monkey (37) and from the investigations of Linghorne and others (38) on RCAF personnel. These investigations demonstrated that the recurrence of previously corrected gingivitis was retarded somewhat by the daily dietary intake of 75 mg of ascorbic acid in comparison with subjects receiving 25 or 10 mg of ascorbic acid. Without attempting to discuss all of the implications of this particular study, suffice it to note that gingivitis recurred (although more slowly) despite large intakes of ascorbic acid. Obviously there are causative factors other than vitamin C deficiency. Hence, gingivitis alone is not a valid criterion of ascorbic acid deficiency within the population. The gums, like other oral structures, are subject to many varieties of trauma and numerous gingival diseases are recognized by dentists and other oral diagnosticians. It is regretted that the guidance of those professionally trained persons with the best backgrounds in oral pathology has seldom been sought by nutrition investigators.

To summarize to this point: 1) biomicroscopic examination is of very limited value in assessing nutritional level of individuals. It would seem to be useless in nutrition surveys as a generally applied procedure. Its usefulness is limited to the study either of individual cases of suspected ariboflavinosis or to observations of tissue changes which occur in controlled experimental investigations such as that of the Vitamin C Sub-Committee (39). 2) A corollary of this conclusion is that estimates of the prevalence of nutritional deficiencies based upon this method should be discounted. 3) Clinical signs of nutritional deficiency are not specific and for them to have usefulness in the detection of nutritional deficiencies within the population, they must be interpreted in relation to the medical (and dietary) history, other physical findings, laboratory studies, and the results of therapeutic tests. 4) The nutrition investigator must recognize the limitations of his own training and background in clinical medicine, dentistry, ophthalmology and allied fields and work with competent individuals who are trained in these disciplines. Only by so doing will he make the greatest contribution to our total knowledge and avoid repetition of some of the expensive mistakes of the past.

Some of the signs widely used to 'judge nutri-

tional status' appear to have been incorporated into surveys without clearly defined rationale. For example, it has repeatedly been suggested that alterations in the texture, pigment or amount of hair, particularly in children, may be due to malnutrition, and these qualitative alterations have been listed in tables of clinical findings useful in the evaluation of nutritional status (40, 41). Hair changes are indeed oftentimes noted in malnourished children (42, 43). By analogy to experience with animals, unkempt hair might be considered as a manifestation of illness due to deficiency disease. However, such changes in the human have not been linked with any specific nutrient, and from the analogy with animals, one might predict that if alterations in hair texture and color be indeed evidences of malnutrition they could arise from a great variety of deficiencies. Momentary reflection upon the problem indicates that numerous non-nutritional factors related to the hygiene of the scalp and hair influence hair texture.

The significance of some additional skin changes deserves mention. Keratotic lesions have been discussed. In Kwashiorkor a 'crazy pavement' dermatosis occurs (44). It would not be surprising if some uninitiated enthusiastic worker reports a series of patients with congenital ichthyosis as having 'subclinical Kwashiorkor'. Another skin lesion of some interest is petechiae. These small, usually perifollicular 'spots' are seen in early scurvy, where they are usually distributed over the dependent portions of the body or, in infants, in areas of trauma. Petechiae resulting from other causes, however, have similar distributions. For example, patients with idiopathic or orthostatic purpura exhibit petechiae of similar distribution to those seen in early adult scurvy despite high ascorbic acid levels in the serum. They do not respond to massive therapy with vitamin C or numerous other therapeutic agents, nutritional and non-nutritional. Petechiae are seen in toxic manifestations, allergic states and in blood dyscrasias. In some of the latter, they may be associated with gingivitis to present a more confusing diagnostic problem. Petechiae also occur in a number of infectious diseases and may be seen in limited numbers in older persons with senile atrophy of the skin. Obviously, evaluation of this sign must be made in relation to other findings.

The skin lesions of pellagra are usually symmetrically distributed over the exposed surfaces. The reason for this is that the pellagra is photosensitive (45) and the skin changes are a mani-

festation of this. It is apparent, therefore, that very similar lesions will be produced in other photosensitive states, such as lupus erythematosus, in extensive vitiligo following exposure to the sun, ordinary sunburn, and so on.

So-called 'seborrheic dermatitis,' particularly of the face, nasolabial angle, and ears may result from a deficiency of riboflavin or niacin. However, indistinguishable lesions occur not infrequently in absence of deficiency states.

Neurologic manifestations of deficiency diseases include changes in the tendon reflexes and impairment of vibratory sense, both of which may result from peripheral neuritis. Peripheral neuritis may be a manifestation of thiamine deficiency, due to pernicious anemia, or have other etiologies. Under well-controlled conditions, repeated observations of these changes may provide some useful information regarding the development of deficiency diseases (46), especially if these observations be coupled with therapeutic testing. However, the multiplicity of factors affecting these two signs, together with the alterations which occur with advancing age, and the failure of the response to thiamine of the commonly occurring neurologic 'abnormalities' (47) demonstrate the restricted value of such observations for the determination of thiamine nutriture within a population.

I would be the last to deny that physical signs are an essential part of nutrition surveys when the observed signs are placed in proper relationship to known medical conditions which may result in these signs, along with well-conceived laboratory studies and dietary and therapeutic investigations. However, without such sound medical appraisal of the meaning of these signs, one should not make claims of the existence of widespread deficiency states based upon the physical findings alone.

Another word of caution concerning the evaluation and comparability of physical signs is necessary. This has to do with the subjectivity inherent in the clinical examination in nutrition—a problem well discussed by Bean (48) who noted that considerable differences may occur in the frequency with which examiners record the presence of a given physical finding within a population group. The interpretation or manner in which an examiner sees a condition or lesion depends, as Bean noted, very much upon the physician's own conditioning. For example, in

studying cheilosis the clinicians who reported a very low incidence were familiar with the angular stomatitis or cheilosis which was frequently seen in severely malnourished individuals. They failed to record abnormalities of the lips which were noted by other observers.

In this relation, the following table from data from a study in which I have been interested is pertinent. Observers, whose initial appraisals were all made under one experienced worker, recorded their clinical judgment as to general nutritional status of portions of a population. The ratings were undernourished, adequately nourished and obese. The table presents the percentage judged to be undernourished or obese by a given observer. Great variation is seen in the judgments of these clinicians. Accordingly, we listed these data in approximately the order of

TABLE 1 PHYSICAL FINDINGS BY EXAMINER
EXPRESSED AS PERCENTAGE OF TOTAL
EXAMINATIONS BY THE EXAMINER

EXAMINER	GENERAL NUTRITURE	
	Under	Obese
<i>R C</i>	26.9	9.5
<i>K S</i>	24.3	12.6
<i>E W</i>	11.4	19.8
<i>C W</i>	8.4	16.9
<i>W Mc</i>	14.7	27.2
<i>R P</i>	3.1	13.8

the weight of the examiner. It is apparent that the clinician's concept of normal bore a relationship to his own weight, that is, the heavier physician was inclined to judge fewer people overweight and more people underweight than did the thinner physicians.

Obviously, there is a large and oftentimes immeasurable subjective influence in appraisals of physical signs. This must not be forgotten when one attempts to compare the frequency of occurrence of given lesions in one study with those reported for another. It would be of interest to have data which would test the constancy of the judgment of a single investigator over a period of years. I would predict that changes in the criteria employed by a given observer would be found.

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EVALUATION OF DIETARY SURVEY METHODS¹

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THE ADVANCEMENT of the cause of nutrition depends upon a better understanding of body needs and of how these needs can be met through an appropriate food intake. Dietary surveys serve both of these objectives in a variety of ways. They serve to chart food consumption patterns which can be studied in relation to economic and educational status, cultural backgrounds and food habits, available food supplies and other factors. They provide the basis for more effective programs of nutrition education and for evaluating the results. On a national or regional basis, they furnish needed information for planning food production programs.

With respect to the topic of this symposium, they serve in the appraisal of the state of nutrition of individuals, families and other groups, through a comparison of the data obtained with some standard or yardstick of adequacy. They do not measure nutritional status directly. Rather they provide presumptive evidence, as well as yielding data which can assist in the interpretation of the results of more direct measures. In the past some workers have used the data from dietary surveys to draw conclusions regarding nutritional status, and particularly the extent of malnutrition, far more broadly than the methods used and their bases warranted. On the other hand, others who are particularly impressed by the limitations of the methods have expressed the view that surveys can have little value in the appraisal of nutritional health. Neither extreme viewpoint is justifiable. Surveys can contribute greatly if they are properly conducted and the results interpreted with a full recognition of the limitations involved.

Dietary surveys take many different forms, both with respect to the methods of obtaining data on food and nutrient intake, and to procedures for evaluating their meaning with reference to nutritional status. The various procedures have recently been comprehensively and critically reviewed in a section of the report of the Com-

mittee on Nutrition Surveys of the Food and Nutrition Board (1). This report should be carefully read by anyone who is planning work in the field. The present talk deals with certain aspects which are particularly pertinent in the use of dietary surveys as an aid in appraising nutritional status.

Whatever method of obtaining food intakes is used, some yardstick of adequacy is required to evaluate them. The reliability of the yardstick is thus of basic importance. At the present time there are certainly different concepts as to what constitutes malnutrition and, thus, as to what is an appropriate standard. In this country, assuming that the foods are translated into nutrients, it has become customary to use the Recommended Dietary Allowances. A direct comparison is made where the intakes are expressed as nutrients. Otherwise, servings or other measures of the foods themselves are compared with a recommended dietary pattern based on the Allowances. It is important, therefore, to recognize that these Allowances are higher than average needs, "to cover substantially all individual variations in the requirements of normal people." Parenthetically, the question arises as to what the word, normal, means. Not all individuals who fail to meet the Allowances can be considered to be malnourished. Some have failed to recognize this point in interpreting intake data.

Here also lies a limitation of food or nutrient intake data as measures of individual nutritional status. One does not know whether a particular individual needs the full amounts specified or whether substantially lesser intakes of some of the nutrients would suffice. As an illustration, in a small sample in Canada, Pett found that nearly 50 per cent of the adults were in calcium balance on an intake which was only half the 1945 Recommended Allowance and thus only 40 per cent of the present one (2). It may be considered that those 'normal' individuals who fully meet the Allowances or more are adequately nourished. Certainly, also, there is a level below which inadequacy can be assumed. In between lies a

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zone of uncertainty for the individual which further research may allow. The Allowances provide no data for several nutrients now known to be essential. It is possible that a diet may meet all the stated Allowances adequately yet fail to provide all the nutritional requirements for health. The Allowances are based to a large extent on the findings obtained in biochemical studies and in medical inspections for signs and symptoms of deficiencies, which have been previously discussed in this symposium. Thus, the reliability of these findings must be considered in using the Allowances as a yardstick. The previous discussions have indicated that these biochemical and medical measures are still subject to much uncertainty as regards their significance. All of these considerations should rule out generalizations regarding the incidence of malnutrition from dietary data alone, but they do indicate the value of such data for the support and interpretation of other measures.

It is clear that individual data are needed in any appraisal of the status of the individual. Repeatedly it has been shown that the food intake of the family may not be distributed among its members in proportion to individual needs. Whatever method is used for the individual, the goal must be the actual food intake. Waste must be accounted for. It is agreed that the most accurate method of arriving at the actual nutrient intake is a laboratory analysis of duplicate meals, together with an analysis of the food not eaten, if any. The time, expense and special techniques involved limit the use of this method to a comparatively few individuals. It is not practicable for use in dietary surveys in the broad sense.

Thus, the reliability of other methods of assessing individual nutrient intakes is a question of special importance. The most commonly used procedure is the calculation of the nutrients consumed by use of food tables giving average composition. The question of the applicability of these calculated values as a measure of the intakes of individuals in specific dietary situations is an important one. Several recent studies, such as those reported and reviewed by Kiser and associates (3), Bransby and associates (4), and others, have led many to conclude that calculated values are not sufficiently reliable to assess the adequacy of the diet of an individual. As is to be expected, variations are greater in the case of certain vitamins than for other nutrients. Obviously, tables giving average composition cannot accurately

reflect the actual nutrient intake from the food supply consumed in specific situations. Many factors cause the composition of staple foods to deviate from average values. Production, processing and storage variables are of particular importance for certain foods and in certain localities. Variable losses in home preparation must be expected, even though they are accounted for on an average basis.

These facts should cause us to consider how the influences of the variables in current tables of average composition can be minimized in future work, rather than arbitrarily to rule out calculated values for the individual. Advantage can frequently be taken of data for the composition of the local food supply at the season in question or of its nature as actually consumed, which may be more applicable than the average data. In a recent study by Hunter, *et al* (5), in Canada, in which the food composition data selected were those considered most likely to apply to the conditions in question, reasonably good agreement was obtained, with a few exceptions, between calculated and analyzed values. The revised tables of composition, now in the process of publication by the Bureau of Human Nutrition and Home Economics, will certainly provide data which are more applicable to the foods now used and to specific situations as well.

Comparative studies of calculated and analyzed values should be continued with a fuller recognition of the possibilities of lessening the discrepancies previously reported. Not all of the differences should be blamed on the limitations of the average data on composition. Analyzed values may be subject to errors in measuring the food actually consumed and also to errors in carrying out the analytical methods. Unless the food intake data themselves represent reasonably well what was actually eaten by the individual, nutrient calculations not only are not worthwhile, but also may lead to very erroneous conclusions. Records consisting of weights or measures of food eaten provide a more accurate basis than those expressed as servings.

The discussion thus far has dealt with procedures which give the greatest promise of reliability for appraising the nutritional status of the individual and for the support of and correlation with biochemical and physical findings obtained with the same subjects. Obviously it is impracticable to use them for any large segment of the population and for the broader purpose thus

served. The usefulness of family data for periodically assessing the food intakes, food habits and dietary trends of various segments of the population in relation to economic status, region, family size, food supplies available and other factors is well established. We are all aware of the large and important contributions which the Bureau of Human Nutrition and Home Economics are making along these lines.

With both family and individual food records the question as to whether or not the methods of collecting and evaluating the data can be shortened and still accomplish the purpose is of real importance in the interest of studying large groups. The calculation of nutrient intakes, food by food, is a very time-consuming process. The use of the so-called short method in which certain foods are grouped together greatly lessens the work involved. Several reports have indicated close agreement between certain short methods and the conventional long one. It has been shown, however, that short methods fall down where, in the diets under study, the foods making up a given group are consumed in quite a different proportion from that assumed in the grouping made. An intake of turnip greens as the sole representative of the green, leafy and yellow vegetable group should not be subjected to the same conversion factors as a weighed mixture of spinach, carrots, lettuce etc.

The recording of food intakes by servings is much simpler than by weight, and cooperation in obtaining records which can be considered reliable is more readily obtained on this basis. They are subject to more error, however, as a basis for calculating nutrient intakes. Anyone who has worked with data on food intakes recorded by the subject knows that large errors must have been made by some individuals in keeping the records, although the mean values may be representative of the group as a whole. The low individual records are of most concern, but one does not know to what extent they represent merely omissions from the record rather than dietary inadequacy. Diet histories are very useful as a supplementary measure and as a means of obtaining a general picture of dietary habits, but they hardly suffice in themselves to provide a quantitative picture of food and nutrient intake. Much depends on the skill and experience of the interviewer and the time with each individual. Diet histories can be taken so rapidly and poorly as to result in generally erroneous rather than useful information. Either in taking diet histories or in

tabulating food intake records, an increasingly used method is to score the diet for adequacy in terms of specific food groups, viz two servings daily of fruits and vegetables high in C, one or more servings of fruits and vegetables high in carotene, etc. This system may involve a minimum of error for a diet following the conventional pattern on which the scoring is based. Large errors are possible under other conditions, for there are many different food combinations which can provide an adequate diet.

Much has been written about the relative usefulness of one-, 3- and 7-day records. No generalizations seem worthwhile except to say that a one-day record unaccompanied by any other measure is likely to be meaningless for the individual. Even a week's record has limitations. Seasonal differences have a large influence on the current picture obtained, particularly in rural areas in certain parts of the country. Nutritional status is also a reflection of the previous as well as the current diet. As pointed out by Smith (6) in discussing his survey of maternal nutrition in Holland, "nutrition, good or bad, is the sum of highly variable quantitative and chronological factors. The results of any form of dietary inadequacy must vary in accordance with its extent in time."

The previous discussion is not intended to imply that the shorter methods of collecting and evaluating food data are not useful for various purposes. There are several published reports which show their value (7-9). The studies by Burke and associates showing the influence of nutrition during pregnancy on the condition of the infant at birth are an excellent example. The reports of the survey made under the direction of Harvard workers in New York State following the war, to determine general nutritional status and dietary trends, illustrate how various methods may be used to this end and critically discuss the limitations of each (10, 11). Irrespective of time and cost considerations, the use of more detailed methods has disadvantages in that it may limit the cooperating subjects to a non-representative sample and also result in poor cooperation in the actual recording of data. While contacts by the nutritionists with the subjects both before and during the study, in which its importance is explained, helps greatly in obtaining the desired cooperation, they also may stimulate the subjects to consume a better than usual diet.

Whatever method of arriving at food intakes is

employed, the use of appropriate sampling methods to obtain a representative picture of the population under study is basic. So also is the use of appropriate statistical methods in assessing the significance of the results. These requirements have frequently not been met.

In this talk the limitations of various dietary survey methods have been stressed because it seems important that they be borne in mind in interpreting the conclusions from published results. The discussion should indicate that there is a large background of knowledge and experience of which advantage can and should be taken in making future surveys more reliable and useful. Otherwise, a waste of effort and funds will result and erroneous conclusions will be added to the literature. The effective investigator will be the one who fully understands the advantages and limitations of the various procedures, chooses the one best suited to his specific objective and interprets his results accordingly.

There should be periodic group conferences of workers in the field, such as the U S D A "Conference on Study of Food Intake," held in October 1948, and the one held at the Children's Fund of Michigan last November dealing with the evaluation of methods of studying the relation of nutrition to maternal and infant health. Such conferences provide the opportunity for the critical comparison of procedures and data. They can also serve to promote joint activities to test the correspondence of the data obtained with a given procedure in the hands of different workers.

The results of their deliberations and joint activities should be published to enable others to take advantage of them. By making use of past experience and by critical group examination of current activities, dietary surveys can continue to contribute largely to the advancement of nutrition. Certainly, the methods have limitations but what other method concerned with nutritional status does not?

With respect to the general topic of this symposium, it is clear that dietary records should accompany the appraisal of nutritional status by more direct methods, where possible. Dietary data should help interpret the nutritional significance of the physical signs of deficiency, which are mostly non-specific. They should also aid in clarifying the meaning of various nutrient blood levels since some of them are directly related to current dietary intakes.

In the present situation there is a need for further coordinated studies in which the 3 measures are used with the same subjects, in the interest of evaluating the significance and usefulness of the data of each. Such studies should serve to establish more reliable criteria for evaluating nutritional status by each method. Such continuing research would promote what Dr. King, in a symposium on nutrition surveys in the Federation program 5 years ago, referred to as the most seriously needed study, namely, "to find, through long periods of time, the degree of correlation that exists between the health records of individuals and their dietary habits."

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STANDARDIZATION OF DEFINITIONS AND SYMBOLS IN RESPIRATORY PHYSIOLOGY

ON APRIL 19, 1950, a group of physiologists met in Atlantic City to discuss the possibility of establishing a systematic set of symbols for use in teaching and research publications relating to respiratory physiology. The need for such a set of symbols is very great. During the last few years, a number of mathematical generalizations have been developed to describe respiratory processes and these generalizations now form the framework for research and instruction in respiratory physiology in medical schools, clinical laboratories and military establishments throughout the country. While there is general agreement

literature without sacrificing flexibility desired for development of new ideas and new equations. The following definitions and symbols represent the fruit of these discussions and they are presented here with the hope that authors and editors will use them as a reference source to achieve uniformity and clarity in terminology in the field of respiratory physiology.

SUBDIVISIONS OF THE LUNG VOLUME

The proposed definitions of lung volume are shown diagrammatically in figure 1. The primary subdivisions of the lung volume shown at right apply to all levels of respiratory effort and con-

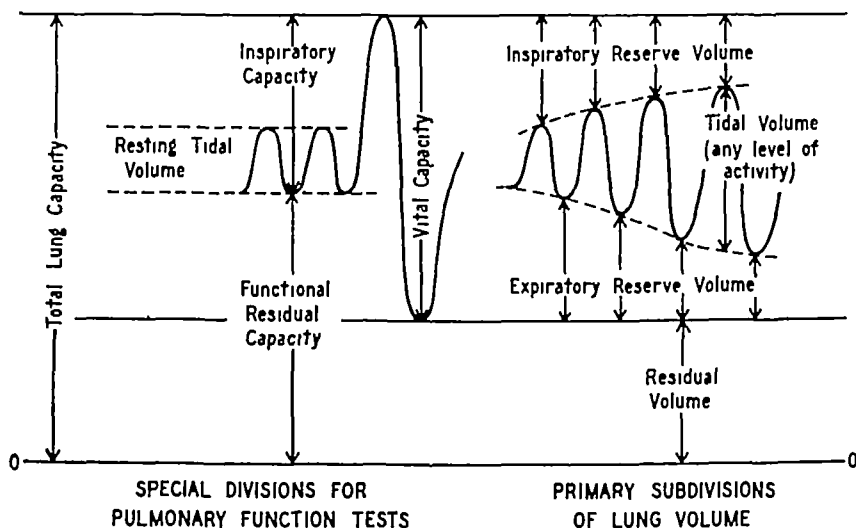


FIG 1 SUBDIVISIONS OF THE LUNG VOLUME

All gas volumes corrected to body temperature, pressure and saturated with water vapor BTPS. Dimensions to be specified in each publication (e.g., liters, ml, cu in., etc.) See table 1 for comparison with the various terminologies now in the literature.

among physiologists concerning the important variables and equations, there has been no effort to reach agreement on definitions and symbols for textbooks or for research publications. Students trained with one set of symbols are bewildered by the multitude of different terminologies in the current literature. Even experts in the field find difficulty in deciphering the equations published by their colleagues from other laboratories.

The discussions at Atlantic City were concerned with two main problems: a) definition of lung volumes and b) recommendation of a set of symbols which would provide uniformity for the

tain no overlapping volumes. The 'capacity' terms at left each include two or more of the primary subdivisions and are specially convenient for clinical applications where gasometric measures are most simply made from the expiratory position of the resting patient.

Table 1 contains a comparison of the proposed definitions with various current terminologies. The references have been taken from eight widely used textbooks or handbooks and provide a sample of the diverse definitions now in the literature. It will be noted that use of the term 'air' to denote volume is avoided in the new terminology. The terms 'complemental air' and 'supplemental air'

are replaced by 'inspiratory reserve volume' and 'expiratory reserve volume' which have functional meanings and are therefore more easily

TABLE 1 SUBDIVISIONS OF THE LUNG VOLUME
COMPARISON OF PROPOSED TERMINOLOGY
WITH PREVIOUS TERMINOLOGIES

PROPOSED TERM	PREVIOUS TERMS WITH REFERENCES
I Inspiratory re-serve volume	Complemental air (3, 4, 7) Complementary air (5) Complemental air minus tidal air (1, 2) Inspiratory capacity minus tidal volume (6)
II Expiratory re-serve volume	Supplemental air (4, 7) Reserve air (1, 2, 3, 5, 7, 8)
III Tidal volume	Tidal air (All references except 6)
IV Residual volume	Residual air (1, 2, 4, 5, 7, 8) Residual capacity (6)
V Vital capacity	Vital capacity (All references)
VI Inspiratory capacity	Complemental air (1, 2) Complementary air (8)
VII Functional residual capacity	Functional residual air (1, 2, 7) Midcapacity (1, 4) Normal capacity (3)

remembered The old controversy as to whether 'complemental air' should include tidal 'air' has been avoided by introducing the term 'inspiratory capacity' to denote the sum of a resting tidal volume and the inspiratory reserve volume

A SET OF SYMBOLS FOR USE IN RESPIRATORY
PHYSIOLOGY

In designing a set of symbols for general use in respiratory physiology it was our aim to provide a systematic method requiring as few special definitions as possible The method of choice was to adopt single large capital letters to denote general respiratory variables such as pressure, volume concentration etc, and to modify each variable with one or more small type symbols denoting location, molecular species or special

TABLE 2 SYMBOLS FOR RESPIRATORY
PHYSIOLOGY

	SYMBOL	DEFINITION DIMENSIONS AND CONDITIONS MUST BE SPECIFIED IN EACH PUBLICATION
I General variables	V	Gas volume in general Pressure, temperature and percentage saturation with water vapor must be stated
	V	Gas volume per unit time
	P	Gas pressure in general
	F	Fractional concentration in dry gas phase
	Q	Volume flow of blood
	C	Concentration in blood phase
	f	Respiratory frequency—breaths per unit time
	R	Respiratory exchange ratio in general (volume CO ₂ /volume O ₂)
II Symbol for the gas phase (SMALL CAPS)	D	Diffusing capacity in general (volume per unit time per unit pressure difference)
	I	Inspired gas
	E	Expired gas
	A	Alveolar gas
	T	Tidal gas
	D	Dead space gas
	B	Barometric
III Symbol for the blood phase	b	Blood in general
	a	Arterial (exact location to be specified in each publication)
	v	Venous (exact location to be specified in each publication)
	c	Capillary (exact location to be specified in each publication)
IV Special symbols and abbreviations	\bar{X}	Dash above any symbol indicates a mean value
	X	Dot above any symbol indicates a time derivative
	s	Subscript to denote the steady state
	STPD	Standard temperature, pressure, dry (0°C, 760 mm Hg)
	BTPS	Body temperature, pressure, saturated with water
	ATPD	Ambient temperature, pressure, dry
	ATPS	Ambient temperature, pressure, saturated with water

TABLE 3 RESPIRATORY EQUATIONS EXAMPLES OF USE OF NEW TERMINOLOGY

- I Calculation of oxygen consumption or carbon dioxide production from analysis of volume and composition of expired gas while breathing any mixture of oxygen and carbon dioxide in nitrogen or other neutral gas

V_{O_2}

$$= V_E \frac{[F_{I_{O_2}}(1 - F_{E_{CO_2}}) - F_{E_{O_2}}(1 - F_{I_{CO_2}})]}{(1 - F_{I_{O_2}} - F_{I_{CO_2}})} \quad (1)$$

V_{CO_2}

$$= V_E \frac{[F_{E_{CO_2}}(1 - F_{I_{O_2}}) - F_{I_{CO_2}}(1 - F_{E_{O_2}})]}{(1 - F_{I_{O_2}} - F_{I_{CO_2}})} \quad (2)$$

where all volumes are corrected to STPD
The quotient of *equations 2 and 1* yields the respiratory exchange ratio for the expired gas

$$R_E = \frac{V_{CO_2}}{V_{O_2}} = \frac{[F_{E_{CO_2}}(1 - F_{I_{O_2}}) - F_{I_{CO_2}}(1 - F_{E_{O_2}})]}{[F_{I_{O_2}}(1 - F_{E_{CO_2}}) - F_{E_{O_2}}(1 - F_{I_{CO_2}})]} \quad (3)$$

For the special case where carbon dioxide may be considered negligible in the inspired gas, these equations reduce to simpler forms
If measurements are made in the steady state then $R_E = R_S =$ metabolic respiratory quotient

- II Calculation of the pressure of oxygen in alveolar gas while breathing any mixture of oxygen with neutral gas at any barometric pressure

$$P_{A_{O_2}} = F_{I_{O_2}}(P_B - P_{A_{H_2O}}) - P_{A_{CO_2}} \left[F_{I_{O_2}} + \frac{(1 - F_{I_{O_2}})}{R_A} \right]$$

This equation is of special importance in determining equivalent altitudes and oxygen specifications for aircraft. It is frequently called the 'alveolar' equation although it applies equally to any part of expired gas which has undergone respiratory exchange

TABLE 3—Continued

- III The relations between alveolar ventilation and metabolism

$$V_A = \frac{V_{CO_2}}{F_{A_{CO_2}}} = \frac{R_A}{F_{A_{CO_2}}} V_{O_2} = (P_B - P_{A_{H_2O}}) \frac{R_A}{P_{A_{CO_2}}} \dot{V}_{O_2}$$

where

$$V_A = (V_T - V_D)f$$

- IV The Bohr Equation for the respiratory dead space to any gas x

$$V_{D_x} = \frac{(F_{E_x} - F_{A_x})}{(F_{I_x} - F_{A_x})} V_T$$

where all volumes are corrected to BTPS

- V Fick's Principle becomes

$$V_{O_2} = Qb(Ca_{O_2} - C\bar{v}_{O_2})$$

- VI The ratio between alveolar ventilation and blood flow through the lungs (cardiac output) in the steady state

$$\frac{V_A}{\dot{Q}_b} = R_S(P_B - P_{A_{H_2O}}) \frac{(Ca_{O_2} - C\bar{v}_{O_2})}{P_{A_{CO_2}}}$$

- VII The diffusing capacity of the lungs to oxygen as calculated by the Bohr method

$$D_{L_{O_2}} = \frac{V_{O_2}}{P_{A_{O_2}} - \bar{P}_{C_{O_2}}}$$

where the mean oxygen pressure in capillary blood ($\bar{P}_{C_{O_2}}$) is calculated from graphical integration of the oxygen dissociation curve

conditions The following conventions were adopted for symbols denoting location and molecular species

- 1 Localization in the gas phase is represented by a small capital letter immediately following the principal variable. Thus pressure in *alveolar* gas is represented by P_A
- 2 Localization in the blood phase is represented by a lower case letter immediately following the principal variable. Thus pressure in the *arterial* blood is represented by P_a
- 3 Molecular species is denoted by the full chemical symbol to be printed in small capital letters immediately following the principal variable

Thus pressure of carbon dioxide is represented by P_{CO}

- 4 When specification of *both* location and molecular species is required then the first modifying letter will be used for localization and the second for species. Thus the pressure of carbon dioxide in alveolar gas is represented by PA_{CO_2} . In this case, the chemical symbol appears as a subscript.

The full list of symbols which were discussed and approved by the group at Atlantic City is shown in table 2. Some of the commonly used respiratory equations are represented in terms of the new symbols in table 3.

NOTES ON THE USE OF THE PROPOSED SYMBOLS

It is hoped that authors employing the proposed system of symbols will provide a list of definitions similar to those of table 2 as an integral part of each manuscript. We wish to emphasize that the symbols listed in table 2 are sufficient only to describe the principal *existing* respiratory equations, samples of which are illustrated in table 3. New symbols are easily added to the list without altering the method of symbolization. For example, it may be useful to consider separately that part of the total pulmonary blood flow which is effective in the exchange of gases. Suitable subscripts for this purpose (e.g. Q_{L_e} , to de-

note effective flow through lungs) should then be included in the list.

No special difficulties are anticipated in connection with printing of the proposed symbols. However, the proposed notation is not adapted to the typewriter. This is a disadvantage but, in our opinion, should not be a deciding factor in the choice of symbols. Several sets of symbols have been devised which were 'tailor-made' for the typewriter but none of them provide a logical system which is easy to learn and adaptable to future developments in the field.

The following have agreed to follow the above terminology and system of notation wherever possible in their future teaching and research publications:

J. H. Comroe, *University of Pennsylvania*
 A. Cournand, *Columbia University*
 J. K. W. Ferguson, *University of Toronto*
 G. F. Filley, *Trudeau Foundation*
 W. S. Fowler, *University of Pennsylvania*
 J. S. Gray, *Northwestern University*
 H. F. Helmholtz, Jr., *Mayo Clinic*
 A. B. Otis, *University of Rochester*
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 H. Rahn, *University of Rochester*
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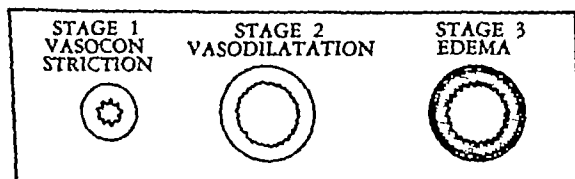
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"Although E.C. 110 (CAFERGONE) was developed primarily for the relief of the migraine attack, it is uniformly effective and has a much wider range of usefulness in the relief of headache of all other types, especially typical and atypical histaminic cephalgia" (Hansel)⁽¹⁾

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Although the cause of migraine is still unknown, the mechanism productive of head pain has been determined⁽²⁾ Today, it has been observed that the head pain in classical migraine and related disorders is produced through abnormal behavior of the cranial vascular system. The affected arteries, principally branches of the external carotids, become constricted in the early stage of the attack. Such vasoconstriction results in pre-headache warning signs such as visual and other sensory disturbances. Later in the attack, these arteries become relaxed and dilated. *At this point, agonizing headache begins.* Exaggerated pulsations and thickening of the affected arterial walls cause stretching of and



BEST RESULTS WITH TREATMENT
IN STAGE 1 OR EARLY STAGE 2

pressure upon adjacent pain-sensitive structures. Headaches of this type may last for a few minutes only or they may last for days. Seizures are usually terminated by severe vomiting.

As a result of recent research, these headaches can be aborted for the great majority of sufferers. *Attention has been centered on the development of an effective oral preparation to relieve vascular headaches.* Cafergone (100 mg caffeine and 1 mg ergotamine tartrate per tablet) is the result of this research. Ergotamine tartrate (Gynergen) has long

been known as a potent vasoconstrictor^(3, 4) Caffeine, when administered orally, also acts as a vasoconstrictor⁽⁵⁾ Simultaneous administration of ergotamine tartrate with caffeine in Cafergone tablets has the added advantage of reducing the usual dose of ergotamine necessary to abort these headaches⁽⁴⁾

These measures will abort vascular headaches for 85-90% of sufferers^(1, 4, 6, 7)

- 1 Give complete physical examination including ancillary tests to rule out other conditions mimicing migraine
- 2 Advise the patient to re-organize his activities where possible
- 3 Improve the general health of the patient.
- 4 Give 2 Cafergone tablets at first sign of impending attack and, if necessary additional 1-tablet doses (up to 6) at half-hour intervals

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Chart, 'Clinical Characteristics of Vascular Headaches'

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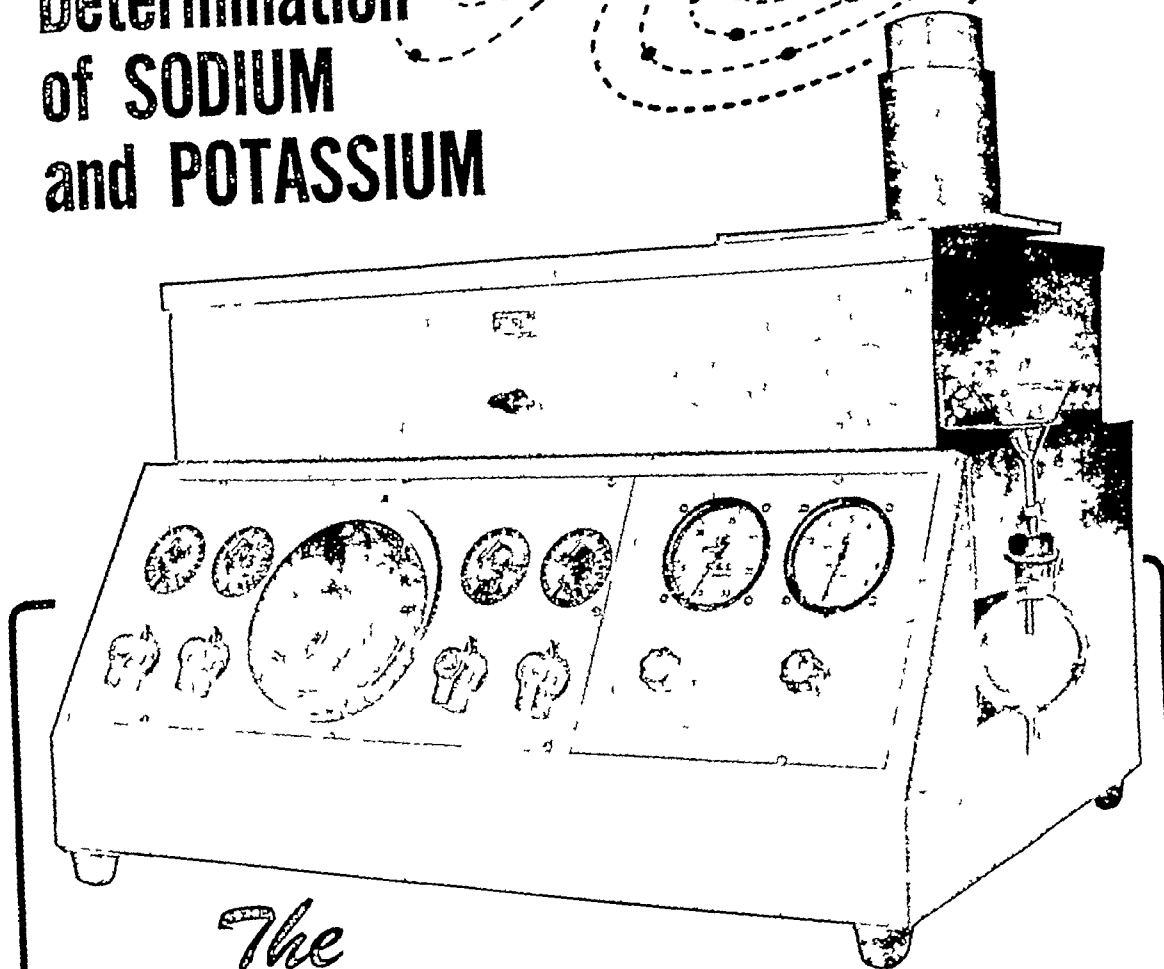
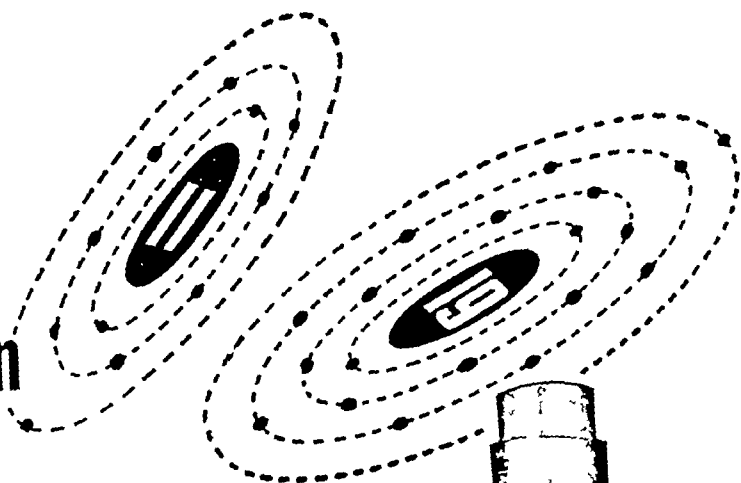
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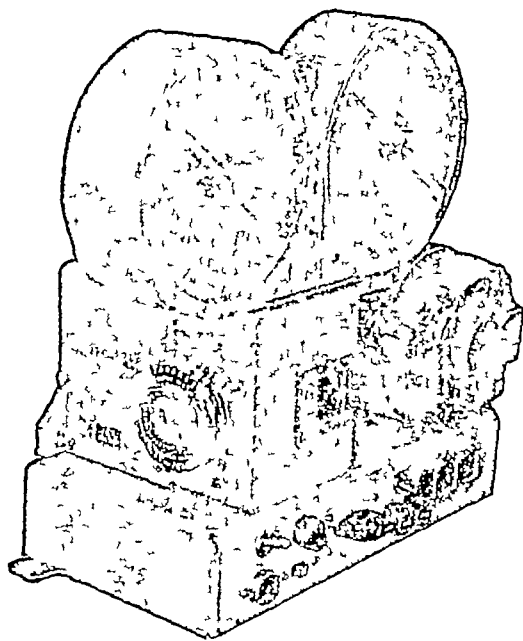
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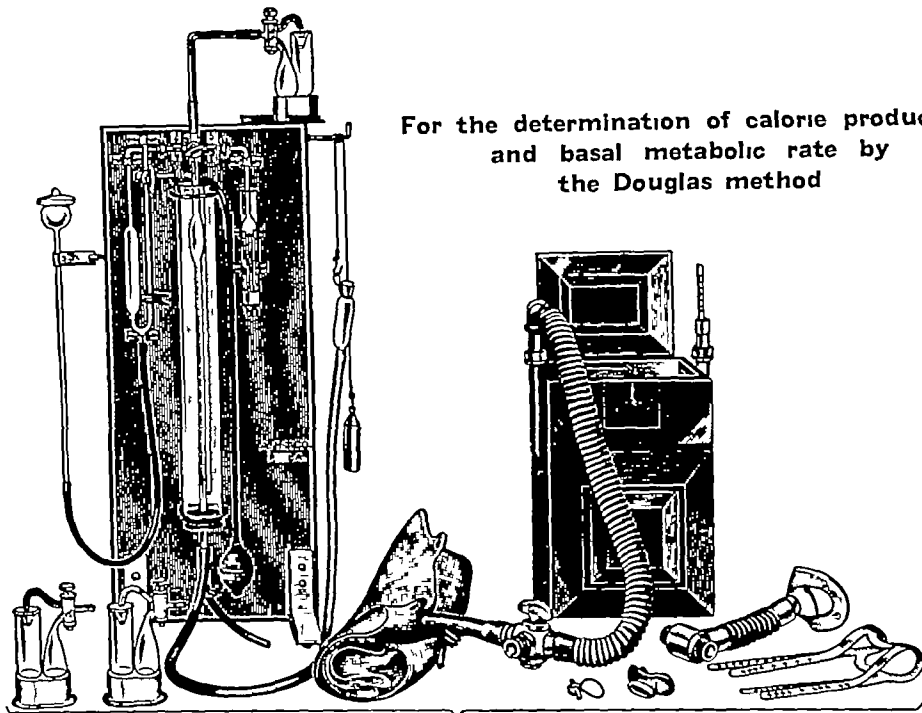
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See T. M. Carpenter, *Carnegie Institution of Washington Publication 216, 1915, p. 67*, Douglas, *Journal of Physiology, 1911, XLII*, Boothby and Sandiford, "*Laboratory Manual of Basal Metabolic Rate Determinations*", and T. M. Carpenter, *Carnegie Institution of Washington Publication No. 303-A, 1924*

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American Society of Biological Chemists
American Society for Pharmacology and Experimental Therapeutics
The American Society for Experimental Pathology
American Institute of Nutrition
The American Association of Immunologists

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2101 Constitution Ave , Washington, D C

FEDERATION PROCEEDINGS is published quarterly by the Federation of American Societies for Experimental Biology. The *March* issue consists of the Program (Part II) of the Annual Meeting of the Federation, and the Abstracts (Part I) of the papers presented at the scientific sessions. Both parts include an author index. The abstracts are arranged alphabetically according to the first author and segregated as to Societies. The *June* and *September* issues contain symposia and other special papers presented at Federation meetings as selected by the Editorial Board. The *December* issue contains the membership list and other matters pertinent to the Constituent Societies of the Federation.

Price Schedule

The subscription price is \$4.50 (\$5.25 foreign) payable in advance. Single issues may be purchased, if ordered in advance, at the following prices: No. 1 (Part I, March, Abstracts), \$3.00; No. 1 (Part II, March, Program), 75¢; Nos. 2 and 3 (June and September), \$1.50 an issue; No. 4 (December), \$2.25 an issue. Subscriptions and orders should be sent to the Federation of American Societies for Experimental Biology, 2101 Constitution Ave., Washington 25, D. C.

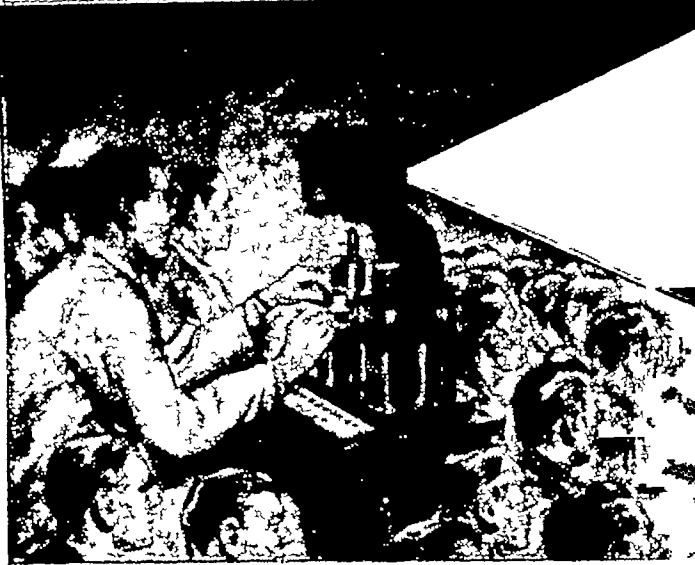
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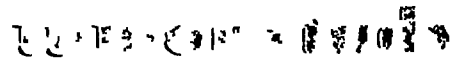
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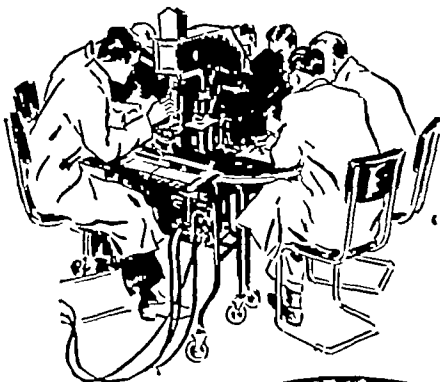
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To facilitate the publication of an accurate and up-to-date Membership List in the December issue of *FEDERATION PROCEEDINGS*, it is requested that *any member whose entry in the December 1950 issue is incorrect* furnish the Secretary of his society with the following data, in duplicate, on 3" x 5" cards: Name, Degree (highest), Mailing Address (street address, city, zone, state), Present Position (title and department or branch), Society membership and year of election to membership in that society. If you are retired, give only degree(s), home address, and society membership(s) with year(s). If you are officially carried on the rolls of your society as a retired or honorary member, please so indicate.

Names and addresses of the Society Secretaries are as follows:

The American Physiological Society—Dr. M. O. Lee, *Executive Secretary*, 2101 Constitution Ave., Washington, D. C.

American Society of Biological Chemists—Dr. E. H. Stotz, University of Rochester School of Medicine and Dentistry, Rochester 7, N. Y.

American Society for Pharmacology and Experimental Therapeutics—Dr. Harvey B. Haag, Medical College of Virginia, Dept. of Pharmacology, Richmond 19, Va.

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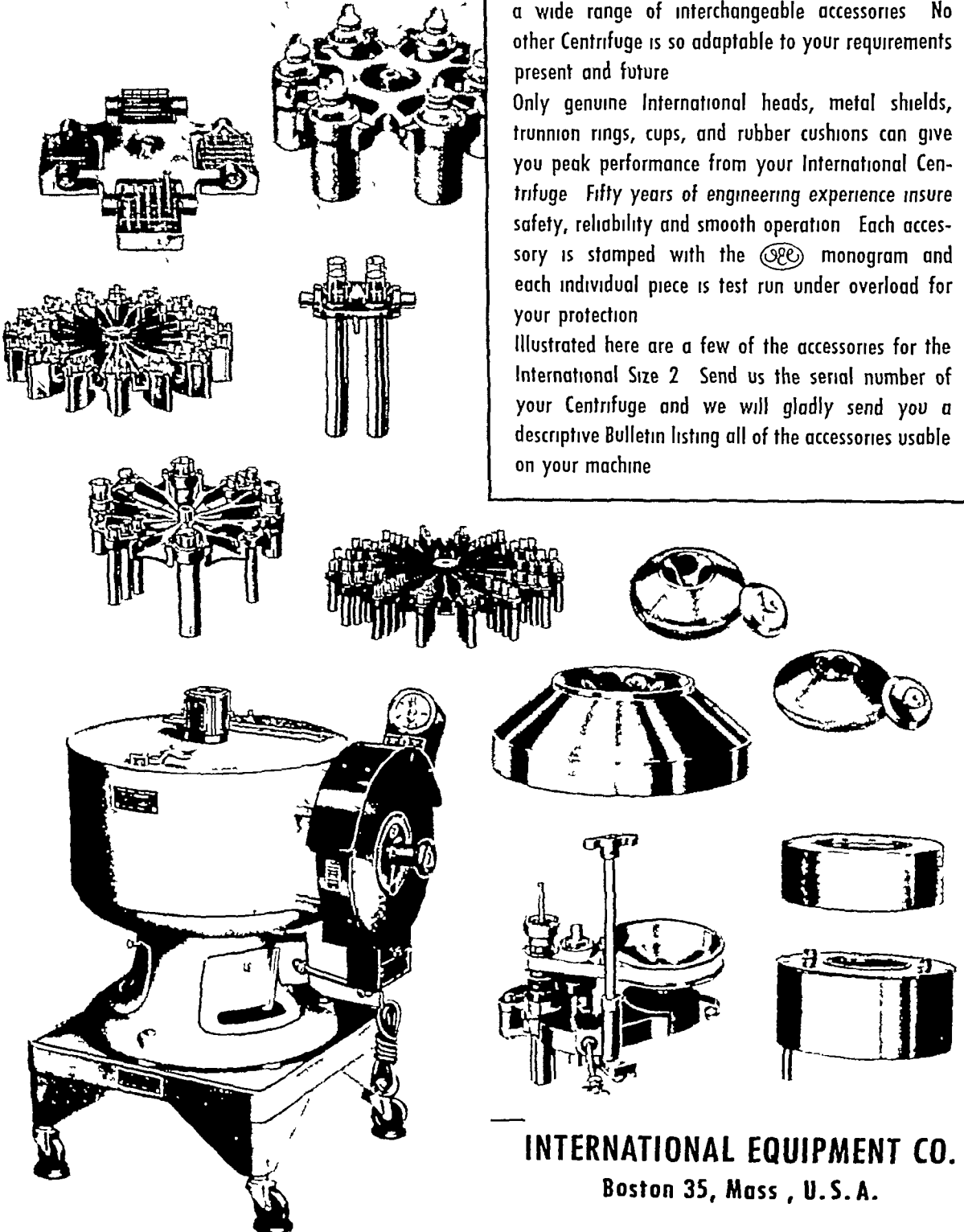
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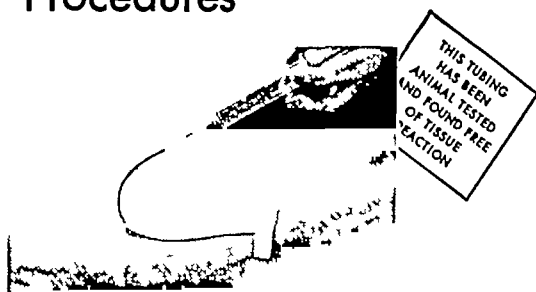
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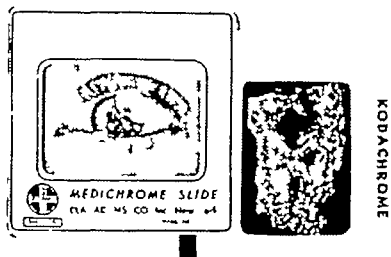


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SPECIAL LITERATURE AVAILABLE

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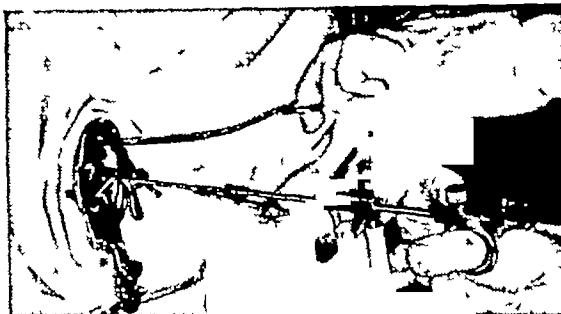
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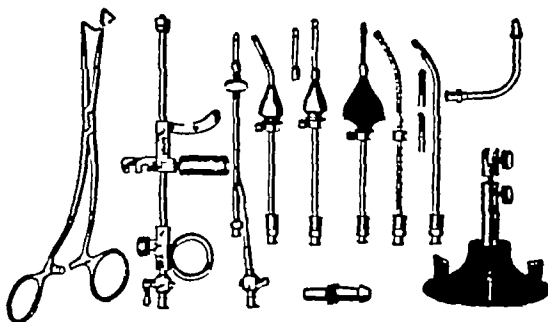


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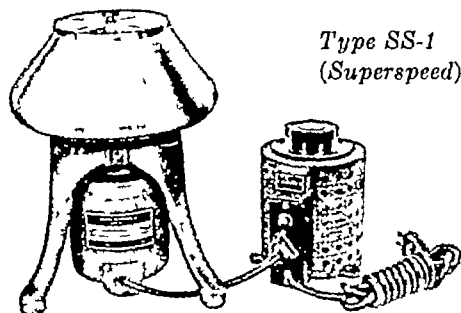
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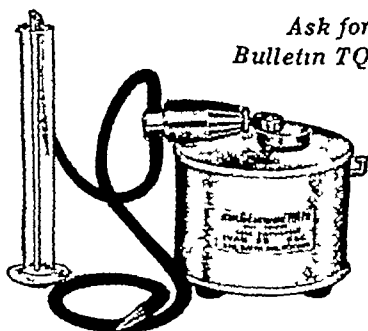
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- 1 Quarterly lists of available personnel
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- 3 Annual lists of available fellowships and exchange positions

This will supplant the previous monthly circulation of applicants alone

The Placement Service makes no recommendations as to either positions or candidates, serving only as an information agency. At the annual Spring Meeting of the Federation interviews between employers and candidates are arranged.

Forms for the registration of applicants, positions, fellowships and exchanges are available upon request from

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GOVERNMENT REGISTRATION OF SCIENTIFIC PERSONNEL

Within the next few months, approximately 150,000 American scientists will be asked to participate in one of the most comprehensive registrations of scientific personnel yet attempted by the Government. Final plans have been completed for this operation by the National Scientific Register, sponsored by the National Security Resources Board. The registration will be accomplished by means of questionnaires with the direct assistance of the principal professional and scientific societies, and will include qualified scientists in the fields of the biological sciences. The National Research Council has assumed the responsibility of conducting the registration of biological scientists, among others.

Provision has been made for such a register in legislation creating the National Science Foundation. However, because of the international situation early in 1950, the NSRB decided that this important function could not be delayed longer, and that some interim action should be taken pending the creating of the NSF.

The project is a voluntary one with the scientists themselves cooperating with the Government. The National Scientific Register at present does not envision a large-scale placement function. Its purpose is to provide urgently needed information on the country's supply of technological personnel, recorded in such a manner that the system will lend itself to whatever type of placement or distribution program might be required in the future. The National Scientific Register project has been given a high priority by the NSRB in view of the critical role played by American science in the last war, and in recognition of the fact that American security and supremacy in a future war will depend to an important degree on the total utilization of scientific and technological skills. *For this reason, it is considered important that every scientist who is circularized in the current registration return his questionnaire as quickly as possible.*

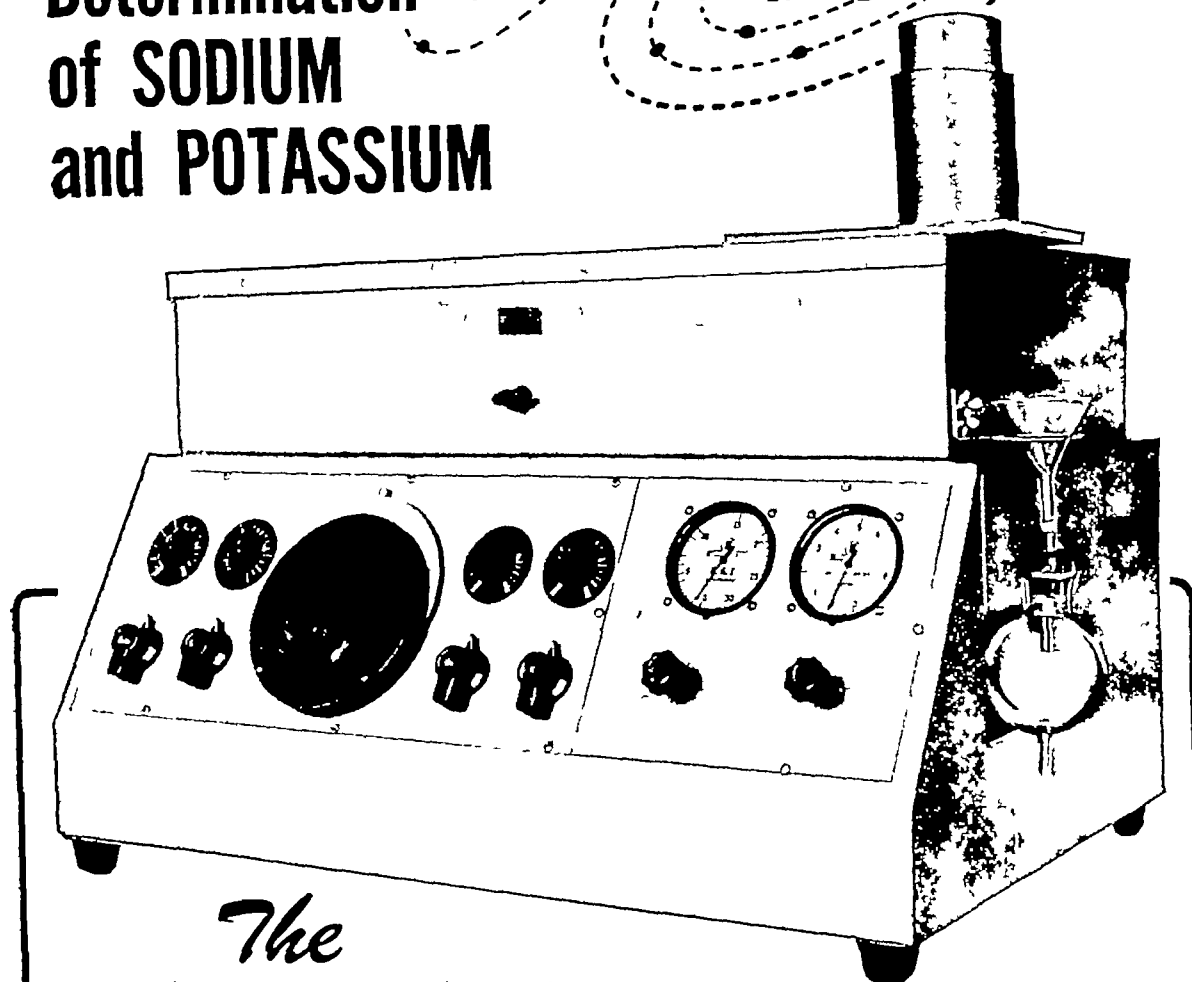
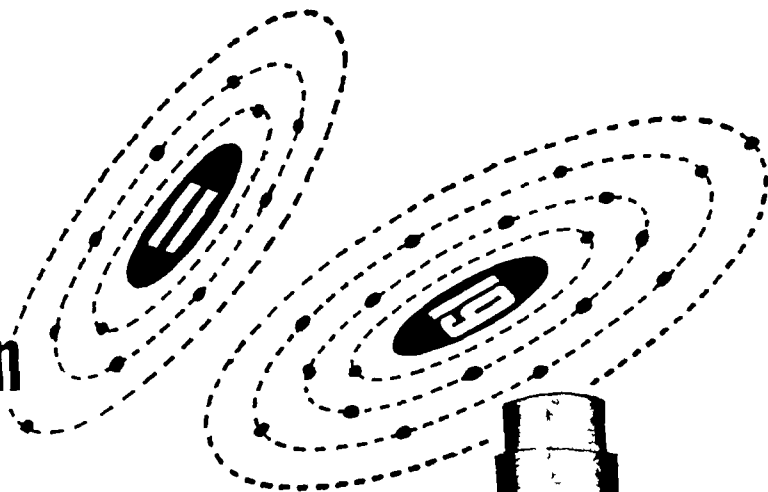
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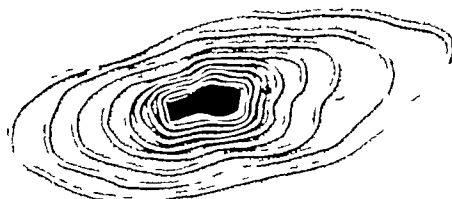
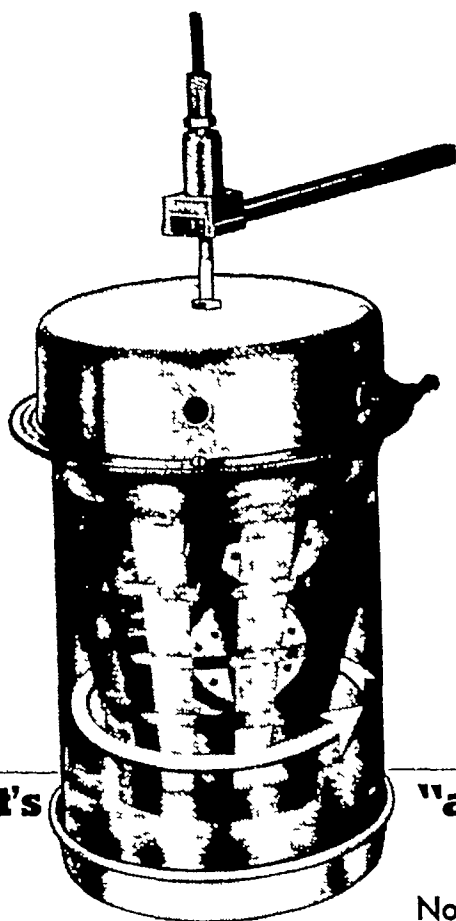
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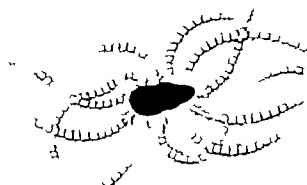
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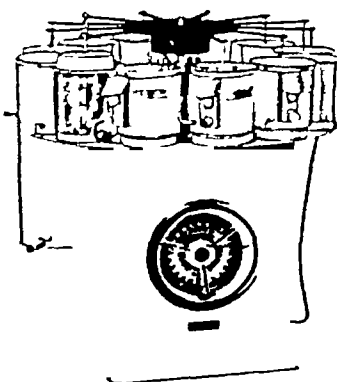
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Volume 9, 1950

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Federation Proceedings

VOLUME 9

December 1950

NUMBER 4

THIRTY-FIFTH ANNUAL MEETING

CLEVELAND, OHIO

April 29-May 3, 1951

THE 1951 Convention of the Federated Societies will be held in Cleveland, Ohio, April 29 to May 3. The scientific sessions of the six constituent Societies will begin at 9 00 A M, Monday, April 30, in the Public Auditorium and will continue through Thursday afternoon, May 3.

Saturday and Sunday, April 28 and 29, will be devoted to meetings of the Society Councils, the Federation Executive Committee and other committees. The Federation Joint Session will be Monday evening, April 30, in the Music Hall of the Auditorium.

Please note that the Federation scientific sessions have been advanced by one day, beginning on Monday rather than Tuesday as in previous years.

REGISTRATION INFORMATION

Registration will open at 9 00 A M, Sunday, April 29, in the Main Lobby of the Public Auditorium and will remain open until 10 00 P M. Registration desks will be open from 8 00 A M to 5 00 P M, Monday, Tuesday, Wednesday, and Thursday. Since the scientific sessions begin at 9 00 A M on Monday, members are urged to complete registration on Sunday if possible. Members of the constituent Societies, guests, and other biologists and physicians who wish to attend the meetings may register. The official badge issued at the registration desk must be worn to secure admission to the scientific sessions and other activities of the Convention. There will be a separate registration desk for ladies and non-professional guests. Programs, abstracts and tickets for various special functions will be on sale near the registration desk.

HEADQUARTERS HOTELS

Hotel Statler American Physiological Society, American Society for Pharmacology and Experimental Therapeutics, American Society for Experimental Pathology.

Hotel Cleveland American Society of Biological Chemists, American Institute of Nutrition, American Association of Immunologists.

Information on hotels and rates and the forms for making reservations will be distributed to members by the Society Secretaries.

EXHIBITS

Industrial Scientific Exhibits as well as Member and Institutional Exhibits will all be located on the Main Arena Floor of the Public Auditorium. Exhibits inspection periods will be as follows: Sunday, April 29, 2 00 P M-8 00 P M; Monday, Tuesday and Wednesday, April 30, May 1 and 2, 8 00 A M-5 00 P M; Thursday, May 3, 8 00 A M-1 00 P M. The exhibits are officially closed Thursday at 1 00 P M.

Due to the great interest which our members and guests have shown and expressed in the exhibits sections since their inauguration at the 1947 meeting, the Exhibits Committee for the 1951 meeting has given much thought to the screening and selection of exhibitors so as to provide exhibits of diversified interest for all our members and guests.

Three manufacturers of soft drinks and cigarettes will provide lounges on the Arena Floor for the convenience of all visitors and will serve complimentary refreshments.

PLACEMENT SERVICE

An office of the Placement Service of the Federation will be located in the South Hall one flight down from the Arena Floor. Applicants for positions should write to the Federation Placement Service, 2101 Constitution Avenue, Washington 25, D C, for application forms to be returned not later than April 15, 1951. Applicants who have previously filed forms with the Service are requested to notify the office and to send additional data not later than April 15, 1951. The application forms, comprising a résumé of education, training, experience, publications and type of position desired, will be on file at the Placement Service office.

Agencies seeking personnel should write to the Federation Placement Service for employers' registration cards on which to describe the positions and to record the Cleveland addresses. The cards should be returned to the Federation office not later than April 15, 1951. Employers who have previously filed cards should notify the office of their Cleveland address not later than April 15.

MOTION PICTURES

Motion pictures will be shown at one session to be scheduled in the program. The title and the

stract of the film must be submitted to the Society Secretaries. Only 16-mm safety film can be shown, equipment for sound projection will be available.

DINNERS AND LUNCHEONS

There will be ample facilities for holding group breakfasts, luncheons and dinners. It is advantageous to schedule all of these through the Feder-

ation Secretary's office. Members and groups desiring to schedule these functions should make their plans early and inform the Federation Secretary of their requirements before March 15, 1951. Specifications should include attendance expected, desirable time and alternatives, conflicts to be avoided, and screen and lantern requirements for the discussion session following the meal.

FEDERATION OF AMERICAN SOCIETIES FOR EXPERIMENTAL BIOLOGY

EXECUTIVE COMMITTEE, 1950-1951

D B DILL, R W GERARD, American Physiological Society
H B VICKERY, E H STOTZ, American Society of Biological Chemists
CARL F SCHMIDT, H B HAAG, American Society for Pharmacology and
Experimental Therapeutics
JAMES F RINEHART, SIDNEY C MADDEN, American Society for Experimental Pathology
W H GRIFFITH, J H ROE, American Institute of Nutrition
GEOFFREY EDSALL, JULES FREUND, American Association of Immunologists
GEOFFREY EDSALL, *Chairman*, Boston Univ School of Medicine, Boston, Mass
C G KING, *Ex-Chairman*
M O LEE, *Federation Secretary*, 2101 Constitution Ave, Washington, D C

STANDING COMMITTEES

Promotion of Biological Research A C IVY,
Chairman, K F MEYER, EPHRAIM SHORR
Secretaries Committee JULES FREUND, *Chairman*,
Immunology, R W GERARD, Physiology,
E H STOTZ, Biochemistry, H B HAAG,
Pharmacology, S C MADDEN, Pathology,
J H ROE, Nutrition
Federation Finance Committee C G KING, *Chair-*
man, S C MADDEN, W O FENN, GEOFFREY
EDSALL, *ex officio*

FORMER EXECUTIVE COMMITTEES

Philadelphia, Dec 28-31, 1913

S J MELTZER, *Chairman* AND A J CARLSON,
Secretary, The Physiological Society A B MACAL-
LUM AND P A SHAFFER, The Biochemical Society
T SOILMANN AND J AUER, The Pharmacological
Society

St Louis, Dec 27-30, 1914

G LUSK, *Chairman*, and P A SHAFFER, *Secre-*
tary, The Biochemical Society T SOILMANN AND
J AUER, The Pharmacological Society R M
PEARCE AND G H WHIPPLE, The Pathological
Society W B CANNON AND A J CARLSON, The
Physiological Society

Boston, Dec 26-29, 1915

TORALD SOILMANN, *Chairman*, and JOHN AUER,
Secretary, The Pharmacological Society THO-
BALD SMITH AND PEYTON ROUS, The Pathological
Society W B CANNON AND C W GREENE, The
Physiological Society WALTER JONES AND P A
SHAFFER, The Biochemical Society

New York, Dec 27-30, 1916

SIMON FLEXNER, *Chairman*, and PEYTON ROUS,
Secretary, The Pathological Society W B CAN-
NON AND C W GREENE, The Physiological So-
ciety WALTER JONES AND STANLEY R BENEDICT,
The Biochemical Society REID HUNT AND J
AUER, The Pharmacological Society

Minneapolis-Rochester, Dec 27-29, 1917

FREDERIC S LEE, *Chairman*, and CHARLES W
GREENE, *Secretary*, The Physiological Society
CARL L ALSBERG, and STANLEY R BENEDICT,
The Biochemical Society REID HUNT AND L G
ROWNTREE, The Pharmacological Society LUD-
VIG HEKTOEN AND HOWARD T KARSNER, The
Pathological Society

Baltimore, April 24-26, 1918

CARL L ALSBERG, *Chairman*, and STANLEY R
BENEDICT, *Secretary*, The Biochemical Society
REID HUNT AND E D BROWN, The Pharmacologi-
cal Society H GIDEON WELLS AND HOWARD T
KARSNER, The Pathological Society FREDERIC S
LEE AND CHARLES W GREENE, The Physiological
Society

Cincinnati, Dec 29-31, 1919

A S LOEVENHART, *Chairman*, and E D
BROWN, *Secretary*, The Pharmacological Society
W G MACCALLUM AND HOWARD T KARSNER,
The Pathological Society WARREN P LOMBARD
AND CHARLES W GREENE, The Physiological So-
ciety STANLEY R BENEDICT AND VICTOR C
MYERS, The Biochemical Society

Chicago, Dec 28-30, 1920

WILLIAM H PARK, *Chairman* and HOWARD T KARSNER, *Secretary*, The Pathological Society WARREN P LOMBARD and CHARLES W GREENE, The Physiological Society STANLEY R BENEDICT and VICTOR C MYERS, The Biochemical Society A S LOEVENHART and EDGAR D BROWN, The Pharmacological Society

New Haven, Dec 28-30, 1921

J J MACLEOD, *Chairman*, and CHARLES W GREENE, *Secretary*, The Physiological Society D D VAN SLYKE and VICTOR C MEYERS, The Biochemical Society C W EDMUNDS and EDGAR D BROWN, The Pharmacological Society F G NOVY and WADE H BROWN, The Pathological Society

Toronto, Dec 27-29, 1922

D D VAN SLYKE, *Chairman*, and VICTOR C MYERS, *Secretary*, The Biochemical Society C W EDMUNDS and EDGAR D BROWN, The Pharmacological Society HOWARD T KARSNER and WADE H BROWN, The Pathological Society J J R MACLEOD and CHARLES W GREENE, The Physiological Society

St Louis, Dec 27-29, 1923

C W EDMUNDS, *Chairman*, and EDGAR D BROWN, *Secretary*, The Pharmacological Society E L OPIE and WADE H BROWN, The Pathological Society A J CARLSON and CHARLES W GREENE, The Physiological Society PHILIP A SHAFFER and VICTOR C MYERS, The Biochemical Society

Washington, Dec 29-31, 1924

ALFRED S WARTHIN, *Chairman*, and E B KRUMBHAAR, *Secretary*, The Pathological Society A J CARLSON and WALTER J MEEK, The Physiological Society P A SHAFFER and D WRIGHT WILSON, The Biochemical Society JOHN AUER and E D BROWN, The Pharmacological Society

Cleveland, Dec 28-30, 1925

A J CARLSON, *Chairman*, and WALTER J MEEK, *Secretary*, The Physiological Society H C SHERMAN and D WRIGHT WILSON, The Biochemical Society JOHN AUER and E D BROWN, The Pharmacological Society GEORGE H WHIPPLE and E B KRUMBHAAR, The Pathological Society

Rochester, N Y, April 14-16, 1927

E C KENDALL, *Chairman*, and F C KOCH, *Secretary*, The Biochemical Society JOHN AUER and E D BROWN, The Pharmacological Society

W H BROWN and E B KRUMBHAAR, The Pathological Society J ERLANGER and W J MEEK, The Physiological Society

Ann Arbor, April 12-14, 1928

CARL VOEGTLIN, *Chairman*, and E D BROWN, *Secretary*, The Pharmacological Society DAVID MARINE and CARL V WELLER, The Pathological Society JOSEPH ERLANGER and WALTER J MEEK, The Physiological Society E V MCCOLLUM and D WRIGHT WILSON, The Biochemical Society

Boston, Aug 19-24, 1929

(The XIIIth International Physiological Congress)

EDWARD B KRUMBHAAR, *Chairman*, and CARL V WELLER, *Secretary*, The Pathological Society JOSEPH ERLANGER and WALTER J MEEK, The Physiological Society E V MCCOLLUM and D WRIGHT WILSON, The Biochemical Society CARL VOEGTLIN and E D BROWN, The Pharmacological Society

Chicago, March 26-29, 1930

WALTER J MEEK, *Chairman*, and ALFRED C REDFIELD, *Secretary*, The Physiological Society W R BLOOR and HOWARD B LEWIS, The Biochemical Society CARL VOEGTLIN and E D BROWN, The Pharmacological Society WILLIAM F PETERSEN and CARL V WELLER, The Pathological Society

Montreal, April 8-11, 1931

W R BLOOR, *Chairman*, and H B LEWIS, *Secretary*, The Biochemical Society GEORGE B WALLACE and E D BROWN, The Pharmacological Society FREDERICK L GATES and C PHILLIP MILLER, The Pathological Society WALTER J MEEK and ARNO S LUCKHARDT, The Physiological Society

Philadelphia, April 27-30, 1932

GEORGE B WALLACE, *Chairman*, and V E HENDERSON, *Secretary*, The Pharmacological Society SAMUEL R HAYTHORN and C PHILLIP MILLER, The Pathological Society WALTER J MEEK and ARNO B LUCKHARDT, The Physiological Society H C BRADLEY and HOWARD B LEWIS, The Biochemical Society

Cincinnati, April 10-12, 1933

PEYTON ROUS, *Chairman*, and C PHILLIP MILLER, *Secretary*, The Pathological Society ARNO B LUCKHARDT and FRANK C MANN, The Physiological Society H C BRADLEY and

HOWARD B LEWIS, The Biochemical Society
WM DEB MACNIDER and V E HENDERSON, The
Pharmacological Society

New York, March 28-31, 1934

ARNO B LUCKHARDT, *Chairman*, FRANK C
MANN, *Secretary*, and ALEXANDER FORBES, *Treas-
urer*, The Physiological Society W M CLARK
and H A MATTILL, The Biochemical Society
W DEB MACNIDER and V E HENDERSON, The
Pharmacological Society CARL V WELLER and
C PHILLIP MILLER, The Pathological Society

Detroit, April 10-13, 1935

W M CLARK, *Chairman*, H A MATTILL, *Secre-
tary*, and C H FISKE, *Treasurer*, The Biochemical
Society CHARLES W GREENE and FRANK C
MANN, The Physiological Society R A HATCHER
and E M K GEILING, The Pharmacological So-
ciety S BURT WOLBACH and SHIELDS WARREN,
The Pathological Society

Washington, March 25-28, 1936

V E HENDERSON, *Chairman*, E M K GEILING,
Secretary, and C M GRUBER, *Treasurer*, The
Pharmacological Society FRANK C MANN and
ANDREW C IVY, The Physiological Society H
B LEWIS and H A MATTILL, The Biochemical
Society OSKAR KLOTZ and SHIELDS WARREN,
The Pathological Society

Memphis, April 21-24, 1937

ALPHONSE R DOCHEZ, *Chairman*, and SHIELDS
WARREN, The Pathological Society FRANK C
MANN and ANDREW C IVY, The Physiological
Society HOWARD B LEWIS and H A MATTILL,
The Biochemical Society V E HENDERSON and
E M K GEILING, The Pharmacological Society
D R HOOKER, *Secretary*

Baltimore, March 30-April 2, 1938

WILLIAM T PORTER, *Honorary President*, WAL-
TER E GARREY, *Chairman*, and ANDREW C IVY,
The Physiological Society GLENN E CULLEN and
H A MATTILL, The Biochemical Society ARTHUR
L TATUM and G PHILIP GRABFIELD, The Pharma-
cological Society C PHILLIP MILLER and PAUL
R CANNON, The Pathological Society D R
HOOKER, *Secretary*

Toronto, April 26-29, 1939

GLENN E CULLEN, *Chairman*, and CHARLES G
KING, The Biochemical Society ARTHUR L TATUM
and G PHILIP GRABFIELD, The Pharmacological
Society C PHILLIP MILLER and PAUL R CANNON,

The Pathological Society WALTER E GARREY
and ANDREW C IVY, The Physiological Society
D R HOOKER, *Secretary*

New Orleans, March 13-16, 1940

E M K GEILING, *Chairman*, and G PHILIP
GRABFIELD, The Pharmacological Society ERN-
EST W GOODPASTURE and PAUL R CANNON, The
Pathological Society ANDREW C IVY and PHILIP
BARD, The Physiological Society WILLIAM C
ROSE and CHARLES G KING, The Biochemical
Society D R HOOKER, *Secretary*

Chicago, April 15-19, 1941

SHIELDS WARREN, *Chairman*, and H P SMITH,
The Pathological Society THORNE M CARPENTER
and L A MAYNARD, The Institute of Nutrition
ANDREW C IVY and PHILIP BARD, The Physio-
logical Society WILLIAM C ROSE and CHARLES G
KING, The Biochemical Society E M K GEIL-
ING and G PHILIP GRABFIELD, The Pharmacologi-
cal Society D R HOOKER, *Secretary*

Boston, March 31-April 4, 1942

ALBERT G HOGAN, *Chairman*, and ARTHUR H
SMITH, The Institute of Nutrition PHILIP BARD
and CARL J WIGGERS, The Physiological Society
RUDOLPH J ANDERSON and ARNOLD K BALLS,
The Biochemical Society E M K GEILING and
R N BIETER, The Pharmacological Society
JESSE L BOLLMAN and H P SMITH, The Patho-
logical Society SHIELDS WARREN, *Ex-Chairman*
D R HOOKER, *Secretary*

1943, 1944, 1945 The meetings scheduled for
Cleveland were cancelled because of war
conditions

PHILIP BARD, *Chairman*, and WALLACE O FENN,
The Physiological Society E A DOISI and
ARNOLD K BALLS, The Biochemical Society E
K MARSHALL, JR and RAYMOND N BIETER, The
Pharmacological Society BALDWIN LUCKÉ and
H P SMITH, The Pathological Society LEONARD
A MAYNARD and ARTHUR H SMITH, The Institute
of Nutrition JACQUES J BRONFENBRENNER and
ARTHUR F COCA, The Association of Immunolo-
gists D R HOOKER, *Secretary*

Atlantic City, March 11-15, 1946

PHILIP BARD, *Chairman*, WALLACE O FENN,
The Physiological Society A BAIRD HASTINGS
and ARNOLD K BALLS, The Biochemical Society
ERWIN E NELSON and RAYMOND N BIETER, The
Pharmacological Society BALDWIN LUCKÉ and H
P SMITH, The Pathological Society WILLIAM C
ROSE and H E CARTER, The Institute of Nutri-

tion JACQUES J BRONFENBRENNER and ARTHUR F COCA, The Association of Immunologists D R HOOKER, *Secretary*

Chicago, May 18-22, 1947

A BAIRD HASTINGS, *Chairman*, and OTTO A BESSEY, The Biochemical Society MAURICE H SEEVERS and HARVEY B HAAG, The Pharmacological Society PAUL R CANNON and FRIEDA S ROBSCHUIT-ROBBINS, The Pathological Society ARTHUR H SMITH and H E CARTER, The Institute of Nutrition MICHAEL HEIDELBERGER and ARTHUR F COCA, The Association of Immunologists WALLACE O FENN and MAURICE B VISSCHER, The Physiological Society WILLIAM H CHAMBERS, *Secretary*

Atlantic City, March 15-19, 1948

MAURICE H SEEVERS, *Chairman*, and HARVEY B HAAG, The Pharmacological Society DOUGLAS H SPRUNT and FRIEDA S ROBSCHUIT-ROBBINS, The Pathological Society R M BETHKE and H E CARTER, The Institute of Nutrition LLOYD D FELTON and ARTHUR F COCA, The Association of Immunologists WALLACE O FENN and MAURICE B VISSCHER, The Physiological Society HANS T

CLARKE and OTTO A BESSEY, The Biochemical Society WILLIAM H CHAMBERS, *Secretary*

Detroit, April 18-22, 1949

H P SMITH, *Chairman*, and FRIEDA S ROBSCHUIT-ROBBINS, The Pathological Society E M NELSON and J H ROE, The Institute of Nutrition MICHAEL HEIDELBERGER and JULES FREUND, The Association of Immunologists MAURICE B VISSCHER and D B DILL, The Physiological Society HANS T CLARKE and OTTO A BESSEY, The Biochemical Society CARL A DRAGSTEDT and HARVEY B HAAG, The Pharmacological Society M O LEE, *Federation Secretary*

Atlantic City, April 17-21, 1950

C G KING, *Chairman*, and J H ROE, The Institute of Nutrition THOMAS FRANCIS, JR, and JULES FREUND, The Association of Immunologists CARL J WIGGERS and D B DILL, The Physiology Society CARL F CORI and RICHARD W JACKSON, The Biochemistry Society CARL F SCHMIDT and H B HAAG, The Pharmacology Society JOHN G KIDD and SIDNEY C MADDEN, The Pathology Society M O LEE, *Federation Secretary*

FEDERATION BY-LAWS

BY-LAWS

Adopted at the Washington Meeting, 1936, and amended at the Boston Meeting, 1942

1 The Presidents and Secretaries of the Constituent Societies, the Chairman of the Executive Committee of the preceding year and the Federation Secretary shall form the Executive Committee of the Federation

2 The Chairmanship of the Executive Committee shall be held in turn by the Presidents of the Constituent Societies, who shall succeed one another annually in the order of seniority of the Societies

3 The Executive Committee shall appoint annually from the membership of the Federation a Secretary-Treasurer, to be known as the Federation Secretary

4 The Federation Secretary shall (a) Keep the minutes of the Executive Committee and distribute copies to the Secretaries of the Constituent Societies (b) Make arrangements for the Annual Meeting with the Local Committee, with the approval of the Executive Committee (c) Print in convenient combined form and distribute to the membership of the Federation the programs of the Constituent Societies as received from their respective Secretaries (d) Undertake such other duties to be decided upon from time to time by the

Executive Committee, as do not conflict with the complete autonomy of the Constituent Societies

5 The Executive Committee shall control all monies in the hands of the Federation Secretary, who shall make an annual report to the Executive Committee for audit and approval The expenses of the Federation Secretary, as authorized by the Executive Committee, shall be the first charge on such monies and if insufficient for the purpose the Executive Committee shall prorate such expenses to the Constituent Societies of the Federation in proportion to their respective memberships

The Executive Committee may appropriate Federation monies annually for the uses of Local Committees and for the uses of other authorized Committees but in the latter cases an audit of expenditures shall be made and approved before such committees are discharged

6 The Executive Committee shall determine the place of the Annual Meeting, and the time shall be determined by the Local Committee, preferably within the period of March fifteenth to May first

7 The Local Committee at the place of meeting of the Federation shall charge such fee for registration as may be approved by the Executive Committee The monies thus collected shall be used to defray the expenses of the Local Com-

mittee and the remainder, after such expenses have been met, shall be turned over to the Federation Secretary

8 The Executive Committee shall consider measures of advantage to the Federation as a whole. Any Constituent Society may refer similar measures to the Executive Committee. No action, however, shall be taken by the Executive Committee unless specifically authorized by all the Constituent Societies.

9 The Chairman of the Executive Committee may appoint committees when the purposes of such committees have been approved by all the Constituent Societies of the Federation. Such committees shall be appointed for a term of one year, but may be continued and their members reappointed. Such committees shall report in writing to the Executive Committee, which shall in turn report thereon to the Constituent Societies either for information or recommendation. The Secretaries of the Constituent Societies shall report the recommendations of their respective Societies to the Executive Committee for final action.

10 All individuals whose names appear on the program by invitation or introduction and those registering from any recognized biological laboratory may be enrolled as Associate Members of the Federation for that Annual Meeting. Such Associate Members may enjoy all the privileges of the Annual Meeting except that of voting.

11 No person may present orally more than one paper during all of the scientific sessions of the

Constituent Societies at the time of the Annual Meeting except upon invitation of the Executive Committee or a Council. Papers must be submitted to the Secretary of the Society of which the proposer is a member. The proposer may request transfer to another program, but this may only be done with the consent of the Secretary of the Society concerned. Any Secretary who regards any paper submitted to him as better suited to the program of another Society may arrange this transfer with the Secretary of the Society concerned, if it be possible. Such transfer shall be indicated on the program.

12 Abstracts not to exceed two hundred and fifty words in length, of papers approved for presentation at all of the scientific sessions of all the Constituent Societies at the Annual Meeting, shall receive publication in the *Federation Proceedings*.

13 A Control Committee, consisting of at least one representative of each Constituent Society as designated by the several Councils, shall have editorial control over the *Federation Proceedings* which shall be financed as required by an annual assessment of all the members of each Constituent Society.

14 The Control Committee shall have power to choose certain additional papers presented at the Annual Meetings and from other sources, including material heretofore published in the *Federation Yearbook*, for publication in the *Federation Proceedings*.

PROPOSED FEDERATION CONSTITUTION AND BY-LAWS

To the Members of the Constituent Societies

The Federation Executive Committee in 1948 appointed a committee (W. H. Chambers, M. O. Lee and the Society Secretaries) to prepare a draft of a Constitution and a revision of the By-Laws of the Federation. The draft was submitted previous to the 1950 Spring meeting to the Executive Committee, which made various changes and submitted it as a proposed Constitution and By-Laws to the constituent Societies for their consideration. The Executive Committee then voted to publish the proposed Constitution and By-Laws, with further changes suggested by the Societies, in *Federation Proceedings*, so that the mem-

bers of each Society could examine it before their Society takes action.

In the draft below, certain provisions are given in an alternative form as the result of suggestions that have not yet had consideration by the whole Executive Committee. At the coming 1951 Federation meeting these and other suggestions will be considered by the Federation Executive Committee and a final form of the proposed Constitution and By-Laws may be referred to the Societies for their action as to adoption.

Suggestions for changes or additions are solicited and may be sent to the Secretary of your Society or to the Federation Secretary.

CONSTITUTION

ARTICLE I *Name*

The name of this organization is the FEDERATION OF AMERICAN SOCIETIES FOR EXPERIMENTAL BIOLOGY.

ARTICLE II *Purpose*

(Alternative 1) The purposes of the Federation are to bring together investigators in the biological and medical sciences in the fields repre-

sented by its constituent Societies and to disseminate information on the results of biological and medical research through publications and scientific meetings

(*Alternative 2*) The objects of the Federation are to bring together investigators in the biological and medical sciences in the fields represented by the member Societies, to disseminate information on the results of biological research through publications and scientific meetings, and to serve such other purposes as may be approved by the member Societies

[*Alternative 2* is proposed in order to permit the continuation of such Federation activities as the Placement Service]

ARTICLE III *Membership*

SECTION 1 The constituent members of the Federation are The American Physiological Society, Inc, American Society of Biological Chemists, Inc, American Society for Pharmacology and Experimental Therapeutics, Inc, The American Society for Experimental Pathology, Inc, American Institute of Nutrition, Inc, The American Association of Immunologists

SECTION 2 Other Societies in the field of experimental biological science, whose purposes are consonant with those of the Federation, may upon application for membership be admitted, but only by the unanimous consent of the Societies that are members of the Federation at the time of such application

Any constituent Society may withdraw from membership in the Federation one year following written notification to each of the other constituent Societies of its intention to discontinue membership

ARTICLE IV *Management*

(*Alternative 1*) The general management of the Federation shall be vested in a Federation Council consisting of the President and Secretary of each constituent Society and the Chairman of the Federation Council for the preceding year

The Chairmanship of the Federation Council shall be held in turn by the Presidents of the constituent Societies, who shall succeed one another annually in the order of seniority of the Societies in the Federation

(*Alternative 2*) The general management of the Federation shall be vested in a Federation Council consisting of a Chairman and the President and Secretary of each member Society

Each Society, in its turn as determined by seniority in the Federation shall furnish for a one-year term the Federation Council Chairman, who shall have been the President of that Society during the preceding year

[The intent of *Alternative 2* is to insure that the Chairman of the Federation Council shall have served at least one year as a member of the Council previous to becoming its Chairman]

ARTICLE V *Amendments*

Amendments to the Constitution may be entertained only when presented by a constituent Society Any constituent Society may propose amendments to the Constitution to the Federation Council at any annual meeting If the proposed amendment is approved by a majority of the members of the Federation Council, it shall be published in the *Federation Proceedings* and referred to the constituent Societies for ratification at the next annual meeting The proposed amendment shall be adopted if it is ratified by all of the constituent Societies within a period of two years after its publication

(*Alternative 1*) By-laws may be adopted, amended or repealed at any annual meeting by the approval of all of the constituent Societies

(*Alternative 2*) By-laws may be adopted, amended or repealed at any annual meeting by the approval of a majority of the constituent Societies

BY-LAWS

ARTICLE I *The Federation Council*

SECTION 1 The Federation Council shall consider matters affecting the Federation as a whole and matters referred to it by any constituent Society

SECTION 2 The Federation Council shall appoint annually from the membership of the constituent Societies a Secretary-Treasurer, to be known as the Federation Secretary, who may present matters to the Federation Council for decision at its executive sessions or by mail, but who shall not be entitled to vote The Federation Secretary shall be compensated as an employee of the Federation

SECTION 3 The Federation Council shall control all monies of the Federation It shall determine the annual Federation assessment per member of the constituent Societies The assessment shall be applied to the total membership of each Society as of July 1 of each year and shall be due the Federation on that date The Federation Council shall set the subscription rates of Federation publications after considering the recommendations of the Managing Editor, and shall set the fees of the Placement Service The Federation Council may appropriate Federation monies for the use of its authorized committees and for other purposes of the Federation

SECTION 4 The Federation Council shall determine the time and place of the Annual Meeting

upon recommendation of the Federation Secretary. The suggested time shall be during the second, third or fourth week of April.

SECTION 5 *Method of Voting Questions and motions, except those pertaining to Article III, and Article V of the Constitution, put to the Federation Council shall be decided by one of the following three methods*

(a) By majority vote of the members of the Federation Council present. The presence of nine members representing four or more Societies shall constitute a quorum.

(b) By vote by Societies, whereby the two representatives of each Society shall cast a single ballot on behalf of their Society, and a unanimous vote shall be necessary for approval.

(c) By vote of Societies, whereby the matter shall be referred to the Societies for decision. In such case the vote of each Society shall be reported to the Federation Council and recorded. Approval by all of the member Societies shall be necessary for action by the Federation Council.

The method prescribed under (a) of this section shall be followed on all questions in executive session or by mail ballot, unless the two representatives of any Society jointly request that method (b) or (c) of this section be followed. If requests by the representatives of two or more Societies differ as to whether method (b) or (c) shall be used, the method prescribed in (c) shall be followed.

(*Alternative* Omit the words "or by mail ballot" in the last paragraph of Section 5 and add the following as a new paragraph at the end of Section 5.)

Matters referred to the Federation Council for vote by mail ballot shall require unanimous approval if there has been no opportunity for discussion, if such opportunity has been provided either in a Council session or by mail, a majority vote shall prevail unless alternate method (b) or (c) of voting is requested. In either case a mail vote shall require at least nine replies.

SECTION 6 In the absence of the Chairman of the Federation Council at any meeting, the Past-Chairman shall preside. In the absence of the President and/or Secretary of a Society, that Society may authorize a designated alternate to represent it. (*Alternative*) Change "Past Chairman" in Section 6 to "prospective Chairman."

ARTICLE II *Federation Secretary*

The Federation Secretary shall act as Secretary to the Federation Council and be responsible for all Federation records, he shall act as Treasurer of the Federation and shall submit to the Federation Council not later than February of each year financial statements for the previous calendar year audited by a reputable firm of

certified public accountants, he shall be responsible for making the arrangements for the Annual Meeting of the Federation, he shall act as managing editor of the publications of the Federation and be responsible for their business management and for carrying out the editorial policies, he shall supervise the Federation Placement Service and make an annual report on its activity to the Federation Council, he shall perform such other duties as may be prescribed by the Federation Council and which do not conflict with the constitution and by-laws of the constituent Societies.

ARTICLE III *Committees and Representatives*

SECTION 1 The Chairman of the Federation Council shall appoint members of Standing Committees (except the Secretaries Committee), representatives of the Federation to other organizations, and members of such other committees as may be designated by the Federation Council. Members of the committees shall be appointed for specific terms and may be reappointed. Committees shall report in writing through their Chairmen to the Federation Council, which shall in turn report thereon to the constituent Societies for their information, recommendation or action. Any committee using authorized funds of the Federation for its purposes shall have an audit of its expenditures made and approved before it is discharged.

SECTION 2 A Secretaries Committee composed of the Secretaries of the constituent Societies and the Federation Secretary shall be a standing Committee of the Federation. This committee shall meet at the call of the Federation Secretary and shall be responsible for arranging the program of the annual meeting, for setting, upon the recommendation of the Federation Secretary, the registration fees for the annual meeting, and for such other duties as the Federation Council may designate.

(*Alternative 1*) The Chairman of the Secretaries Committee shall be the Secretary of the Society whose President is the Chairman of the Federation Council.

(*Alternative 2*) The Chairman of the Secretaries Committee shall be the Secretary of the Society which furnishes the Chairman of the Federation Council.

ARTICLE IV *Meetings*

SECTION 1 The Federation shall hold an Annual Meeting for the presentation of papers at the scientific sessions of the constituent Societies and the general sessions of the Federation and for the transaction of business by the Federation Council.

SECTION 2 No person may present orally more than one paper during all of the scientific sessions.

of the constituent Societies at the Annual Meeting, except upon invitation of the Chairman of the Federation Council or the Council of a Society

The oral presentation of any paper shall be by the first-named author except by permission of the Secretary of the Society concerned (*Alternative*) Omit the preceding sentence Each Society may make such additional restrictions on its program as it desires

SECTION 3 Papers to be read at the annual meeting of the Federation must be submitted to the Secretary of a Society of which the proposer of the paper is a member A member of a constituent Society may invite non-members to appear as co-authors of a paper with him, or he may propose a paper by non-member authors whom he introduces

SECTION 4 Each Society shall have responsibility for selecting and arranging its scientific program provided the general regulations of the Federation are followed

The authors of a paper may request its transfer to the program of another Society, but this may be done only with the consent of the Secretaries of the two Societies concerned Secretaries on their own initiative may transfer papers from the program of one Society to that of another All transfers of papers shall be indicated in the program

SECTION 5 The abstracts of papers read at the scientific sessions of all of the constituent Societies at the annual meeting shall be published in *Federation Proceedings* Abstracts shall not exceed 275 words in total length, inclusive of titles, authors' names, laboratory and place of origin

ARTICLE V *Publications*

SECTION 1 The official publications of the Federation shall be the *Federation Proceedings* and such other publications as the Federation Council may authorize *Federation Proceedings* shall be owned and published quarterly by the Federation under the supervision of the managing editor and

the Secretaries Committee The March issue shall consist of Part I, containing the abstracts of papers presented or read by title at the Annual Meeting and Part II, the Program of the Annual Meeting The June and September issues shall contain the papers from the Joint Session and Symposia, and other special papers as may be selected by the Secretaries Committee The December issue shall contain the annual membership list and matters of interest to the constituent Societies

ARTICLE VI *Placement Service*

The Federation shall maintain a Placement Service, under the supervision of the Federation Secretary, the function of which shall be to assist in the placing of professional biologists in suitable positions with universities, hospitals, research foundations, industrial research and other organizations seeking such personnel

PLACEMENT SERVICE

The Federation maintains a service to act as a medium of communication between persons seeking positions for teaching or research and institutions that wish to fill vacancies in these sciences

The service does not undertake to recommend or to pass judgment upon applicants It aims merely to serve as a clearing-house for such information as above stated and to bring into touch with one another candidates for positions and employers

Individuals, whether members of the Federation or not, universities, other institutions and organizations desiring to avail themselves of the Service may receive such information as is available By action of the Executive Committee in 1947, a registration fee of one dollar is required of each applicant for a position

All communications should be addressed to Federation Placement Service, 2101 Constitution Ave., Washington 25, D C

THE AMERICAN PHYSIOLOGICAL SOCIETY, INC

Founded December 30, 1887, Incorporated June 2, 1923

OFFICERS, 1950-1951

President—H C BAZETT* (April to July, 1950)
D B DILL, Medical Division, Army Chemical Center, Md

President-Elect—D B DILL (April to July, 1950) Office vacant since July 1950

Past-President—CARL J WIGGERS, Western Reserve University, Cleveland, Ohio

Council—H C BAZETT* (April to July, 1950), D B DILL, C J WIGGERS, R W GERARD (1953), *Secretary-Treasurer*, E M LANDIS (1951), F A HITCHCOCK (1952), E F ADOLPH (1953)

Executive Secretary—M O LEE, 2101 Constitution Ave, Washington, D C

Members of Joint Finance Committee (with Federation)—W O FENN, D B DILL

Board of Publication Trustees—W O FENN, *Chairman* (1952), FRANK C MANN (1951), R F PITTS (1953)

Representative on the Division of Biology and Agriculture, National Research Council—MAURICE B VISSCHER (1952)

Representative on the Division of Medical Sciences, National Research Council—HALLOWELL DAVIS (1953)

Representatives on the Council of the American Association for the Advancement of Science—J H BODINE, (1952), LEIGH CHADWICK (1952)

Historian—WALTER J MEEK

Contributing Editor to Scientific Monthly—JOHN FIELD II

PAST OFFICERS

Organization Meeting, December 30, 1887

S WEIR MITCHELL, *President*

H N MARTIN, *Secretary*

1888 H P BOWDITCH, *President*, H N MARTIN, *Secretary-Treasurer*, J G CURTIS, H C WOOD, H SEWALL, *Councilors* 1889 S WEIR MITCHELL, *President*, H N MARTIN, *Secretary-Treasurer*, H P BOWDITCH, J G CURTIS, H C WOODS, *Councilors* 1890 S WEIR MITCHELL, *President*, H N MARTIN, *Secretary-Treasurer*, H P BOWDITCH, J G CURTIS, H H DONALDSON, *Councilors* 1891 H P BOWDITCH, *President*, H N MARTIN, *Secretary-Treasurer*, R H CHITTENDEN, J G CURTIS, H N DONALDSON, *Councilors* 1892 H P BOWDITCH, *President*, H N MARTIN, *Secretary-Treasurer*, R H CHITTENDEN, J G CURTIS, W H HOWELL, *Councilors* 1893 H P BOWDITCH, *President*, W P LOMBARD, *Secretary-*

Treasurer, R H CHITTENDEN, J G CURTIS, W H HOWELL, *Councilors* 1894 H P BOWDITCH, *President*, W P LOMBARD, *Secretary-Treasurer* R H CHITTENDEN, W H HOWELL, J W WARREN, *Councilors* 1895 H P BOWDITCH, *President*, F S LEE, *Secretary-Treasurer*, R H CHITTENDEN, W H HOWELL, W P LOMBARD, *Councilors* 1896 R H CHITTENDEN, *President*, F S LEE, *Secretary-Treasurer*, H P BOWDITCH, W H HOWELL, J W WARREN, *Councilors* 1897 R H CHITTENDEN, *President*, F S LEE, *Secretary-Treasurer*, H P BOWDITCH, W H HOWELL, W P LOMBARD, *Councilors* 1898 R H CHITTENDEN, *President*, F S LEE, *Secretary-Treasurer*, H P BOWDITCH, W H HOWELL, W P LOMBARD, *Councilors* 1899 R H CHITTENDEN, *President*, F S LEE, *Secretary-Treasurer*, W H HOWELL, W P LOMBARD, W T PORTER, *Councilors* 1900 R H CHITTENDEN, *President*, F S LEE, *Secretary-Treasurer*, W H HOWELL, W P LOMBARD, W T PORTER, *Councilors* 1901 R H CHITTENDEN, *President*, F S LEE, *Secretary-Treasurer*, W H HOWELL, W P LOMBARD, W T PORTER, *Councilors* 1902 R H CHITTENDEN, *President*, F S LEE, *Secretary-Treasurer*, W H HOWELL, W P LOMBARD, W T PORTER, *Councilors* 1903 R H CHITTENDEN, *President*, F S LEE, *Secretary-Treasurer*, W H HOWELL, W P LOMBARD, W T PORTER, *Councilors* 1904 R H CHITTENDEN, *President*, W T PORTER, *Secretary-Treasurer*, F S LEE, W P LOMBARD, W H HOWELL, *Councilors* 1905 W H HOWELL, *President*, L B MENDEL, *Secretary*, W B CANNON, *Treasurer*, R H CHITTENDEN, S J MELTZER, *Councilors* 1906 W H HOWELL, *President*, L B MENDEL, *Secretary*, W B CANNON, *Treasurer*, A B MACALLUM, S J MELTZER, *Councilors* 1907 W H HOWELL, *President*, L B MENDEL, *Secretary*, W B CANNON, *Treasurer*, J J ABEL, G LUSK, *Councilors* 1908 W H HOWELL, *President*, R HUNT, *Secretary*, W B CANNON, *Treasurer*, J J ABEL, G LUSK, *Councilors* 1909 W H HOWELL, *President*, R HUNT, *Secretary*, W B CANNON, *Treasurer*, A J CARLSON, W P LOMBARD, *Councilors* 1910 W H HOWELL, *President*, A J CARLSON, *Secretary*, W B CANNON, *Treasurer*, J ERLANGER, F S LEE, *Councilors* 1911 S J MELTZER, *President*, A J CARLSON, *Secretary*, W B CANNON, *Treasurer*, J ERLANGER, F S LEE, *Councilors* 1912 S J MELTZER, *President*, A J CARLSON, *Secretary*, W B CANNON, *Treasurer*, J ERLANGER, F S LEE, *Councilors* 1913 S J MELTZER, *President*, A J CARLSON, *Secretary*, J ERLANGER, *Treasurer*, W B CANNON,

* Deceased

F S LEE, Councilors 1914 W B CANNON, President, A J CARLSON, Secretary, J ERLANGER, Treasurer, F S LEE, S J MELTZER, Councilors 1915 W B CANNON, President, C W GREENE, Secretary, J ERLANGER, Treasurer, W E GARREY, W H HOWELL, J J R MACLEOD, W J MEEK, Councilors 1916 W B CANNON, President, C W GREENE, Secretary, J ERLANGER, Treasurer, W E GARREY, W H HOWELL, J J R MACLEOD, W J MEEK, Councilors 1917 F S LEE, President, C W GREENE, Secretary, J ERLANGER, Treasurer, W B CANNON, W H HOWELL, J J R MACLEOD, W J MEEK, Councilors 1918 F S LEE, President, C W GREENE, Secretary, J ERLANGER, Treasurer, W B CANNON, W H HOWELL, J J R MACLEOD, W J MEEK, Councilors 1919 W P LOMBARD, President, C W GREENE, Secretary, J ERLANGER, Treasurer, W B CANNON, Y HENDERSON, J J R MACLEOD, W J MEEK, Councilors 1920 W P LOMBARD, President, C W GREENE, Secretary, J ERLANGER, Treasurer, W B CANNON, J J R MACLEOD, Y HENDERSON, C J WIGGERS, Councilors 1921 J J R MACLEOD, President, C W GREENE, Secretary, J ERLANGER, Treasurer, J A E EYSTER, Y HENDERSON, C J WIGGERS, A J CARLSON, Councilors 1922 J J R MACLEOD, President, C W GREENE, Secretary, J ERLANGER, Treasurer, Y HENDERSON, C J WIGGERS, A J CARLSON, J A E EYSTER, Councilors 1923 A J CARLSON, President, C W GREENE, Secretary, J ERLANGER, Treasurer, C J WIGGERS, A B LUCKHARDT, J A E EYSTER, J R MURLIN, Councilors 1924 A J CARLSON, President, W J MEEK, Secretary, C K DRINKER, Treasurer, A B LUCKHARDT, J A E EYSTER, J R MURLIN, W E GARREY, Councilors 1925 A J CARLSON, President, W J MEEK, Secretary, C K DRINKER, Treasurer, J A E EYSTER, J R MURLIN, W E GARREY, JOSEPH ERLANGER, Councilors 1926 J ERLANGER, President, W J MEEK, Secretary, A FORBES, Treasurer, J R MURLIN, W E GARREY, A B LUCKHARDT, C J WIGGERS, Councilors 1927 J ERLANGER, President, W J MEEK, Secretary, A FORBES, Treasurer, W E GARREY, A B LUCKHARDT, C J WIGGERS, R GESELL, Councilors 1928 J ERLANGER, President, W J MEEK, Secretary, A FORBES, Treasurer, A B LUCKHARDT, C J WIGGERS, R GESELL, A J CARLSON, Councilors 1929 W J MEEK, President, ALFRED C REDFIELD, Secretary, A FORBES, Treasurer, C J WIGGERS, R GESELL, A J CARLSON, J R MURLIN, Councilors 1930 W J MEEK, President, ARNO B LUCKHARDT, Secretary, A FORBES, Treasurer, R GESELL, A J CARLSON, J R MURLIN, E G MARTIN, Councilors 1931 W J MEEK, President, ARNO B LUCKHARDT, Secretary, ALEXANDER FORBES, Treasurer, A J CARLSON, J R MURLIN, E G MARTIN, JOHN

TAIT, Councilors 1932 ARNO B LUCKHARDT, President, FRANK C MANN, Secretary, ALEXANDER FORBES, Treasurer, E G MARTIN, W J MEEK, J R MURLIN, JOHN TAIT, Councilors 1933 ARNO B LUCKHARDT, President, FRANK C MANN, Secretary, ALEXANDER FORBES, Treasurer, HERBERT S GASSER, ERNEST G MARTIN, W J MEEK, JOHN TAIT, Councilors 1934 CHARLES W GREENE, President, FRANK C MANN, Secretary, ALEXANDER FORBES, Treasurer, HERBERT S GASSER, ARNO B LUCKHARDT, W J MEEK, JOHN TAIT, Councilors 1935 FRANK C MANN, President, ANDREW C IVY, Secretary, ALEXANDER FORBES, Treasurer, CHARLES H BEST, HERBERT S GASSER, ARNO B LUCKHARDT, W J MEEK, Councilors 1936 FRANK C MANN, President, ANDREW C IVY, Secretary, WALLACE O FENN, Treasurer, CHARLES H BEST, PHILIP BARD, HERBERT S GASSER, ARNO B LUCKHARDT, Councilors 1937 WALTER E GARREY, President, ANDREW C IVY, Secretary, WALLACE O FENN, Treasurer, CHARLES H BEST, PHILIP BARD, HERBERT S GASSER, ARNO B LUCKHARDT, Councilors 1938 WILLIAM T PORTER, Honorary President, WALTER E GARREY, President, ANDREW C IVY, Secretary, WALLACE O FENN, Treasurer, ARNO B LUCKHARDT, CHARLES H BEST, PHILIP BARD, HERBERT S GASSER, Councilors 1939 ANDREW C IVY, President, PHILIP BARD, Secretary, WALLACE O FENN, Treasurer, CHARLES H BEST, HERBERT S GASSER, ARNO B LUCKHARDT, MAURICE B VISSCHER, Councilors 1940 ANDREW C IVY, President, PHILIP BARD, Secretary, CARL J WIGGERS, Treasurer, CHARLES H BEST, HERBERT S GASSER, ARNO B LUCKHARDT, MAURICE B VISSCHER, Councilors 1941 PHILIP BARD, President, CARL J WIGGERS, Secretary, HALLOWELL DAVIS, Treasurer, CHARLES H BEST, ARNO B LUCKHARDT, MAURICE B VISSCHER, HIRAM E ESSEX, Councilors 1942, 1943, 1944, 1945 PHILIP BARD, President, WALLACE O FENN, Secretary, HALLOWELL DAVIS, Treasurer, CHARLES H BEST, MAURICE B VISSCHER, HIRAM E ESSEX, W F HAMILTON, Councilors 1946 WALLACE O FENN, President, MAURICE B VISSCHER, Secretary, D B DILL, Treasurer, CHARLES H BEST, HIRAM E ESSEX, W F HAMILTON, H C BAZETT, Councilors 1947 WALLACE O FENN, President, MAURICE B VISSCHER, Secretary, D B DILL, Treasurer, EUGENE M LANDIS, HIRAM E ESSEX, W F HAMILTON, H C BAZETT, Councilors 1948 MAURICE B VISSCHER, President, CARL J WIGGERS, President-Elect, WALLACE O FENN, Past-President, W F HAMILTON, H C BAZETT, EUGENE M LANDIS, D B DILL, Councilors 1949 CARL J WIGGERS, President, H C BAZETT, President-Elect, MAURICE B VISSCHER, Past-President, EDWARD F ADOLPH, EUGENE M LANDIS, D B DILL, R W GERARD, Councilors

CONSTITUTION

I

1 This Society shall be named "THE AMERICAN PHYSIOLOGICAL SOCIETY, INCORPORATED "

2 The Society is instituted to promote the advance of Physiology and to facilitate personal intercourse between American Physiologists

II

1 The Society shall consist of members and honorary members

2 Any person who has conducted and published meritorious original researches in Physiology and who is a resident of North America shall be eligible for membership in the Society

3 Members who have been relieved by the Council of the payment of the annual assessment shall retain all the rights of members

4 Distinguished men of science who have contributed to the advance of Physiology shall be eligible for election as honorary members of the Society. Honorary members shall pay no membership fee. They shall have the right of attending the meetings of the Society, and of taking part in its scientific discussions, but they shall have no vote

III

1 The management of the Society shall be vested in a Council consisting of a President-Elect, President, Past-President for the previous year, and four other members. The President-Elect and one member of the Council shall be chosen by ballot at the Spring meeting. The President-Elect shall automatically assume the duties of President at the adjournment of the annual meeting following his or her election. The four additional members shall be elected for terms of four years and shall not be eligible to succeed themselves except those who have served for two years or less in filling interim vacancies. A person who has once been President shall not be eligible for re-election as President-Elect. The Council shall select from among its own members a Secretary-Treasurer of the Society and Council. It shall also have the power to appoint and to compensate an Executive Secretary of the Society, who shall not be a voting member of the Council but shall assist it in carrying on the functions of the Society, including the receipt and disbursement of funds under the direction of the Council. If the annual meeting is not held all the members of the Council shall continue in office until their successors are chosen in the prescribed manner in succession.

2 The Council shall define the respective duties of the President, Secretary-Treasurer and Executive Secretary

3 If a member of the Council be elected President-Elect, the vacancy on the Council shall be filled by election of the same meeting, the electee to serve the unexpired term. The Council shall have the power to fill all interim vacancies that may occur in its membership or in any Committee or board of the Society except those for which other provisions have been made

IV

1 At least a fortnight before a meeting of the Society the Executive Secretary shall send to each member a notice of the place and time of each meeting, and shall make such other announcements as the Council shall direct

2 The member assessment shall be determined by the Council and shall be due in advance at the time of the annual meeting. No allocation or disbursement of funds of the Society shall be made except upon prior approval of the Council. Appropriations shall be made by the Council for the conduct of the necessary and appropriate business of the Society

3 Any member whose assessment is two years in arrears shall cease to be a member of the Society, unless at the next Spring meeting he shall be reinstated by special vote of the Society, and it shall be the duty of the Secretary-Treasurer to notify the said delinquent of his right to appeal at the said meeting

4 Any member who has retired because of illness or age may, upon application to the Council, be relieved from payment of the annual assessment

V

1 A meeting of the Society for transacting business, the election of officers and members, and presenting communications shall be held in the Spring, except in the case of national emergencies or other exceptional circumstances, when the Council may decide on postponement. The time and place of such meetings shall be determined by the Council in consultation with the Executive Committee of the Federation of American Societies for Experimental Biology

2 A Fall meeting of the Society may be held at a time and place determined by the Council for presenting communications and transacting any business except the election of officers and members. Special meetings may be held at such times and places as the Council may determine

VI

1 Proposed amendments to the Constitution must be brought up at one meeting for preliminary discussion and approval by a majority vote and cannot be adopted except by a two-thirds vote at a business session at the next Spring meeting

Notice of such changes shall be sent to all members at least two weeks prior to the meeting at which they are scheduled for adoption

2 At all business meetings of the Society twenty-five members shall form a quorum

3 By-laws for the conduct of the Society may be adopted, altered, or repealed at any business meeting by two-thirds vote of the ballots cast

VII

1 The Council may, from the names of the candidates proposed in writing by at least two members of the Society, nominate candidates for election to membership. The names of the candidates so nominated and a statement of their qualifications for membership signed by their proposers shall be available for inspection during the business sessions of the Society at which their election is considered. The candidates may be balloted for at any session of the same meeting and a majority vote shall elect. If a Spring meeting is not held, the Society shall elect candidates to membership at the next Spring meeting

2 Honorary members shall be proposed by the Council, and shall be elected by a majority ballot of the members present at the Spring meeting of the Society

VIII §

1 If a majority of the Council shall decide that the interests of the Society require the expulsion of a member, the Secretary-Treasurer shall send a notice of this decision to each member at least two weeks before the next annual meeting. At this meeting the Secretary-Treasurer shall on behalf of the Council, propose the expulsion, and if two-thirds of the members present vote for it, the member shall be expelled, and his assessment for the current year shall be returned to him, and he shall cease to be a member of the Society

IX

1 The official organs of the Society shall be the *American Journal of Physiology*, the *Journal of Applied Physiology*, *Physiological Reviews* and such other publications as the Society shall establish. These the Society shall own and they shall be managed according to the provisions of Article X

X

1 The President of the Society shall appoint, in consultation with the Council and subject to

the approval of the Society, three members of the Society to serve as members of a Board of Publication Trustees

2 The initial appointments shall be for one, two and three years. Thereafter, each member shall be appointed for three years, and shall be eligible for one immediate reappointment. He may be subsequently reappointed, but only after the lapse of at least one year between reappointments

3 The Board of Publication Trustees shall be vested with full power of the Society to control and manage, both editorially and financially, all of the publications owned in whole or in part by the Society, to appoint editorial boards, to appoint and compensate a Managing Editor, and to control all publication funds, none of which, however, may be diverted from support of publications of the Society except by consent of the Council

4 The Board of Publication Trustees shall make a full report to the Council at each annual meeting of the financial condition and publication policy of the Journals or other publications

BY-LAWS

1 All papers read before the Society shall be limited to a length of ten minutes. No person may orally present more than one paper. In case of joint authorship the name of the individual who will orally present the paper shall stand first

2 Abstracts in duplicate, not to exceed two hundred and fifty words in length, of all papers to be presented at the annual meeting of the Society shall be required by the Secretary for publication in the *Federation Proceedings*, in accordance with rules approved by the Council

3 The Council shall upon the request of twenty-five members call a regional meeting of the Society at any time and place, for the reading of papers and the promotion of personal intercourse. Such a request shall be made in writing at least six weeks before the proposed date of meeting. Such meeting shall be held in accordance with the Constitution and By-laws of the Society, and if the regular officers of the Society cannot be present the President shall appoint a committee from among the petitioners to conduct the meeting. The Committee through a Secretary chosen by them shall forward an account of the scientific proceedings of the meeting to the official Secretary of the Society for insertion in the minutes

4 No general business of the Society shall be transacted at such regional meetings

AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, INC

Founded December 6, 1906, Incorporated September 12, 1919

OFFICERS, COMMITTEES AND REPRESENTATIVES FOR 1950-1951

President—H B VICKERY, Connecticut Agricultural Experiment Station, New Haven (1950-51)

Vice President—V DU VIGNEAUD, Cornell University Medical College, New York City (1950-51)

Secretary—E H STOTZ, University of Rochester School of Medicine and Dentistry, Rochester N Y (1950-51)

Treasurer—E A EVANS, University of Chicago, Chicago, Ill (1946-51)

Council—H B VICKERY, V DU VIGNEAUD, E H STOTZ, E A EVANS, JR, H T CLARKE (1949-52), C F CORI (1950-53), J M LUCK (1947-48, 1948-51), Councilors

STANDING COMMITTEES

Editorial Board—R J ANDERSON (1931, *Managing Editor*, 1935-42, 1942—), R M ARCHIBALD (1948-53), E G BALL (1950-55), H E CARTER (1950-55), W M CLARK (1938), H T CLARKE (1937), C F CORI (1941), E A DOISY (1938), V DU VIGNEAUD (1948-53), J S FRUTON (1948-53), W H GRIFFITH (1949-54), A B HASTINGS (1941), H B LEWIS (1938), H S LORING (1950-55), E V MCCOLLUM (1933), S MOORE (1950-55), S OCHOA (1950-55), E E SNELL (1949-54), W M SPERRY (1949-54), E L TATUM (1948-53), H B VICKERY (1941), H G WOOD (1949-54)

Editorial Committee—H A MATTILL (1947-53, *Chairman*, March 3, 1948), W R BLOOR (1947-53), R K CANNAN (1949-55), J T EDSALL* (1948-51), C G KING (1947-53), J M LUCK (1949-55), A N RICHARDS (1945-51), P A SHAFFER (1945-51), D W WILSON (1949-55)

Nominating Committee—H E CARTER (1949-50, 1950-51, *Chairman and Secretary*), K BLOCH (1950-51), H BORSOOK (1950-51), H J DEUEL (1950-51), J T EDSALL (1950-51), D M GREENBERG (1949-50, 1950-51), H B LEWIS (1949-50, 1950-51), J B SUMNER (1950-51), H G WOOD (1949-50, 1950-51)

SPECIAL COMMITTEES

Armed Forces Committee, 1950-51 (March 14, 1946)—W H GRIFFITH, *Chairman*, O A BESSEY, C G KING

Biochemical Nomenclature, 1950-51 (April 15, 1941)—H B VICKERY, *Chairman*, E M NELSON, H J ALMQUIST (American Institute of Nutrition), C A LIEBHJEM (American Institute of Nutrition)

* Elected to fill unexpired term 1945-1951 of V du Vigneaud, resigned January, 1948

Clinical Chemistry, 1950-51 (March 11, 1946)—W M SPERRY, *Chairman*, H T CLARKE, R N HARGER, R M HILL, J REINHOLD, H SOBOTKA, W A WOLFF

Codify Administrative and Business Organization of the Society, 1950-51 (March 14, 1948)—E A EVANS, JR (ex officio *Treasurer*), *Chairman*, O A BESSEY, R W JACKSON

Finance Committee, 1950-51 (reappointed March 13, 1942)—E A EVANS JR (ex officio *Treasurer*), *Chairman*, H B VICKERY (ex officio *President*), E H STOTZ (ex officio *Secretary*)

Professional Training of Biochemists, 1950-51 (November 29, 1945)—C A ELVEHJEM, *Chairman*, W M CLARK, E A DOISY, V DU VIGNEAUD, H B LEWIS

Historian—P A SHAFFER (1946—)

Legal Counselor—LLOYD N SCOTT (1950-51)

REPRESENTATIVES TO OTHER ORGANIZATIONS

American Association for the Advancement of Science (April 18, 1941)—W D ARMSTRONG (1949-51), D BURK (1949-51)

American Documentation Institute (1937)—A SEIDELL (January 1, 1950-53)

International Committee for Biochemistry (1949)—A B HASTINGS (1950-51)

National Research Council—W M CLARK, *Division of Biology and Agriculture* (1948-51), W C STADIE, *Division of Medical Sciences* (1948-51)

PAST OFFICERS

1907 RUSSELL H CHITTENDEN, *President*, J J ABEL, *Vice-President*, W J GIES, *Secretary*, L B MENDEL, *Treasurer*, W JONES, W KOCH, J MARSHALL, T B OSBORNE, *Councilors* 1908 JOHN J ABEL, *President*, OTTO FOLIN, *Vice-President*, WM J GIES, *Secretary*, L B MENDEL, *Treasurer*, A B MACALLUM, A P MATHEWS, F G NOVY, *Councilors* 1909 OTTO FOLIN, *President*, T B OSBORNE, *Vice-President*, WM J GIES, *Secretary*, L B MENDEL, *Treasurer*, J J ABEL, P A LEVEE, G LUSK, *Councilors* 1910 THOMAS B OSBORNE, *President*, L B MENDEL, *Vice-President*, A N RICHARDS, *Secretary*, WALTER JONES, *Treasurer*, A B MACALLUM, A P MATHEWS, V C VAUGHAN, *Councilors* 1911 LAFAYETTE B MENDEL, *President*, A B MACALLUM, *Vice-President*, A N RICHARDS, *Secretary*, WALTER JONES, *Treasurer*, WM J GIES, A S LOEVENHART, P A SHAFFER, *Councilors* 1912 ARCHIBALD B MACALLUM, *President*, G LUSK, *Vice-President*, A N RICHARDS, *Secretary*, WALTER JONES, *Treasurer*, H P ARMSBY, L B

MENDEL, H G WELLS, Councilors 1913 ARCHIBALD B MACALLUM, President, G LUSK, Vice-President, P A SHAFFER, Secretary, D D VAN SLYKE, Treasurer, H P ARMSBY, L B MENDEL, H G WELLS, Councilors 1914 GRAHAM LUSK, President, C L ALSBERG, Vice-President, P A SHAFFER, Secretary, D D VAN SLYKE, Treasurer, J J ABEL, A B MACALLUM, T B OSBORNE, Councilors 1915 WALTER JONES, President, C L ALSBERG, Vice-President, P A SHAFFER, Secretary, D D VAN SLYKE, Treasurer, OTTO FOLIN, G LUSK, L B MENDEL, Councilors 1916 WALTER JONES, President, F P UNDERHILL, Vice-President, S R BENEDICT, Secretary, D D VAN SLYKE, Treasurer, OTTO FOLIN, A B MACALLUM, P A SHAFFER, Councilors 1917 CARL L ALSBERG, President, A P MATHEWS, Vice-President, S R BENEDICT, Secretary, H C BRADLEY, Treasurer, L J HENDERSON, P A SHAFFER, F P UNDERHILL, Councilors 1918 CARL L ALSBERG, President, A P MATHEW, Vice-President, S R BENEDICT, Secretary, H C BRADLEY, Treasurer, W J GIES, ANDREW HUNTER, E V MCCOLLUM, Councilors 1919 STANLEY R BENEDICT, President, D D VAN SLYKE, Vice-President, V C MYERS, Secretary, H C BRADLEY, Treasurer, ANDREW HUNTER, E V MCCOLLUM, L B MENDEL, Councilors 1920 STANLEY R BENEDICT, President, D D VAN SLYKE, Vice-President, V C MYERS, Secretary, H C BRADLEY, Treasurer, OTTO FOLIN, WALTER JONES, L B MENDEL, Councilors 1921 DONALD D VAN SLYKE, President, P A SHAFFER, Vice-President, V C MYERS, Secretary, H C BRADLEY, Treasurer, S R BENEDICT, OTTO FOLIN, WALTER JONES, Councilors 1922 DONALD D VAN SLYKE, President, P A SHAFFER, Vice-President, V C MYERS, Secretary, W R BLOOR, Treasurer, S R BENEDICT, H C BRADLEY, A P MATHEWS, Councilors 1923 PHILIP A SHAFFER, President, H C SHERMAN, Vice-President, V C MYERS, Secretary, W R BLOOR, Treasurer, H C BRADLEY, ANDREW HUNTER, A P MATHEWS, Councilors 1924 PHILIP A SHAFFER, President, HENRY C SHERMAN, Vice-President, D WRIGHT WILSON, Secretary, WALTER R BLOOR, Treasurer, OTTO FOLIN, ANDREW HUNTER, VICTOR C MYERS, Councilors 1925 HENRY C SHERMAN, President, EDWARD C KENDALL, Vice-President, D WRIGHT WILSON, Secretary, WALTER R BLOOR, Treasurer, OTTO FOLIN, LAFAYETTE B MENDEL, PHILIP A SHAFFER, Councilors 1926 EDWARD C KENDALL, President, ELMER V MCCOLLUM, Vice-President, FRED C KOCH, Secretary, GLENN E CULLEN, Treasurer, J B COLLIP, EDWARD A DOISY, ALBERT P MATHEWS, Councilors 1927 E V MCCOLLUM, President, W R BLOOR, Vice-President, D WRIGHT WILSON, Secretary, G E CULLEN, Treasurer, E A DOISY, F C KOCH, D D

VAN SLYKE, Councilors 1928 E V MCCOLLUM, President, W R BLOOR, Vice-President, D WRIGHT WILSON, Secretary, G E CULLEN, Treasurer, WM M CLARK, F C KOCH, D D VAN SLYKE, Councilors 1929 W R BLOOR, President, H C BRADLEY, Vice-President, H B LEWIS, Secretary, G E CULLEN, Treasurer, W M CLARK, C L A SCHMIDT, P A SHAFFER, Councilors 1930 W R BLOOR, President, H C BRADLEY, Vice-President, H B LEWIS, Secretary, G E CULLEN, Treasurer, W M CLARK, P A SHAFFER, D W WILSON, Councilors 1931 H C BRADLEY, President, W M CLARK, Vice-President, H B LEWIS, Secretary, C H FISKE, Treasurer, W C ROSE, P A SHAFFER, D W WILSON, Councilors 1932 H C BRADLEY, President, W M CLARK, Vice-President, H B LEWIS, Secretary, C H FISKE, Treasurer, P E HOWE, W C ROSE, D W WILSON, Councilors 1933 W M CLARK, President, H B LEWIS, Vice-President, H A MATTILL, Secretary, C H FISKE, Treasurer, H C BRADLEY, P E HOWE, W C ROSE, Councilors 1934 W M CLARK, President, H B LEWIS, Vice-President, H A MATTILL, Secretary, C H FISKE, Treasurer, H C BRADLEY, E A DOISY, P E HOWE, Councilors 1935 H B LEWIS, President, G E CULLEN, Vice-President, H A MATTILL, Secretary, C H FISKE, Treasurer, H C BRADLEY, J B COLLIP, E A DOISY, Councilors 1936 H B LEWIS, President, G E CULLEN, Vice-President, H A MATTILL, Secretary, A B HASTINGS, Treasurer, J B COLLIP, E A DOISY, W C ROSE, Councilors 1937 G E CULLEN, President, W C ROSE, Vice-President, H A MATTILL, Secretary, A B HASTINGS, Treasurer, E A DOISY, H B LEWIS, H B VICKERY, Councilors 1938 G E CULLEN, President, W C ROSE, Vice-President, CHARLES G KING, Secretary, A B HASTINGS, Treasurer, H B LEWIS, H A MATTILL, H B VICKERY, Councilors 1939 W C ROSE, President, R J ANDERSON, Vice-President, CHARLES G KING, Secretary, A B HASTINGS, Treasurer, H B LEWIS, H A MATTILL, G E CULLEN, Councilors 1940 WILLIAM C ROSE, President, RUDOLPH J ANDERSON, Vice-President, CHARLES G KING, Secretary, A B HASTINGS, Treasurer, H A MATTILL, GLENN E CULLEN, E A DOISY, Councilors 1941 R J ANDERSON, President, E A DOISY, Vice-President, A K BALLS, Secretary, W C STADIE, Treasurer, H B LEWIS, W C ROSE, Councilors 1942 R J ANDERSON, President, E A DOISY, Vice-President, A K BALLS, Secretary, W C STADIE, Treasurer, W C ROSE, C A KING, H T CLARKE, Councilors 1943 E A DOISY, President, A B HASTINGS, Vice-President, A K BALLS, Secretary, W C STADIE, Treasurer, W C ROSE, H T CLARKE, R J ANDERSON, Councilors 1944 E A DOISY,

President, A B HASTINGS, Vice-President, A K BALLS, Secretary, W C STADIE, Treasurer, R J ANDERSON, H T CLARKE, V DU VIGNEAUD, Councilors 1945 A B HASTINGS, President, H T CLARKE, Vice-President, A K BALLS, Secretary, W C STADIE, Treasurer, R J ANDERSON, C F CORI, V DU VIGNEAUD, Councilors 1946 A B HASTINGS, President, H T CLARKE Vice-President, OTTO A BESSEY, Secretary, E A EVANS, JR, Treasurer, V DU VIGNEAUD, C F CORI, A K BALLS, Councilors 1947 HANS T CLARKE, President, CARL F CORI, Vice-President, OTTO A BESSEY, Secretary, E A EVANS, JR Treasurer, A K BALLS, A BAIRD HASTINGS, J MURRAY LUCK, Councilors 1948 HANS T CLARKE, President, CARL F CORI, Vice-President, OTTO A BESSEY, Secretary, E A EVANS, JR, Treasurer, A K BALLS, A BAIRD HASTINGS, J MURRAY LUCK, Councilors 1949 CARL F CORI, President, H B VICKERY, Vice-President, R W JACKSON, Secretary, E A EVANS, JR, Treasurer, R W JACKSON, J M LUCK, H B VICKERY, Councilors

CONSTITUTION

FROM THE ARTICLES OF INCORPORATION

1 The name of the proposed corporation is "AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, INCORPORATED"

2 The purposes for which this corporation is formed are to further the extension of biochemical knowledge and to facilitate personal intercourse between American investigators in biological chemistry

BY-LAWS

ARTICLE I *Membership*

SECTION 1 *Eligibility for Membership* Qualified investigators who have conducted and published meritorious original investigations in biological chemistry shall be eligible for membership in the Society

SECTION 2 *Nomination* Nominations for membership shall be made and seconded by members of the Society on blanks furnished by the Secretary. Nominations shall be submitted to the Council who shall determine eligibility and make recommendation to the Society at a regular meeting

SECTION 3 *Election to Membership* A A nominee for membership may be voted for by ballot at any meeting of the Society after the Council has reported its findings on his eligibility. The eligible candidate shall be reported by the Council as "eligible" or as "eligible and indorsed" B A majority of the ballots cast shall elect

SECTION 4 *Forfeiture* A Any member who may grant the use of his name for (a) the advertisement of a patent medicine, a proprietary food preparation or any other commercial article of doubtful

value to the public or possibly harmful to the public health, or (b) who may concede its use for the purpose of encouraging the sale of individual samples (of any such product) that he has not examined, shall forfeit his membership

B The Council shall have authority to announce forfeiture of membership, provided that the copy of the charges, together with a written notice of a hearing thereon by the Council at a place and time specified in such notice, shall have been delivered to the member charged with violating the preceding section either personally or mailed to him at his last known address at least thirty days before the date of such hearing

SECTION 5 *Expulsion* Upon the recommendation of the Council any member may be expelled by a majority vote of the total membership at a meeting of the Society, provided that a copy of the charges against him, together with a written notice of a hearing thereon by the Council at a place and time specified in such notice shall have been delivered to him personally or mailed to him at his last known address at least thirty days before the date of such hearing

ARTICLE II *Meetings and Quorum*

SECTION 1 *Annual* The annual meeting of the Society shall be held on the date fixed by the Certificate of Incorporation

SECTION 2 *Special* A special meeting may be called at any time by the President, or in case of his absence or disability, by the Vice-President, and must be called at the request of a majority of the Council or fifteen members of the Society. A notice specifying the purpose of such meeting shall be mailed to each member at least ten days previous thereto. The Council shall select the places at which meetings shall be held

SECTION 3 *Quorum* Fifteen members shall constitute a quorum at all meetings of the Society, but in absence of a quorum any number shall be sufficient to adjourn to a fixed date

ARTICLE III *Officials*

SECTION 1 *Officers* The officers shall be a President, a Vice-President, a Secretary, and a Treasurer, who shall be elected annually by the members of the Society

SECTION 2 *Council* A The Officers so elected and three additional members, one of whom shall be elected at each annual meeting of the Society to serve a three year term, shall constitute the Board of Directors of the corporation and shall be known as "The Council" (When this provision is first put into effect three members will need to be elected for a one, a two and a three year period)

B No two members of the Council may be from the same institution and none of the officers shall

elected shall be eligible for re-election for more than two years except the Secretary and Treasurer, who shall be eligible for re-election for five years. The three additional members of the Council shall be ineligible for re-election (until after the lapse of one year)

SECTION 3 *Duties of Officers* The powers and duties of the officers elected by the Society shall be such as usually devolve upon their respective positions

SECTION 4 *Assistant Treasurer* A The Council may from time to time appoint a trust company, or some member of the Society, to serve during the pleasure of the Council as Assistant Treasurer, and to act as depositary of the investments and income of the "Christian A Herter Memorial Fund" and of such other funds as the Society may from time to time commit to its or his charge

B The Assistant Treasurer shall have and exercise the following powers and duties, viz, the custody and safe-keeping of securities and cash belonging to the "Christian A Herter Fund" and the collection of income and other moneys due to the Fund, with power to receipt for the same and to endorse for deposit all checks payable to the Society or the Treasurer, or to the Journal of Biological Chemistry for income or other moneys due to the Fund, the investment or reinvestment of the capital of the Fund, subject to the approval of the Council, the disbursement of principal under the direction of the Council and the disbursement of income under the direction of the Editorial Board of the Journal of Biological Chemistry, such disbursement to be made under a resolution of the Council or Board, or with the approval of two members of either the Council or Board, as the case may be. The Assistant Treasurer shall keep books of account and render statements, annually or oftener upon the request of the Council or Board setting forth the condition of the Fund and the receipts and disbursements since the date of the preceding statement

ARTICLE IV *The Council*

SECTION 1 *Powers* The general management of the Society during the intervals between meetings shall be vested in the Council, which shall regularly perform the ordinary duties of an executive committee and possess all the powers conferred upon the Board of Directors of a membership corporation by the Membership Corporation Law of the State of New York

SECTION 2 *Reports* The Council shall report to the Society as promptly as possible its findings on the eligibility of candidates for membership, and on all charges of a violation of these By-Laws

SECTION 3 *Journal of Biological Chemistry* The Council shall have power to appoint the persons to act as proxies for the Society at all meetings of

the stockholders of the "Journal of Biological Chemistry" (a corporation) of which all the stock is owned by the Society, and also to designate the persons to be elected as Directors of such corporation

SECTION 4 *Herter Fund* It shall be the duty of the Council to see that the "Christian A Herter Memorial Fund" is administered in accordance with the terms of the Trust Agreement, Dated May 16, 1911, executed by the Journal of Biological Chemistry and the donors of said Fund

ARTICLE V *Nominating Committee*

SECTION 1 *Membership* A The Nominating Committee shall consist of nine members from nine different institutions elected at each annual meeting to serve for the ensuing year. Members who have served on the Nominating Committee for two consecutive years shall be ineligible for re-election until after the lapse of one year

B The member of the Nominating Committee who is elected to the Committee by the largest number of votes shall become Chairman and Secretary of the Committee

SECTION 2 *Nomination of Officials* A The Nominating Committee shall make at least one nomination for each of the four offices and for each of the three additional positions in the Council to be filled by vote of the members

B The nominations by the Nominating Committee must be transmitted to the Secretary at least one month before the annual meeting at which they are to be considered

C The Secretary shall send to every member, at least two weeks before the annual meeting, two copies of the list of nominees presented to him by the Nominating Committee and at the same time shall notify all the members that they may vote by proxy

D At the opening of the first executive session of the ensuing annual meeting the Secretary shall formally present the regular nominations for the Nominating Committee

E Additional nominations for the offices and for membership in the Council may be made by any member at the opening of the first executive session of any annual meeting

F Nominations for membership on the Nominating Committee shall be made by or for individual members, either in person or by proxy, and not otherwise, at the opening of the first executive session of any annual meeting

SECTION 3 *Election of Officials* A The Secretary shall receive and present to the tellers, appointed by the President to take charge of the election, all signed ballots forwarded by absent members. When such ballots are presented to the tellers the Secretary shall announce the names of the mem-

bers voting by proxy, and he shall record the same names in the minutes of the meeting

B All elective officials shall be selected by ballot at the close of the first executive session of each annual meeting

C A majority of the votes cast shall be necessary to elect an official

D Elective officials shall take office on July 1st following the annual meeting

SECTION 4 *Filling of Vacancies* A The Nominating Committee shall fill all vacancies in elective positions except such as may occur at a meeting of the Society

B The President of the Society shall fill all vacancies in appointive positions

ARTICLE VI *Financial*

SECTION 1 *Dues* Annual assessments shall be determined by majority vote at the annual meetings, upon the recommendation of the Council, and shall be due January 15th in each year. Members who have reached the age of 65 years, or who have become incapacitated, may, by vote of the Council, be exempted from the payment of dues

SECTION 2 *Expenditures* No expenditures from the general funds of the Society except those required in the performance of the ordinary official duties shall be made except by vote of the Society or the Council, but this section shall not apply to expenditures from the "Christian A. Herter Memorial Fund"

SECTION 3 *Privileges of Membership Begin with Payment of Dues* Candidates for membership, if elected, shall not be entitled to any of the privileges of membership, before they pay the dues of the fiscal year succeeding their election

SECTION 4 *Penalty for Non-Payment of Dues* A Members in arrears for dues for a period of three consecutive years shall thereupon forfeit their membership

B Delinquent members may be reinstated by the Council provided all indebtedness to the Society is liquidated

SECTION 5 *Herter Fund* The "Christian A. Herter Memorial Fund" shall be held and invested separately from the general funds of the Society and the income thereof shall be expended under the direction of the Editorial Board exclusively for the maintenance and support of the Journal of Biological Chemistry, subject to the supervision and control of the Editorial Committee in accordance with the terms of the Trust Agreement mentioned in ARTICLE IV, SECTION 4, and the provisions of ARTICLE XII of the By-Laws

ARTICLE VII *Journal of Biological Chemistry*

SECTION 1 *Editorial Committee* There shall be an Editorial Committee consisting of nine members of the Society who shall be nominated by the

Nominating Committee and elected by the Society in the same manner as officers. The nine members first elected shall divide themselves by lot into three classes of three in each class, to serve for two, four, and six years respectively, and thereafter three members shall be elected at each alternate annual meeting of the Society to succeed the members of the outgoing class and to serve for a term of six years. Members of the Committee shall be eligible to re-election

SECTION 2 *Powers of Committee* The Committee shall have power to elect an Editorial Board and shall have final authority in matters pertaining to the general policy of the Journal

SECTION 3 *Editorial Board* The members of the Board shall hold office until their successors are elected and shall appoint a Managing Editor from among their own number who shall have direct responsibility and authority for the active editorial conduct of the Journal, and who shall have discretionary power in arranging the details as to the conduct of the Journal. The expenditures of the income of the "Christian A. Herter Memorial Fund" shall be under the direction of the Board, and the approval of any two members of the Board shall be a sufficient warrant to authorize payments from such income

ARTICLE VIII *Papers on Scientific Subjects*

SECTION 1 *Presentation of Papers* The Secretary shall request each member who signifies his intention of reading a paper at any session to specify the length of time which its presentation will require. The time thus specified shall be printed on the official program, and the presiding officer shall have no authority to extend it unless a majority of the members present signify their wish to the contrary. In the absence of any specification of time required not more than ten minutes shall be allotted for the reading of any one paper

SECTION 2 *Number of Papers* No member shall be permitted to present more than one paper, either alone or in collaboration, until every member shall have had the opportunity of presenting one paper

ARTICLE IX *Corporate Seal*

SECTION 1 The corporate seal of the corporation shall be a circle surrounded by the words, "AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS," and including the word, "INCORPORATED"

ARTICLE X *Amendments*

SECTION 1 *Amendments* These By-Laws after having been approved by the Council and adopted by the Society at its first annual meeting shall not be amended except as hereinafter provided

SECTION 2 *Manner of Presentation Proposed*

amendments to the By-Laws must be sent to the Secretary at least one month before the date of the meeting at which they are to be considered and must be indorsed in writing by at least three members

SECTION 3 *Notice of Intended Amendments*
Secretary shall give every member notice of proposed amendments at least two weeks before the meeting at which they are to be considered and shall notify all members that they may vote by proxy

SECTION 4 *Adoption of Amendments* A The Secretary shall receive and present to the tellers

appointed by the President all signed ballots forwarded by absent members When such ballots are presented to the tellers, the Secretary shall announce the names of members voting by proxy, and he shall record the same names in the minutes of the meeting

B Votes upon amendments shall be cast at the opening of the second executive session of the meeting at which they are considered

C Affirmative votes from three-fifths of the members voting shall be required for the adoption of an amendment

AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, INC

Founded December 28, 1908 Incorporated June 19, 1933

OFFICERS, 1950-1951

President—CARL F SCHMIDT, University of Pennsylvania School of Medicine, Philadelphia

Vice-President—McKEEN CATTELL, Cornell University Medical College, New York City

Secretary—HARVEY B HAAG, Medical College of Virginia, Richmond

Treasurer—K K CHEN, Lilly Research Laboratories, Indianapolis, Ind

Council—CARL F SCHMIDT, McKEEN CATTELL, HARVEY B HAAG, K K CHEN AND LOUIS S GOODMAN, THOMAS C BUTLER, ARTHUR C DE GRAFF, Councilors

Membership Committee—ROBERT P WALTON (1950), JOHN C KRANTZ, JR (1951) J H COMROE, JR (1952)

Nominating Committee—OTTO KRAYER, *Chairman*, W T SALTER, WALTON VAN WINKLE, JR, ALFRED GILMAN, M H SEEVERS

Historian—WILLIAM DEB MACNIDER

Committee on International Pharmacological Congress—M L TAINTER, *Chairman*, OTTO KRAYER, H B VAN DYKE

Representatives to the Council of the American Association for the Advancement of Science—ARNOLD D WELCH, HARVEY B HAAG

PAST OFFICERS

1909 J J ABEL, President, REID HUNT, Secretary, A S LOEVENHART, Treasurer, S J MELTZER, T SOLLMANN, C W EDMUNDS, A C CRAWFORD, Councilors 1910 J J ABEL, President, REID HUNT, Secretary, A S LOEVENHART, Treasurer, A C CRAWFORD, G B WALLACE, Councilors 1911 J J ABEL, President, REID HUNT, Secretary, A S LOEVENHART, Treasurer, G B WALLACE, W DEB MACNIDER, Councilors 1912 J J ABEL, President, J AUER, Secretary, A S LOEVENHART, Treasurer, G B WALLACE, REID HUNT, Councilors 1913 T SOLLMANN, President, J AUER, Secretary, A S LOEVENHART, Treasurer, J J ABEL, W DEB MACNIDER, Councilors 1914 T SOLLMANN, President, J AUER, Secretary, W DEB MACNIDER, Treasurer, J J ABEL, A S LOEVENHART, Councilors 1915 T SOLLMANN, President, J AUER, Secretary, W DEB MACNIDER, Treasurer, WORTH HALE, D E JACKSON, Councilors 1916 REID HUNT, President, J AUER, Secretary, W DEB MACNIDER, Treasurer, A D HIRSCHFLDER, G B ROTH, Councilors 1917 REID HUNT, President, L G ROWANTREE, Secretary, W DEB MACNIDER,

Treasurer, J AUER, CARL VOEGTLIN, Councilors 1918 REID HUNT, President, E D BROWN, Secretary, W DEB MACNIDER, Treasurer, HUGH McGUIGAN, CARL VOEGTLIN, Councilors 1919 A S LOEVENHART, President, E D BROWN, Secretary, W DEB MACNIDER, Treasurer, REID HUNT, E K MARSHALL, JR, Councilors 1920 A S LOEVENHART, President, E D BROWN, Secretary, W DEB MACNIDER, Treasurer, D E JACKSON, E K MARSHALL, JR, Councilors 1921 C W EDMUNDS, President, E D BROWN, Secretary, HUGH McGUIGAN, Treasurer, JOHN AUER, J P HANZLIK, Councilors 1922 C W EDMUNDS, President, E D BROWN, Secretary, HUGH McGUIGAN, Treasurer, J P HANZLIK, H G BARBOUR, Councilors 1923 C W EDMUNDS, President, E D BROWN, Secretary, HUGH McGUIGAN, Treasurer, J P HANZLIK, H G BARBOUR, Councilors 1924 JOHN AUER, President, E D BROWN, Secretary, A L TATUM, Treasurer, J P HANZLIK, H G BARBOUR, Councilors 1925 JOHN AUER, President, E D BROWN, Secretary, A L TATUM, Treasurer, H G BARBOUR, W DEB MACNIDER, Councilors 1926 JOHN AUER, President, E D BROWN, Secretary, A L TATUM, Treasurer, H G BARBOUR, W DEB MACNIDER, Councilors 1927 CARL VOEGTLIN, President, E D BROWN, Secretary, A L TATUM, Treasurer, V E HENDERSON, C W EDMUNDS, Councilors 1928 CARL VOEGTLIN, President, E D BROWN, Secretary, A L TATUM, Treasurer, V E HENDERSON, C W EDMUNDS, Councilors 1929 CARL VOEGTLIN, President, E D BROWN, Secretary, O H PLANT, Treasurer, V E HENDERSON, C W EDMUNDS, Councilors 1930 GEORGE B WALLACE, President, E D BROWN, Secretary, O H PLANT, Treasurer, H G BARBOUR, C M GRUBER, Councilors 1931 GEORGE B WALLACE, President, VELYTEN E HENDERSON, Secretary, O H PLANT, Treasurer, PAUL D LAMSON, WILLIAM DEB MACNIDER, Councilors 1932 WM DEB MACNIDER, President, A N RICHARDS, Vice-President, V E HENDERSON, Secretary, O H PLANT, Treasurer, G B ROTH, A L TATUM, Councilors 1933 WM DEB MACNIDER, President, A L TATUM, Vice-President, V E HENDERSON, Secretary, O H PLANT, Treasurer, C M GRUBER, G B ROTH, Councilors 1934 R A HATCHER, President, A L TATUM Vice President, E M K GEILING, Secretary, O H PLANT, Treasurer, WM DEB MACNIDER R L STEHLF, Councilors 1935 V E HENDERSON, President, O H PLANT, Vice

President, E M K GEILING, Secretary, C M GRUBER, Treasurer, FLOYD DE EDS, M S DOOLEY, Councilors 1936 V E HENDERSON, President, O H PLANT, Vice-President, E M K GEILING, Secretary, C M GRUBER, Treasurer, C W EDMUNDS, G B WALLACE, Councilors 1937 A L TATUM, President, E M K GEILING, Vice-President, G P GRABFIELD, Secretary, C M GRUBER, Treasurer, V E HENDERSON, M H SEEVERS, Councilors 1938 A L TATUM, President, E M K GEILING, Vice-President, G P GRABFIELD, Secretary, C M GRUBER, Treasurer, E K MARSHALL, JR, C F SCHMIDT, Councilors 1939 O H PLANT, President, E M K GEILING, Vice-President, G P GRABFIELD, Secretary, E E NELSON, Treasurer, A L TATUM, C A DRAGSTEDT, Councilors 1940 E M K GEILING, President, C F SCHMIDT, Vice-President, G PHILIP GRABFIELD, Secretary, E E NELSON, Treasurer, B H ROBBINS, C H THIENES, Councilors 1941 E M K GEILING, President, C F SCHMIDT, Vice-President, RAYMOND N BIETER, Secretary, E E NELSON, Treasurer, E G GROSS, R G SMITH, Councilors 1942 E K MARSHALL, JR, President, CARL A DRAGSTEDT, Vice-President, RAYMOND N BIETER, Secretary, E E NELSON, Treasurer, MCK CATTELL, R G SMITH, Councilors 1943 E K MARSHALL, JR, President, CARL A DRAGSTEDT, Vice-President, RAYMOND N BIETER, Secretary, E E NELSON, Treasurer, MCK CATTELL, R G SMITH, Councilors 1944, 1945 E E NELSON, President, C M GRUBER, Vice-President, R N BIETER, Secretary, McKEEN CATTELL, Treasurer, HARRY BECKMAN, NATHAN B EDDY, Councilors 1946 MAURICE H SEEVERS, President, H B VAN DYKE, Vice-President, HARVEY B HAAG, Secretary, McKEEN CATTELL, Treasurer, HAMILTON H ANDERSON, JOHN C KRANTZ, JR, Councilors 1947 MAURICE H SEEVERS, President, CARL A DRAGSTEDT, Vice-President, HARVEY B HAAG, Secretary, K K CHEN, Treasurer, HAMILTON H ANDERSON, JOHN C KRANTZ, JR, Councilors 1948 CARL A DRAGSTEDT, President, H B VAN DYKE, Vice-President, HARVEY B HAAG, Secretary, K K CHEN, Treasurer, ARTHUR C DEGRAFF, ROBERT A WOODBURY, GORDEN A ALLES, Councilors 1949 CARL F SCHMIDT, President, J C KRANTZ, JR, Vice-President, HARVEY B HAAG, Secretary, K K CHEN, Treasurer, THOMAS C BUTLER, ARTHUR C DEGRAFF, ROBERT A WOODBURY, Councilors

CONSTITUTION

ARTICLE I *Name*

The name of this organization shall be the "AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, INCORPORATED"

ARTICLE II *Objects*

The purpose of this Society shall be to promote these branches of science and to facilitate personal intercourse between investigators who are actively engaged in research in these fields

ARTICLE III *Membership*

SECTION 1 Any person who has conducted and published a meritorious investigation in pharmacology or experimental therapeutics, and who is an active investigator in one of these fields, shall be eligible to membership, subject to the conditions of the other sections of Article III

SECTION 2 A Candidates for membership to this Society shall be proposed by two members who are not members of the Council The names so proposed shall be sent to the Secretary at least three months prior to the Annual Meeting

B The Membership Committee shall investigate the qualifications of the candidates and report to the Council

C Candidates reported upon by the Membership Committee to the Council may be recommended for admission by the Council only provided they have been approved by four-fifths of the combined membership of the Membership Committee and the Council

D The names of the candidates recommended for admission by the Council shall be posted by the Secretary not later than the day preceding the election for members

E The election of members shall be by individual ballot, one opposing vote in every eight cast shall be sufficient to exclude a candidate from membership

SECTION 3 *Forfeiture of Membership*

A Any member whose assessment is three years in arrears shall cease to be a member of the Society, unless he shall be reinstated by a special vote of the Council, and it shall be the duty of the Treasurer to inform the Secretary that he may notify the said delinquent of his right to appeal to the Council

B If the Council shall decide that it is for the best interests of the Society that a member be expelled, the member shall be notified and given an opportunity of a hearing before the Council Upon the recommendation of the Council the member then may be expelled by a three fourths vote of those present at a regular meeting of the Society

SECTION 4 *Honorary Members*

A Distinguished men of science who have contributed to the advance of pharmacology or experimental therapeutics shall be eligible for election as honorary members of the Society

B Nominations for honorary members shall take the same course as nominations for ordinary

members (Art III, Sec 2), but their election shall require the unanimous vote of the members present at the election

C Honorary members shall pay no membership fee They shall have the right to attend all meetings of the Society, and to take part in its discussions, but they shall have no vote

D The conditions for continuation of membership shall be the same for honorary as for ordinary members (Art III, Sec 3), except that forfeiture for arrears of fees does not apply to honorary members

ARTICLE IV *Officers and Elections*

SECTION 1 *Officers and Committees*

A The management of the Society shall be vested in a Council of seven officers consisting of a President, President-Elect, Secretary, Treasurer of the Society, and three Councilors-at-Large

B The President-Elect, Secretary, Treasurer and one Councilor-at-Large shall be elected at each annual meeting The Secretary and Treasurer may succeed themselves The President-Elect shall assume the duties of President at the adjournment of the next annual meeting following his election No person who has served as President is eligible for re-election as President-Elect

C The three Councilors-at-Large shall serve for a period of three years, and shall not be eligible for immediate re-election

D There shall be a Membership Committee, consisting of three members No two members shall be from the same institution The election of the Membership Committee shall be held annually at the time when the election of officers occurs At the first meeting of the Society under this constitution, one member shall be elected to serve on the Committee for three years, one for two years, and one for one year, and subsequently one member shall be elected each year to serve for a period of three years

E There shall be a Nominating Committee of five members No two members shall be from the same institution Members of the Nominating Committee shall serve for one year They are eligible for re election, but shall not hold membership in the Committee for more than two consecutive years

F No person may hold dual membership on the Council, Membership or Nominating Committees

G Members of the Nominating Committee are not eligible to nomination for other office

SECTION 2 *Nomination of Officials and Committeemen*

A The Nominating Committee shall make at least one nomination for each office and for the position on the Membership Committee to be filled by vote of the members The nominations so made

shall be transmitted to the Secretary and by him in turn to the members, at least one month before the annual meeting

B Nominations for membership on the Nominating Committee shall be made by individual members at the time of the annual election The five nominees who receive the highest numbers of votes shall be declared elected The Nominating Committee shall select its own Chairman who shall serve as Secretary to the Committee

SECTION 3 *Election of Officials and Committeemen*

A At the opening of the first executive session of the annual meeting the Secretary shall give to each member present a printed ballot showing the nominations of the Nominating Committee After accepting additional nominations from individual members present a complete list of nominees shall be posted A preliminary vote shall then be taken and the tellers, appointed by the President to conduct the election, shall post immediately a final list showing the two nominees for each office receiving the highest number of votes At the close of the first session, a final vote shall be taken A majority of votes cast shall be necessary to a choice

B Such vacancies as may occur in the offices and in the various committees in the interval between annual meetings shall be filled by a majority vote of the Council

ARTICLE V *Meetings*

SECTION 1 The annual meeting of the Society shall be held at a time and place determined by the Council in consultation with the Executive Committee of the Federation of American Societies for Experimental Biology

SECTION 2 Special meetings may be held at such times and places as the Council may determine

SECTION 3 At least four weeks before the annual meeting the Secretary shall send to each member a notice of the time and place of such meeting and shall make such announcements as the Council may direct

ARTICLE VI *Financial*

SECTION 1 The annual assessment shall be determined by majority vote at the annual Spring meeting upon the recommendation of the Council, and shall be due at the close of each such meeting

SECTION 2 Beyond the ordinary expenditures required by the routine business of the Society no money shall be disbursed save by the authority of the Council or Society

SECTION 3 The treasurer shall make an annual report to the Society

SECTION 4 All publication funds shall be kept in a separate account subject to the control of the Board of Publications Trustees except that

none of these funds may be diverted from the support of publications of the Society except by consent of the Council or the Society. A financial report shall be made by the Board at each annual meeting.

ARTICLE VII *Quorum*

Ten members shall constitute a quorum for the transaction of business.

ARTICLE VIII *By-Laws*

By-Laws shall be adopted, altered or repealed at any meeting by two-thirds vote of the ballots cast.

ARTICLE IX *Amendments*

SECTION 1 Intended amendments to the Constitution shall be sent to the Secretary at least one month before the date of the meeting at which they are to be considered, and must be indorsed in writing by at least three members.

SECTION 2 The Secretary shall give all members due notice of proposed amendments.

SECTION 3 A four-fifths vote of the members present shall be required for the adoption of an amendment.

ARTICLE X *Official Publications*

SECTION 1 The President of the Society shall appoint, in consultation with the Council, and subject to the approval of the Society, three members of the Society to serve as members of a Board of Publications Trustees, these members shall elect a Managing Editor for each of the official journals of the Society, and each Managing Editor, during the term of his service, shall act as an additional voting member of the Board of Publications Trustees and shall participate in all the activities of the Board except that of election of managing editors of the journals of the Society.

SECTION 2 The members of the Board of Publications Trustees, hereinafter termed "The Board," shall each serve for a term of three years, shall be subject to reappointment, and may hold office concurrently in the Society. At the first appointment, however, one member shall be appointed for three years, one member for two years, and one member for one year, in order that in the future, appointments may be made annually in rotation. The Board shall meet at least once annually (a quorum shall consist of three members) and shall report directly to the Society.

SECTION 3 The special functions of The Board shall be to consider and to investigate thoroughly all matters pertaining to the fiscal and editorial policies of the journals which may come to the

Society or to its Council, to the Managing Editors, and to the members of The Board. The Board shall (1) administer the finances of the journals, (2) establish the publication policies of the journals, and (3) elect a Managing Editor for each of the journals of the Society (as described in Sec 1).

SECTION 4 The Managing Editor of each journal shall nominate to The Board, members of the Society acceptable to him as Associate Editors. In the event that the Society should develop an International Journal, foreign Associate Editors who are not members of the Society are eligible for nomination and appointment to the Editorial Board of said Journal. From those nominated, The Board shall elect, for each journal, Associate Editors in such number as they shall consider adequate to fulfill the duties of that Editorial Board. The Managing Editor and each Associate Editor shall serve for three years, subject to reappointment, and may hold office concurrently in the Society. In the choice of The Boards of Editors, The Board is charged with the responsibility of obtaining editors with expert knowledge in the several fields of pharmacological research and with evidenced ability for critical and grammatical expression.

SECTION 5 The Boards of Editors of the journals shall meet on call of the respective Managing Editor, if possible just prior to or during the regular meetings of the Society, and may make recommendations to The Board concerning the improvement of the publication policies of the journals.

BY-LAWS

1 Papers to be read shall be submitted by the members of the Society to the Secretary, who, with the President, shall be empowered to arrange the program. No person may orally present more than one paper. In case of joint authorship, the name of the individual who will orally present the paper shall stand first. Papers not read shall appear on the program as read by title.

2 An abstract of a paper to be read before the Society shall be sent to the Secretary with the title. As early as possible after each meeting, the Secretary shall edit and publish the *Proceedings* of the Society together with abstracts in a publication authorized by the Society.

3 All applications for membership shall be accompanied by a copy of as many reprints as possible of the published work of the applicant.

4 Any member who has been an active member for thirty years, or who has retired for disability or age, may upon notification to the Treasurer be relieved from payment of dues.

THE AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY, INC.

Founded December 29, 1913

OFFICERS, 1950-1951

President—JAMES F RINEHART, University of California Medical School, San Francisco

Vice-President—F S ROBSCHT-ROBBINS, University of Rochester School of Medicine and Dentistry, Rochester, N Y

Secretary-Treasurer—SIDNEY C MADDEN, Brookhaven National Laboratory, Upton, Long Island, N Y

Councilors—D MURRAY ANGEVINE (1949-51), RUSSELL L HOLMAN (1950-52), JAMES F RINEHART, F S ROBSCHT-ROBBINS, SIDNEY C MADDEN

Representative on the Division of Medical Sciences of the National Research Council—JOHN G KIDD (1949-52)

Representatives on the Council of the American Association for the Advancement of Science—MALCOLM H SOULE (1949-52), E B KRUMBHAAR (1949-52)

Representatives on the Eli Lilly Award Committee (jointly with the Society of American Bacteriologists)—*For nominations* PAUL R CANNON (1951) *For award* PEYTON ROUS (1951)

Representative on the Committee for the Placement Service—DOUGLAS H SPRUNT

PAST OFFICERS

1914 R M PEARCE, President, JOHN F ANDERSON, Vice-President, G H WHIPPLE, Secretary-Treasurer, HARVEY CUSHING, DAVID MARINE, Councilors 1915 THEOBALD SMITH, President, G H WHIPPLE, Vice-President, PEYTON ROUS, Secretary-Treasurer, DAVID MARINE, R M PEARCE, Councilors 1916 SIMON FLEXNER, President, LEO LOEB, Vice-President, PEYTON ROUS, Secretary-Treasurer, DAVID MARINE, R M PEARCE, Councilors 1917 LUDVIG HEKTOEN, President, LEO LOEB, Vice-President, HOWARD T KARSNER, Secretary-Treasurer, PAUL A LEWIS, L G ROWNTREE, Councilors 1918 H GIDEON WELLS, President, W G MACCALLUM, Vice-President, HOWARD T KARSNER, Secretary-Treasurer, L G ROWNTREE, LUDVIG HEKTOEN, Councilors 1919 W G MACCALLUM, President, WILLIAM H PARK, Vice-President, HOWARD T KARSNER, Secretary-Treasurer, LUDVIG HEKTOEN, E L OPIE, Councilors 1920 WILLIAM H PARK, President, F G NOVY, Vice-President, HOWARD T KARSNER, Secretary-Treasurer, E L OPIE, WADE H BROWN, Councilors 1921 F G NOVY, President, HOWARD T KARSNER, Vice-President, WADE H BROWN, Secretary-Treasurer, PAUL A LEWIS

A R DOCHEZ, Councilors 1922 HOWARD T KARSNER, President, EUGENE L OPIE, Vice-President, WADE H BROWN, Secretary-Treasurer, A R DOCHEZ, GEORGE H WHIPPLE, Councilors 1923 EUGENE L OPIE, President, ALDRED S WARTHIN, Vice-President, WADE H BROWN, Secretary-Treasurer, GEORGE H WHIPPLE, H GIDEON WELLS, Councilors 1924 ALDRED S WARTHIN, President, GEORGE H WHIPPLE, Vice-President, EDWARD B KRUMBHAAR, Secretary-Treasurer, H GIDEON WELLS, FREDERICK L GATES, Councilors 1925 GEORGE H WHIPPLE, President, WADE H BROWN, Vice-President, EDWARD B KRUMBHAAR, Secretary-Treasurer, FREDERICK L GATES, DAVID MARINE, Councilors 1926 WADE H BROWN, President, DAVID MARINE, Vice-President, EDWARD B KRUMBHAAR, Secretary-Treasurer, FREDERICK L GATES, WILLIAM F PETERSEN, Councilors 1927 DAVID MARINE, President, EDWARD B KRUMBHAAR, Vice-President, CARL V WELLER, Secretary-Treasurer, WILLIAM F PETERSEN, FREDERICK L GATES, Councilors 1928 EDWARD B KRUMBHAAR, President, WILLIAM F PETERSEN, Vice-President, CARL V WELLER, Secretary-Treasurer, FREDERICK L GATES, SAMUEL R HAYTHORN, Councilors 1929 WILLIAM F PETERSEN, President, FREDERICK L GATES, Vice-President, CARL V WELLER, Secretary-Treasurer, SAMUEL R HAYTHORN, PEYTON ROUS, Councilors 1930 FREDERICK L GATES, President, SAMUEL R HAYTHORN, Vice-President, C PHILLIP MILLER, Secretary-Treasurer, PEYTON ROUS, CARL V WELLER, Councilors 1931 SAMUEL R HAYTHORN, President, PEYTON ROUS, Vice-President, C PHILLIP MILLER, Secretary-Treasurer, CARL V WELLER, S BURT WOLBACH, Councilors 1932 PEYTON ROUS, President, CARL V WELLER, Vice-President, C PHILLIP MILLER, Secretary-Treasurer, S BURT WOLBACH, OSKAR KLOTZ, Councilors 1933 CARL V WELLER, President, S BURT WOLBACH, Vice-President, C PHILLIP MILLER, Secretary-Treasurer, OSKAR KLOTZ, ALPHONSE R DOCHEZ, Councilors 1934 S BURT WOLBACH, President, OSKAR KLOTZ, Vice-President, SHIELDS WARRFAN, Secretary-Treasurer, C PHILLIP MILLER, ALPHONSE R DOCHEZ, Councilors 1935 OSKAR KLOTZ, President, ALPHONSE R DOCHEZ, Vice-President, SHIELDS WARREN, Secretary-Treasurer, MORTON McCUTCHEON, C PHILLIP MILLER, Councilors 1936 ALPHONSE R DOCHEZ, President, C PHILLIP MILLER, Vice President, SHIELDS WARRFAN, Secretary-Treasurer, MORTON McCUTCHEON, ERNEST W GOODPASTURE, Councilors 1937 C PHILLIP MILLER, President, MORTON McCUTCHEON, Vice-

President, PAUL R CANNON, Secretary-Treasurer, ERNEST W GOODPASTURE, SHIELDS WARREN, Councilors 1938 MORTON McCUTCHEON, President, ERNEST W GOODPASTURE, Vice-President, PAUL R CANNON, Secretary-Treasurer, SHIELDS WARREN, JESSE L BOLLMAN, Councilors 1939 ERNEST W GOODPASTURE, President, SHIELDS WARREN, Vice-President, PAUL R CANNON, Secretary-Treasurer, JESSE L BOLLMAN, BALDUIN LUCKÉ, Councilors 1940 SHIELDS WARREN, President, JESSE L BOLLMAN, Vice-President, H P SMITH, Secretary-Treasurer, BALDUIN LUCKÉ, PAUL R CANNON, Councilors 1941 JESSE L BOLLMAN, President, BALDUIN LUCKÉ, Vice-President, H P SMITH, Secretary-Treasurer, PAUL R CANNON, DOUGLAS H SPRUNT, Councilors 1942, 1943, 1944, 1945 BALDUIN LUCKÉ, President, PAUL R CANNON, Vice-President, H P SMITH, Secretary-Treasurer, DOUGLAS H SPRUNT, FRIEDA S ROBSCHUIT-ROBBINS, Councilors 1946 PAUL R CANNON, President, DOUGLAS H SPRUNT, Vice-President, FRIEDA S ROBSCHUIT-ROBBINS, Secretary-Treasurer, H P SMITH, JOHN G KIDD, Councilors 1947 DOUGLAS H SPRUNT, President, H P SMITH, Vice-President, FRIEDA S ROBSCHUIT-ROBBINS, Secretary-Treasurer, JAMES F RINEHART, JOHN G KIDD, Councilors 1948 H P SMITH, President, JOHN G KIDD Vice President, FRIEDA S ROBSCHUIT-ROBBINS Secretary-Treasurer, JAMES F RINEHART, SIDNEY C MADDEN, Councilors 1949 JOHN G KIDD, President JAMES F RINEHART, Vice-President, SIDNEY C MADDEN, Secretary-Treasurer, F S ROBSCHUIT ROBBINS, D MURRAY ANGEVINE, Councilors

CONSTITUTION

ARTICLE I *Name*

The Society shall be named "THE AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY"

ARTICLE II *Object*

The object of this Society is to bring the productive investigators in pathology, working essentially by experimental methods, in closer affiliation with the workers in the other fields of experimental medicine

ARTICLE III *Time and Place of Meeting*

The Society shall meet at the same time and place as the Federation of American Societies for Experimental Biology, which comprises at present the American Physiological Society, the American Society of Biological Chemists, the American Society for Pharmacology and Experimental Therapeutics, the American Society for Experimental Pathology, the American Institute of Nutrition and the American Association of Immunologists

ARTICLE IV *Membership*

SECTION 1 Any American investigator who, through the use of experimental methods, has, within three years prior to his candidacy, contributed meritorious work in pathology, is eligible to membership

SECTION 2 It shall be the policy of the Society to restrict its membership to as small numbers as is compatible with the maintenance of an active existence

SECTION 3 There shall be two classes of members active and honorary members

Active members Candidates for active membership shall be nominated at or before an annual meeting by two members of the Society. The nominators shall present to the Secretary in writing evidence of the candidate's qualifications for membership. Nominations approved by the Council shall be presented to the Society for election at the next annual meeting following nomination. For election a favorable ballot by a majority of the members present is necessary.

Honorary members These may be elected from the active list or from the group of distinguished investigators at home or abroad who have contributed to the knowledge of pathology by experimental study. They shall be elected only by the unanimous vote of the members present at time of nomination.

SECTION 4 Active members shall pay such annual dues as are determined upon, from year to year, by the Council. Honorary members shall pay no dues, are not eligible to office, and have no vote in the business affairs of the Society, but they shall have all the privileges of the active members in the scientific proceedings.

SECTION 5 Upon failure of an active member to pay dues for two years, notice shall be given to the member by the Secretary. At the end of the third year, if dues are still unpaid, such failure constitutes forfeiture of membership.

SECTION 6 A motion for expulsion of a member must be thoroughly investigated by the Council, at this investigation the accused shall be afforded a hearing or may be represented by a member. Expulsion can be accomplished only after a unanimous vote by the Council in favor of expulsion, sustained by a four-fifths vote of the members present at the meeting.

ARTICLE V *Officers*

The management of the Society shall be vested in a Council of five members, consisting of a President, a Vice-President, a Secretary-Treasurer, and two other members who shall be nominated by the Council and elected by the Society. Officers are elected by a majority vote and remain in office until July 1 following the Federation

Meeting Vacancies shall be filled by the Council for the unexpired term

The President and Vice-President shall hold office for one year and are ineligible for re-election during the following year The Secretary-Treasurer is eligible for re-election Councilors shall hold office for two years and are elected on alternate years At the first election one Councilor shall be elected for a short term of one year

ARTICLE VI *Quorum*

SECTION 1 Three constitute a quorum of the Council The Council decides by a majority vote

SECTION 2 A quorum of the Society for transaction of business shall be one-fourth of the total membership In all questions brought before the Society a majority vote of those present shall decide, except as elsewhere provided for

ARTICLE VII *Annual Meeting*

SECTION 1 Papers shall be limited to ten minutes However, on motion and with unanimous consent, the time may be prolonged by a period not exceeding five minutes The Council may make provision for longer papers on suitable occasions

SECTION 2 The subjects of papers must be confined to experimental work in pathology In doubtful cases a liberal interpretation by the President and Secretary may prevail The Council may invite, however, presentations dealing with any subject which it considers of considerable interest to the Society

ARTICLE VIII *Change of Constitution*

A motion concerning a change of the Constitution must be presented to the Council in writing by three members, and must be communicated to the members by the Secretary at least four weeks before the annual meeting At this meeting such a change may be established when accepted by a four-fifths vote of the members present

BY-LAWS

1 There must be in each year at least one meeting of the Council, which shall take place not later than the evening before the annual meeting

2 At the end of the first session of the annual meeting the Secretary shall read the report of the Council This report shall include (1) names of persons recommended for membership, (2) nominations for offices, (3) matters of general interest The Secretary shall exhibit in a conspicuous place the names of candidates for membership recommended by the Council, together with the evidence of the qualifications of the candidates

3 The election of officers and of new members, changes in the Constitution, etc., shall be voted upon at the end of the first session

4 Changes in the By-Laws may be determined by a majority vote of those present

5 In the year that a new Secretary-Treasurer is elected the incoming Council Member elected that year, or another member of the Council, shall become Assistant Secretary-Treasurer for the duration of the term of the Secretary-Treasurer

AMERICAN INSTITUTE OF NUTRITION, INC
Founded April 11, 1933, Incorporated November 16, 1934
Member of Federation 1940

OFFICERS, 1950-1951

President—W H GRIFFITH, University of Texas Medical School, Galveston

Vice-President—C M McCAY, Cornell University, Ithaca, N Y

Secretary—J H ROE, George Washington University School of Medicine, Washington, D C

Treasurer—N B GUERRANT, Pennsylvania State College, State College, Pa

Council—E N TODHUNTER, A H SMITH, W C RUSSELL, W H GRIFFITH, C M McCAY, J H ROE, N B GUERRANT

Nominating Committee—W J DARBY, *Chairman*, O A BESSEY, A G HOGAN, L A MAYNARD, A H SMITH

Committee on Registry of Nutritional Pathology—R E JOHNSON, *Chairman*, O A BESSEY, P L PHILLIPS, W H SEBRELL

Representatives on Committee on Biochemical Nomenclature (joint with American Society of Biological Chemists)—H J ALMQUIST, C A ELVEHJEM

PAST-OFFICERS

1933 L B MENDEL, President, H C SHERMAN, Vice-President, J R MURLIN, Secretary-Treasurer, E F DuBois, M S ROSE, Councilors 1934 J R MURLIN, President, E F DuBois, Vice-President, ICIE G MACY, Secretary, W M BOOTHBY, Treasurer, A H SMITH, AGNES FAY MORGAN, R M BETHKE, Councilors 1935 J R MURLIN, President, E F DuBois, Vice-President, ICIE G MACY, Secretary, G R COWGILL, Treasurer, A H SMITH, R M BETHKE, L A MAYNARD, Councilors 1936 E F DuBois, President, MARY SWARTZ ROSE, Vice-President, G R COWGILL, Treasurer, ICIE G MACY, Secretary, R M BETHKE, L A MAYNARD, C A ELVEHJEM, Councilors 1937 MARY S ROSE, President, E V McCOLLUM, Vice-President, G R COWGILL, Treasurer, ICIE G MACY, Secretary, L A MAYNARD, C A ELVEHJEM, P E HOWE, Councilors 1938 E V McCOLLUM, President, T M CARPENTER, Vice-President, G R COWGILL, Treasurer, L A MAYNARD, Secretary, C A ELVEHJEM, P E HOWE, HELEN S MITCHELL, Councilors 1939 H C SHERMAN, President, T M CARPENTER, Vice-President, G R COWGILL, Treasurer, L A MAYNARD, Secretary, P E HOWE, HELEN S MITCHELL, A H SMITH, Councilors 1940 THORNE M CARPENTER, President, A G HOGAN, Vice-President, L A MAYNARD, Secretary, W H SEBRELL, JR, Treasurer, HELEN S MITCHELL,

ARTHUR H SMITH, LYDIA J ROBERTS, Councilors 1941 A G HOGAN, President, L A MAYNARD, Vice-President, ARTHUR H SMITH, Secretary, W H SEBRELL, JR, Treasurer, T H JUKES, LYDIA J ROBERTS, H B LEWIS, Councilors 1942 L A MAYNARD, President, H B LEWIS, Vice-President, ARTHUR H SMITH, Secretary, W H SEBRELL, JR, Treasurer, LYDIA J ROBERTS, GENEVIEVE STEARNS, T H JUKES, Councilors 1943 H B LEWIS, President, ICIE G MACY-HOOBLER, Vice-President, ARTHUR H SMITH, Secretary, LYDIA J ROBERTS, GENEVIEVE STEARNS, T H JUKES, Councilors 1944 ICIE G MACY-HOOBLER, President, Wm C ROSE, Vice-President, ARTHUR H SMITH, Secretary, E M NELSON, Treasurer, GENEVIEVE STEARNS, T H JUKES and C A ELVEHJEM, Councilors 1945 Wm C ROSE, President, ARTHUR H SMITH, Vice-President, H E CARTER, Secretary, E M NELSON, Treasurer, T H JUKES, C A ELVEHJEM, D W WOOLLEY, Councilors 1946 ARTHUR H SMITH, President, R M BETHKE, Vice-President, H E CARTER, Secretary, E M NELSON, Treasurer, C A ELVEHJEM, D W WOOLLEY, H J ALMQUIST, Councilors 1947 R M BETHKE, President, E M NELSON, Vice-President, H E CARTER, Secretary, N R ELLIS, Treasurer, D W WOOLLEY, H J ALMQUIST, A D HOLMES, Councilors 1948 E M NELSON, President, C G KING, Vice-President, J H ROE, Secretary, N R ELLIS, Treasurer, H J ALMQUIST, A D HOLMES, E N TODHUNTER, Councilors 1949 C G KING, President, W H GRIFFITH, Vice-President, J H ROE, Secretary, N R ELLIS, Treasurer, A D HOLMES, E N TODHUNTER, A H SMITH

CONSTITUTION

1 The name of the proposed society is the "AMERICAN INSTITUTE OF NUTRITION"

2 The purposes of the society are to further the extension of the knowledge of nutrition and to facilitate personal contact between investigators in nutrition and closely related fields of interest

3 The management of the American Institute of Nutrition shall be vested in a council consisting of the President, Vice-President, Secretary, Treasurer and three additional members

BY-LAWS

ARTICLE I Membership

SECTION 1 *Eligibility for membership* Members Qualified investigators who have independently

conducted and published meritorious original investigations in some phase of the chemistry or physiology of nutrition and who have shown a professional interest in nutrition for at least 5 years shall be eligible for membership in the Society

SECTION 2 *Nomination* Nominations for membership shall be made and seconded by members of the Society on blanks furnished by the Secretary. Nominations shall be submitted to the Council who shall determine eligibility and make recommendation to the Society at a regular meeting

SECTION 3 *Election to membership* A A nominee for membership may be voted for by ballot at any meeting of the Society after the Council has reported its findings on his eligibility B A majority of the ballots cast shall elect

SECTION 4 *Forfeiture* If a majority of the Council after due notice to the member in question and opportunity for a hearing, shall decide that the interests of the Society require the expulsion of a member, the Secretary shall send a notice of this decision to each member at least two weeks before the next annual meeting At this meeting the Secretary shall, on behalf of the Council, propose the expulsion, and if two-thirds of the members present vote for it, the member shall be expelled, his assessment for the current year shall be returned to him, and he shall cease to be a member of the Society

ARTICLE II *Meetings and Quorum*

SECTION 1 *Annual* The annual meeting of the Society shall be held on the date fixed by the Certificate of Incorporation

SECTION 2 *Special* A special meeting may be called at any time by the President, or in case of his absence or disability, by the Vice-President, and must be called at the request in writing of a majority of the Council or fifty members of the Society Notice specifying the purpose of such meeting shall be mailed to each member at least ten days previous thereto The Council shall select the places at which meetings shall be held

SECTION 3 *Quorum* Thirty members shall constitute a quorum at all meetings of the Society, but in the absence of a quorum any number shall be sufficient to adjourn to a fixed date

ARTICLE III *Officials*

SECTION 1 *Officers* The officers shall be a President, and a Vice President who shall be elected annually, and a Secretary and Treasurer, each of whom shall be elected to serve for a term of three years These officers shall be elected by the members of the Society Their terms of office shall commence on July 1 of the year in which they are elected

SECTION 2 *Council* The officers so selected and three additional members, one of whom shall be elected at each annual meeting to serve a term of three years, shall constitute a Board of Trustees and shall be known as "The Council" (When this provision is first put into effect one member shall be elected for 1 year, one for 2 years and the third for 3 years)

SECTION 3 *Duties of Officers* The powers and duties of the officers elected by the Society shall be such as usually devolve upon their respective positions

ARTICLE IV *The Council*

SECTION 1 *Powers* The general management of the Society during the intervals between meetings shall be vested in the Council, which shall regularly perform the ordinary duties of an executive committee and possess all the powers conferred upon the Board of Trustees of an educational institution chartered by the Education Department of the University of the State of New York A permanent charter was issued to the American Institute of Nutrition under date of November 16, 1934

SECTION 2 *Reports* The Council shall report to the Society its findings on the eligibility of candidates for membership, and on all charges of a violation of these By-Laws

ARTICLE V *Nominating Committee*

SECTION 1 *Membership* A The Nominating Committee shall consist of five members appointed for the coming year by the retiring President Members who have served on the Nominating Committee for two consecutive years shall be ineligible for reappointment until after a lapse of one year B The President shall designate one member to be Chairman of the Nominating Committee

SECTION 2 *Nomination of Officials* A The Nominating Committee shall make at least one nomination for each of the four offices, for each of the additional positions on the Council to be filled by vote of the members and for each of the positions on the Editorial Board to be vacated at the time of the annual meeting Any member of the Institute may submit nominations to the Nominating Committee for its consideration along with those nominations made by the members of the Nominating Committee B The nominations by the Nominating Committee shall be transmitted to the Secretary at least six weeks before the annual meeting at which they are to be considered C The Secretary shall send to every member, at least two weeks before the annual meeting a printed ballot containing the list of nominees and space for such additional names as the mem-

ber wishes to propose, and at the same time shall notify the members that they may vote by mail, returning to the Secretary the marked ballot in the envelope provided, at such a time and place as the Secretary may designate, or the ballot may be delivered to the Secretary at the beginning of the business session at which the elections are to take place

SECTION 3 *Election of officials* A At the beginning of the business session the Secretary shall present to the tellers, appointed by the President, the ballots submitted by the members and the ballots shall be counted forthwith B A majority of votes cast shall be necessary to elect an official

SECTION 4 *Filling of Vacancies* A The Nominating Committee shall fill all vacancies in elective positions except such as may occur at a meeting of the Society B The President of the Society shall fill all vacancies in appointive positions

ARTICLE VI *Financial*

SECTION 1 *Dues* The dues shall be the annual cost of subscription to *The Journal of Nutrition* for members plus an annual assessment which shall be determined by majority vote at the annual meetings, upon recommendation of the Council, and shall be due within a month after the annual meeting A member on attaining the age of 65 may elect to be relieved from all financial obligations to the Institute including subscription to *The Journal of Nutrition*

SECTION 2 *Expenditures* No expenditures from the general funds of the Society except those required in the performance of the ordinary official duties shall be made except by vote of the Society or the Council

SECTION 3 *Penalty for non-payment of dues* A Members in arrears for dues for two consecutive years shall forfeit their membership B Delinquent members may be reinstated by the Council provided all indebtedness to the Society is liquidated

ARTICLE VII *The Journal of Nutrition*

SECTION 1 The American Institute of Nutrition designates *The Journal of Nutrition* as its official organ of publication

SECTION 2 In accordance with the expressed wish of the Wistar Institute of Anatomy and

Biology, owner and publisher of *The Journal of Nutrition*, the American Institute of Nutrition shall nominate members of the Editorial Board for its official organ A The editorial management of *The Journal of Nutrition* shall be vested in an Editorial Board consisting of an Editor and twelve Board Members B The Editor shall be chosen by the Editorial Board to serve a term of five years beginning July 1 of the year in which he is chosen, and shall be eligible for reelection The Editor shall have the power to designate one of the Board Members to serve as his assistant, and such an appointee shall be called Associate Editor C Three members of the Institute shall be nominated by the Nominating Committee for membership on the Editorial Board each year to serve a term of four years, replacing three retiring members and taking office May 1 of the year in which they are elected In the event of a vacancy in the membership of the Editorial Board occurring through death or other reason, the Nominating Committee, for each such vacancy to be filled shall make an additional nomination In this event the nominees elected who receive the greatest number of votes shall serve the longest term of vacancies to be filled D Retiring members of the Editorial Board shall not be eligible for renomination until one year after their retirement

ARTICLE VIII *Papers on Scientific Subjects*

SECTION 1 The Secretary shall be authorized to arrange programs for the scientific sessions at the annual meetings

ARTICLE IX *Changes in Constitution and By-Laws*

SECTION 1 Proposed changes in the Constitution and By-Laws must be sent in writing to the Secretary at least one month before the date of the meeting at which they are to be considered, and must be signed by at least three members The Secretary shall send a printed copy of any proposed change to each member at least two weeks before the next meeting and shall notify all members that they may vote by proxy

SECTION 2 If at this meeting two-thirds of the votes cast shall favor the proposed change, it shall be made

THE AMERICAN ASSOCIATION OF IMMUNOLOGISTS

Founded June 19, 1913, Member of Federation 1942

OFFICERS, 1950-1951

President—GEOFFREY EDSALL, Boston University School of Medicine, Boston, Mass

Honorary President—ARTHUR F COCA, Oradell, N J

Vice-President—COLIN M MACLEOD, New York University College of Medicine, New York City

Secretary-Treasurer—JULES FREUND, Public Health Research Institute, New York City

Council—THOMAS FRANCIS, JR, JOHN F ENDERS, THOMAS P MAGILL, MICHAEL HEIDELBERGER, GEOFFREY EDSALL, COLIN M MACLEOD, JULES FREUND

PAST OFFICERS

Presidents—1913 GERALD B WEBB 1915 JAMES W JOBLING 1916 RICHARD WEIL 1917 JOHN A KOLMER 1918 WILLIAM H PARK 1919 HANS ZINSSER 1920 RUFUS I COLE 1921 FREDERICK P GAY 1922 GEORGE W MCCOY 1923 H GIDEON WELLS 1924 FREDERICK G NOVY 1925 WILFRED H MANWARING 1926 LUDVIG HEKTOEN 1927 KARL LANDSTEINER 1928 EUGENE L OPIE 1929 OSWALD T AVERY 1930 STANHOPE BAYNE-JONES 1931 ALPHONSE R DOCHEZ 1932 AUGUSTUS B WADSWORTH 1933 THOMAS M RIVERS 1934 FRANCIS G BLAKE 1935 WARFIELD T LONGCOPE 1936 SANFORD B HOOKER 1937 CARL TENBROECK 1938 DONALD T FRASER 1939 GEORGE P BERRY 1940 PAUL R CANNON 1941 KARL F MEYER 1942-1945 JACQUES J BRONFENBRENNER 1945-1947 MICHAEL HEIDELBERGER 1947 LLOYD D FEITON 1948 MICHAEL HEIDELBERGER 1949 THOMAS FRANCIS, JR

Vice-Presidents—1913-1915 GEORGE W ROSS 1915 GEORGE P SANBORN 1916 JOHN A KOLMER 1947 MICHAEL HEIDELBERGER 1948 LLOYD D FEITON 1949 MICHAEL HEIDELBERGER

Secretary—1913-1918 MARTIN J SYNOTT

Treasurer—1913-1918 WILLARD J STONE

Secretary Treasurer—1918-1947 ARTHUR F COCA 1948-1949 JULES FREUND

CONSTITUTION

(As revised, 1949)

ARTICLE I Name

This association shall be called THE AMERICAN ASSOCIATION OF IMMUNOLOGISTS

ARTICLE II Object

The purpose of the Association shall be to advance knowledge of immunology and related disci-

plines, and to facilitate interchange of ideas and information among investigators in the various fields

ARTICLE III Members

SECTION 1 The Association shall consist of active members, members emeriti and honorary members

SECTION 2 Any qualified person engaged in the study of problems related to the purpose of the Association may apply for active membership. Candidates for active membership shall be nominated by two members of the Association on blanks furnished by the Secretary. Applications must be accompanied by letters of recommendation of the sponsors, a curriculum vitae, and a list of reprints of publications. The Council shall determine eligibility and post a list of candidates at the annual meeting. The membership shall elect new members by majority vote.

SECTION 3 Failure to pay dues for three successive years shall annul membership. The Council may reinstate a member if an acceptable explanation is submitted and all indebtedness to the Association is liquidated. Payment of such indebtedness may be waived by unanimous vote of the Council if circumstances justify such action.

SECTION 4 If a two-thirds majority of the Council decides that the best interests of the Association require the expulsion of a member, the Secretary shall notify the affected member in writing of the charges. The Council shall allow a reasonable time for the presentation of his defense before acting. Upon recommendation of a two-thirds majority of the Council, the Secretary shall send a notice of the decision to each active member at least six weeks before the next annual meeting. At this meeting the Secretary shall, on behalf of the Council, propose the expulsion, and on a two-thirds vote of the members present, the member shall be expelled, his assessment for the current year shall be returned, and he shall cease to be a member of the Association.

SECTION 5 An active member of the Association for twenty years upon retirement because of age or disability may elect to accept the status of member emeritus. As such he shall retain voting privileges but shall be relieved of all financial obligations to the Association and shall not receive the Journal without payment.

SECTION 6 The Council may nominate for honorary membership persons of extraordinary achievement in the field of endeavor of this Association. Election to honorary membership shall

follow the same procedure as that for election for office in the Association, and the Secretary, on order of the Council, shall place nominations for honorary membership on the annual ballot

ARTICLE IV *Officers*

SECTION 1 The Association shall be governed by a Council, which shall consist of the Officers of the Association and five Councilors, one of whom shall be the Editor-in-Chief of the Journal of Immunology

SECTION 2 The Officers of the Association shall be a President, a Vice President, a Secretary, and a Treasurer. The duties of the two latter may be performed by one or two individuals as determined by vote of the Association (See By-Law # 7)

SECTION 3 The President, the Vice President, the Secretary, and the Treasurer shall be elected at the regular annual meeting of the Association to serve for one year. Only the Secretary and the Treasurer are eligible to serve for successive terms in their respective offices. The term of office shall begin the day after the end of the annual meeting

SECTION 4 One Councilor shall be elected each year to serve for a term of four years. He may however serve in any other elective office immediately after expiration of his term as Councilor

SECTION 5 The President shall appoint a Nominating Committee of three (or more) members not currently serving on the Council of the Association and shall designate the Chairman. The Committee shall submit nominations for the offices of President, Vice President, Secretary, Treasurer, and Councilor. They may nominate the member currently serving as Vice President for the office of President, and the Councilor who is serving his fourth year for the office of Vice President. They shall submit the names of three members of the Association as candidates for the office of Councilor for a four-year term. The Nominating Committee shall confer at least three months before the annual meeting and shall transmit its nominations, over the signatures of the majority of the Committee, to the Secretary at least two months before the annual meeting. Other names may be added to the ballot upon petition of at least twenty-five members of the Association to the Secretary at least two months before the annual meeting. The Secretary shall send to each member of the Association, at least six weeks before the annual meeting, a ballot containing the list of all such nominees

SECTION 6 The members of the Association shall vote on the nominations by secret ballot by mail. All ballots must be in the hands of the Secretary one week before the annual meeting. At the time of the annual meeting the Secretary shall present all the valid sealed ballots received by him to the

tellers appointed by the President. A plurality of votes shall be sufficient for election

SECTION 7 The Vice President shall substitute for the President when necessary. If both the President and Vice President are unable to serve, the senior Councilor shall temporarily assume the duties of President. Should a vacancy occur in the office of Secretary or Treasurer, or Councilor, the Council by a majority vote shall appoint a member to fill the unexpired term until the following election. In the event of a vacancy in the Council, the members shall in the following election choose two Councilors from among six nominees whose names shall be selected by the Nominating Committee and included on the ballot as above. More over, in this event the terms of the four Councilors shall be adjusted so as to provide an orderly progression within the general sense of the Constitution

ARTICLE V *Meetings*

SECTION 1 A regular meeting of the Association shall be held annually at such time and place as the Council shall determine. Regular meetings shall be open to all members of the Association. The members present at the annual meeting of the Association shall constitute a quorum

SECTION 2 A meeting of the Council shall be held prior to the annual business meeting of the Association. A quorum of the Council for this meeting shall be five

SECTION 3 In case of equal division of votes, the President shall cast the decisive ballot

SECTION 4 Minutes of the annual meeting of the Council and of the annual business meeting of the Association shall be published in the form of a news-letter and be distributed only to members of the Association. The annual report of the Treasurer shall be included in this news-letter

ARTICLE VI *Business*

SECTION 1 The fiscal year of the Association shall begin March first

SECTION 2 Annual dues, upon recommendation of the Council, shall be determined by a majority vote at the annual meeting of the Association

ARTICLE VII *Publication*

SECTION 1 The Journal of Immunology, which is the property and official organ of this Association, shall be administered for the Association by an Editorial Board

SECTION 2 An Editor-in-Chief shall be elected from the membership by a majority vote of the Council to serve for four years. His term of office shall be subject to renewal. He shall appoint with the consent of the Council an Editorial Board of four to six members to serve for four years. The Editor-in-Chief and the members of the Edi-

torial Board shall be responsible for the editorial conduct of the Journal

SECTION 3 The Editor-in-Chief shall, with the consent of the Council, appoint from the active members Associate Editors as needed. These Associate Editors are to be chosen because of their ability in specialized fields.

SECTION 4 The Editor-in-Chief shall make a report of his stewardship of the Journal at the annual meeting and in this report shall summarize the editorial situation and all matters dealing with finances. He shall submit to the Council statements relating to the number of manuscripts received, rejected, accepted, and published during the year, changes in editorial personnel of editors, a complete summary of circulation and of finances and of any other information which the Editorial Board may feel to be pertinent or which may be required by the Council.

ARTICLE VIII *Amendments*

SECTION 1 Proposed changes in the Constitution shall be submitted by at least five members in writing through the Secretary to the President. The President shall then appoint a committee of at least three members which shall communicate its recommendations to the President for consideration by the Council. The Council shall then advise the Secretary to submit the recommendations of the committee to the membership of the Association for approval with the annual ballot. A change in the Constitution shall require a two-thirds majority of the votes cast by the members at the annual meeting and shall require confirmation by a majority in a mail vote in which at least 50% shall have participated.

SECTION 2 As an alternative procedure a proposed change in the Constitution may be submitted directly to the Council by petition of one-tenth of the total active membership. Under this circumstance the Council is required to arrange for a vote by the members as heretofore described.

SECTION 3 Proposed changes in By-Laws may be adopted by two-thirds vote of members present at the annual meeting.

BY-LAWS

1 The Past Presidents shall have the right of attending, without vote, the meetings of the Council.

2 The President may appoint a Past President or a Past Councilor as pro tempore Councilor at any stated meeting of the Council at which a quorum is not present.

3 The Council may transact and vote by mail on such business as cannot be conveniently transacted at meetings.

4 The Secretary shall arrange the program for the scientific meetings, with the advice of the other officers of the Association. Any member in good standing shall have the right to submit a paper. The privilege of presentation may be extended to non-members upon recommendation of active members. Papers intended for presentation at the meetings shall conform to the standards of the Journal of Immunology. In case of doubt, the Secretary shall have the right to submit papers to the scrutiny of three or more members of the Editorial Board of the Journal of Immunology whose decision shall be final.

5 Each member who signifies his intention of reading a paper at any session may specify the length of time which its presentation shall require. In the absence of any specification of time required not more than ten minutes shall be allotted for the reading of any one paper. The time allotted shall be printed on the official program, and the presiding officer shall have no authority to extend it unless a majority of the members present signify their wish to the contrary.

6 If by force of circumstances it should be impossible to hold the annual meeting, the election of Officers and Council may be carried out entirely by mail.

7 The offices of Secretary and Treasurer are combined and shall be held by one person.

8 Official or invited addresses presented at the annual meeting shall be given immediate priority in publication, unless the Editor-in-Chief shall be otherwise instructed by majority vote of the Council.

9 Dues are payable on or before January 1st and subscriptions to the Journal will be lapsed if not paid by this date.

10 If the Editor-in-Chief is unable to attend the stated meeting of the Council, he may designate an alternate from the membership of the Editorial Board.

ALPHABETICAL LIST OF MEMBERS OF THE SIX SOCIETIES

- (1) The American Physiological Society
- (2) American Society of Biological Chemists
- (3) The American Society for Pharmacology and Experimental Therapeutics
- (4) The American Society for Experimental Pathology
- (5) The American Institute of Nutrition
- (6) The American Association of Immunologists

*Number and year in parentheses following each name indicate Society affiliation and year of election
R signifies retired status*

HONORARY MEMBERS

- Adrian, E D Cambridge University, Dept of Physiology, Cambridge, England (1, 1946)
- Bremer, Frederick, M D 115 Boulevard de Waterloo, Brussels, Belgium *Prof of Pathology, Univ of Brussels, Dir of Lab of General Pathology, Pres of College des Medecins de l'Agglomeration de Bruxelloise* (1, 1950)
- Chopra, R N, M D, Sc D School of Tropical Medicine, Calcutta, India *Director, Professor of Pharmacology* (3, 1938)
- Coca, Arthur F, A M, M D Pearl River, N Y (6, 1916)
- Dale, H H, O M, G B E, M D, F R C P, F R S The Wellcome Trust, 28 Portman Sq, Malet St, London, W 1, England (3, 1926)
- Hektoen, Ludvig, M D 629 S Wood St, Chicago, Ill *President, Chicago Tumor Institute* (6, 1919)
- Hess, Walter Rudolf, M D Univ of Zurich, Physiological Inst, Zurich, Switzerland *Dir, Swiss Physiological Inst* (1, 1950)
- Hill, Archibald Vivian, C H, O B E, Sc D, L L D, F R S 16 Bishopswood Rd, London N 6, England *Foulerton Research Prof of Royal Society, Univ College, London* (1, 1950)
- Houssay, Bernardo A M D Viamonte 2790, Buenos Aires, Argentina *Director and Professor of Physiology* (1, 1942)
- Lapicque, L The Sorbonne, Laboratory of Physiology, Paris, France (1, 1946)
- Liljestrand, Gornan, M D Caroline Inst, Stockholm, Sweden *Prof of Pharmacology* (1, 1950)
- Loewi, Otto, M D 155 E 93rd St, New York City 28 *Research Professor in Pharmacology N Y U College of Med* (3, 1941)
- McCoy, George Walter, M D Louisiana State University Medical School, New Orleans *Director, Department of Public Health* (6, 1916)
- Novy, Frederick G, M D, Sc D 721 Forest Ave, Ann Arbor, Mich *Dean Emeritus and Professor Emeritus of Bacteriology, University of Michigan Medical School* (6, 1920)

Orbeli, L A Academy of Sciences of the USSR, Moscow, USSR (1, 1946)

Sherrington, Sir Charles S, Sc D, M D "Broomside," Valley Road, Ipswich, England, *Former Waynesfete Professor of Physiology, Oxford University Former President of the Royal Society* (1, 1904)

MEMBERS

- Abbot, Lynn D F, Jr Ph D Med College of Virginia, Richmond 19 *Assoc Prof of Biochemistry* (2, 1948)
- Abramson, David I, M D Univ of Illinois, Dept of Medicine, Chicago 12 *Asst Clin Prof Attending Physician, Hines Veterans Hospital* (1, 1937)
- Abramson, Harold A, M D Mt Sinai Hospital, New York City *Chief, Allergy Clinic, Asst Prof of Physiology, Columbia Univ College of Physicians and Surgeons* (1, 1930, 2, 1934)
- Abreu, Benedict E, Ph D, M D Pitman-Moore Co, Indianapolis, Ind *Pharmacologist, Research Dept* (3, 1941)
- Acheson, George H, M D Univ of Cincinnati College of Medicine, Cincinnati, Ohio *Prof of Pharmacology* (1, 1942, 3, 1945)
- Adams, Georgian, M A, D Sc U S Dept of Agriculture, Washington 25, D C *Sr Exper Station Admin* (5, 1946)
- Adams, John M, M D Univ of California, 405 Hilgard Ave, Los Angeles 24 *Prof of Pediatrics* (4, 1947)
- Adams, Mildred, Ph D Agricultural Research Center, Bureau of Human Nutrition and Home Economics, Beltsville, Md *Biochemist, Food and Nutrition* (2, 1934)
- Adams, R Charles, C M, M D Mayo Clinic, Rochester Minn *Assoc Prof of Anesthesiology Mayo Foundation, Consultant Mayo Clinic Section on Anesthesia* (3, 1942)
- Adams, W Lloyd, M D, Ph D 1300 First Natl Bank Bldg, Lexington 6 Ky *Practicing Ophthalmologist* (3, 1942)
- Adams, Wright R, M D Univ of Chicago Dept

- of Medicine, Chicago 37, Ill *Assoc Prof of Medicine* (1, 1946)
- Addison, William H**, M D Univ of Pennsylvania, Philadelphia *Prof Emeritus of Histology and Embryology* (1R, 1928)
- Ades, Harlow Whiting**, Ph D Box 734, Emory Univ, Ga (1, 1945)
- Adler, Harry F**, Ph D, M D School of Aviation Medicine, Randolph AFB, Tex *Dir Med Sciences Div* (1, 1943)
- Adolph, Edward Frederick**, Ph D, Univ of Rochester School of Medicine and Dentistry, Rochester, N Y *Prof of Physiology* (1, 1921)
- Adolph, William H**, Ph D 25 Maple St, New Haven, Conn *Prof of Biochemistry, Peiping Union Med College, Peiping, China* (2, 1946, 5, 1934)
- Ahlquist, Raymond P**, Ph D Med College of Georgia, Augusta *Prof and Chairman of Dept of Pharmacology* (3, 1945)
- Albanese, Anthony A**, Ph D St Luke's Convalescent Hospital, Nutrition Research Lab, King St, Greenwich, Conn *Chief of Nutritional Research* (2, 1944)
- Albaum, Harry G**, Ph D Brooklyn College, Bedford Ave and Ave H, Brooklyn, N Y *Asst Prof of Biology* (2, 1947)
- Albert, A**, Ph D, M D Mayo Foundation, Rochester, Minn *Research Assoc* (1, 1947)
- Albritton, Errett C**, M D George Washington Univ Med School, 1339 H St, N W, Washington, D C *Prof and Head of Dept of Physiology* (1, 1933)
- Alden, Roland H**, Ph D Univ of Tennessee, College of Medicine, Memphis 3 *Prof and Head, Dept of Microscopic Anatomy* (1, 1949)
- Alexander, Robert S**, Ph D Western Reserve Univ School of Medicine, 2109 Adelbert Rd, Cleveland, Ohio *Instr in Physiology* (1, 1946)
- Algire, Glenn H**, M D Natl Cancer Inst, Bethesda, Md *Sr Surgeon, USPHS* (4, 1945)
- Allan, Frank N**, M D Lahey Clinic, 605 Commonwealth Ave, Boston, Mass *Exec Dir of the Med Dept* (4, 1930)
- Allen, Charles Robert**, Ph D, M D Univ of Texas School of Medicine, Galveston *Assoc Prof of Anesthesiology* (1, 1943)
- Allen, Frank W**, Ph D Univ of California, 1557 Life Science Building, Berkeley *Assoc Prof* (2, 1947)
- Allen, Frederick M**, M D 1031 Fifth Ave, New York City *Prof of Medicine, Polyclinic Med School and Hospital* (1R, 1924, 4, prior to 1920)
- Allen, J Garrott**, M D Univ of Chicago, Univ Clinics, Chicago, Ill *Instr in Surgery* (1, 1943)
- Allen, Lane**, Ph D, M D Med College of Georgia, Univ Place, Augusta *Assoc Prof of Anatomy* (1, 1939)
- Allen, Shannon C**, Ph D U S A F School of Aviation Medicine, Randolph AFB, Tex *Chief, Applied Human Biology Branch* (1, 1945)
- Allen, Thomas H**, Ph D Columbia Univ College of Physicians and Surgeons, Dept of Physiology, 630 W 168th St, New York City 32 *Asst Prof of Physiology* (1, 1947)
- Allen, Willard E**, M D Washington Univ School of Medicine, 630 S Kingshighway, St Louis, Mo *Prof of Obstetrics and Gynecology* (1, 1934)
- Allen, William F**, Ph D Univ of Oregon Med School, Portland *Prof Emeritus of Anatomy* (1R, 1929)
- Alles, Gordon A**, Ph D 770 S Arroyo Parkway, Pasadena, Calif *Lecturer in Pharmacology, Univ of California Med School, and Research Assoc in Biology, California Inst of Technology* (1, 1932, 3, 1941)
- Ailing, Eric L**, M D Univ of Rochester School of Medicine and Dentistry, Rochester 7, N Y *Assoc in Radiology* (4, 1947)
- Allison, James B**, Ph D Rutgers Univ, New Brunswick, N J *Prof of Physiology and Biochemistry, Dir of Bureau of Biological Research* (2, 1946, 5, 1949)
- Almquist, Herman J**, Ph D The Grange Co, Modesto, Calif *Dir of Research and Vice Pres* (2, 1937, 5, 1937)
- Altland, Paul D**, Ph D Natl Insts of Health, Bethesda, Md *Physiologist* (1, 1950)
- Altschul, Aaron M**, Ph D Southern Regional Research Lab, USDA, 2100 R E Lee Blvd, New Orleans, 19, La *Head, Protein and Carbohydrate Div* (2, 1949)
- Alvarez, Walter C**, M D Mayo Clinic, Rochester, Minn *Prof of Medicine, Mayo Foundation* (1R, 1917, 3R, 1921)
- Alving, Alf Sven**, M D Billings Hospital, Univ of Chicago, 950 E 59th St, Chicago, Ill *Assoc Prof of Medicine* (1, 1939)
- Amberg, Samuel**, M D Mayo Clinic, Rochester, Minn *Assoc Prof Emeritus of Pediatrics, Mayo Foundation* (1R, 1903, 2, 1906, 3R, 1909)
- Amberson, William R**, Ph D Univ of Maryland School of Medicine, Baltimore *Prof of Physiology* (1, 1924)
- Ambrose, Anthony M**, Ph D Western Regional Research Lab, 800 Buchanan St, Albany, Calif *Pharmacologist, U S Dept of Agriculture, Pharmacology Research Lab* (3, 1937)
- Ames, Stanley R**, Ph D Distillation Products, Inc, Div of Eastman Kodak Co, Rochester 3, N Y *Sr Research Chemist* (2, 1948)
- Amoss, Harold L**, M D, D P H, Sc D 68 Deerfield Drive, Greenwich, Conn (4, 1922, 6, 1917)
- Andersch, Marie A**, Ph D Univ Hospital, Baltimore, Md *Biochemist, Instr in Medicine, Univ of Maryland* (2, 1940)

- Andersen, Dorothy H , M D Babies Hospital, Broadway and 167th St , New York City *Asst Prof of Pathology, Columbia Univ* (4, 1935)
- Anderson, Carl E , Ph D Univ of North Carolina, School of Medicine, Dept of Biological Chemistry and Nutrition, Chapel Hill *Assoc Prof of Biochemistry* (2, 1950)
- Anderson, Evelyn, M D Ph D Natl Insts of Health, Bethesda 14, Md (1, 1934)
- Anderson, Hamilton H , M S , M D Pharmacology Lab , Univ of California Med School, San Francisco *Prof and Chairman of Div , until June 1951, Office of the Dean, American University of Beirut, Lebanon* (3, 1931)
- Anderson, Oscar Daniel, Ph D Cornell Univ , Dept of Psychology, Ithaca, N Y (1, 1939)
- Anderson, Rubert S , Ph D Univ of South Dakota School of Medicine, Vermillion *Prof of Physiology* (1, 1948)
- Anderson, Rudolph J , Ph D Sterling Lab , Yale Univ , New Haven, Conn *Prof of Chemistry* (2, 1915)
- Anderson, W A D , M A , M D Marquette Univ School of Medicine, Milwaukee, Wis *Prof of Pathology* (4, 1941)
- Anderson, William E , M A Eastern State Farmers' Exchange, Westbrook Farm, Rockville, Conn *Biochemist* (2, 1931, 5, 1933)
- Andervont, H B , Sc D Natl Cancer Inst , Bethesda, Md *Biologist, USPHS* (4, 1939)
- Andrews, James C , Ph D Univ of North Carolina, Chapel Hill *Prof of Biological Chemistry and Nutrition* (2, 1925)
- Andrus, E Cowles, M D 24 E Eager St , Baltimore 2, Md *Asst Visiting Physician, Assoc Prof of Medicine, Johns Hopkins Univ* (1, 1925)
- Anfinsen, Christian B , Jr , Ph D Natl Heart Inst , Bethesda 14, Md *Chief, Section of Cellular Physiology* (2, 1946)
- Angerer, Clifford, Ph D Ohio State Univ , Columbus *Assoc Prof of Physiology* (1, 1943)
- Angervine, D Murray, M D Univ of Wisconsin Med School, Madison *Prof of Pathology* (4, 1940)
- Anker, H S , M D , Ph D Univ of Chicago, Dept of Biochemistry, 947 E 58th St , Chicago 37, Ill *Research Assoc and Asst Prof* (2, 1949)
- Annegers, John H , Ph D , M D Northwestern Univ Med School, Dept of Physiology, 313 E Chicago Ave , Chicago 11, Ill *Asst Prof of Physiology* (1, 1949)
- Ansbacher, Stefan, D Sc P O Box 731, Marion, Ind (2, 1939)
- Anslow, W Parker, Jr , Ph D New York Univ College of Medicine, 477 First Ave , New York City 16 *Asst Prof of Physiology* (2, 1948)
- Anson, Mortimer L , Ph D Thomas J Lipton, Inc , 1500 Hudson St Hoboken, N J *Dir of Chemical Research* (2, 1937)
- Appel, Frank L , D Sc , M D Med College of Virginia, Richmond *Prof of Pathology* (4, 1936)
- Archibald, Reginald M , Ph D , M D Rockefeller Inst for Med Research, 66th St and York Ave , New York City 21 *Member, Physician to Hospital* (2, 1947)
- Arkin, Aaron, M D , Ph D Suite 2006, 25 E Washington St , Chicago, Ill *Rush Prof of Medicine, Univ of Illinois, Prof and Chairman of Dept of Medicine, Cook County Grad School* (1, 1914, 3, 1919)
- Armstrong, Harry G , M D Headquarters US Air Force Washington, 25, D C *Surgeon General* (1, 1948)
- Armstrong, Philip B , M D Syracuse Univ College of Medicine, Syracuse 10, N Y *Prof of Anatomy* (1, 1945)
- Armstrong, W D , M D , Ph D Univ of Minnesota, 17 Med Sciences Bldg , Minneapolis *Prof and Head of Physiological Chemistry* (2, 1938)
- Arnold, Aaron, Ph D Sterling-Winthrop Research Inst , Rensselaer, N Y *Head of Nutritional Research Lab* (5, 1947)
- Arnold, Lloyd, A M , M D 1538 E 57th St , Chicago, Ill (4, 1930, 6, 1925)
- Arnow, L Earle, Ph D , M D Sharp & Dohme, Med Research Div , Glenolden, Pa *Dir of Research* (2, 1940)
- Aronson, Joseph D , M D Univ of Pennsylvania, Phipps Inst , Philadelphia 4 *Prof of Bacteriology* (4, 1927, 6, 1925)
- Artom, Camillo, M D Wake Forest College, Bowman Gray School of Medicine, Winston-Salem, N C *Prof of Biochemistry* (2, 1944)
- Ascham, Leah, Ph D Kansas State College, Manhattan *Prof in School of Home Economics* (5, 1935)
- Asenjo, Conrado F , Ph D School of Medicine, Univ of Puerto Rico, San Juan 22 *Assoc Prof of Biochemistry and Nutrition* (2, 1944)
- Ashburn, Llewellyn L , M D U S Marine Hospital, Baltimore 11, Md *Sr Surgeon, U S Public Health Service* (4, 1947)
- Ashby, Winifred M , Ph D 305 10th St , N E Washington, D C *Sr Scientist, Federal Security Agency* (6, 1923)
- Ashman, Richard, Ph D Louisiana State Univ School of Medicine, New Orleans *Prof of Physiology* (1, 1925)
- Astwood, Edwin Bennet, M D , Ph D Pratt Diagnostic Hospital, 30 Bennet St , Boston, Mass *Research Prof of Medicine, Tufts Med School* (1, 1939)
- Atkin, Lawrence, Ph D The Fleischmann Labs , 510 Grand Concourse, New York City 51 *Asst to Dir of Research* (2, 1946, 5, 1946)
- Aub, Joseph C , M D Massachusetts General Hospital Fruit St , Boston 14 *Prof of Re-*

- search *Medicine, Harvard Med School* (1, 1919, 5, 1933)
- Austin, J Harold**, M D College of Physicians of Philadelphia, 19 S 22nd St, Philadelphia 3, Pa *Prof Emeritus, Research Medicine, Univ of Pennsylvania* (2, 1922)
- Avery, O T**, M D, Sc D Hoods Hill Rd, Nashville, Tenn *Member Emeritus, Rockefeller Inst for Med Research* (4, 1921, 6R, 1920)
- Aviado, Domingo M, Jr**, M D Univ of Pennsylvania Med School, Dept of Pharmacology, Philadelphia *Associate* (3, 1950)
- Axelrod, A E**, Ph D Western Pennsylvania Hospital, Pittsburgh, Pa *Research Biochemist, Research Prof, Dept of Chemistry, Univ of Pittsburgh* (2, 1950, 5, 1949)
- Axelrod, Bernard**, Ph D Western Regional Research Lab, 800 Buchanan St, Albany, Calif *Assoc Chemist, Enzyme Research Div* (2, 1948)
- Axtmayer, Joseph H**, Ph D Univ of Puerto Rico, Rio Piedras *Prof of Chemistry* (5, 1935)
- Bach, L M N**, Ph D Tulane Univ School of Medicine, New Orleans, La *Prof of Physiology* (1, 1948)
- Bachem, Albert**, Ph D Univ of Illinois College of Medicine, Chicago 12 *Prof of Biophysics* (1, 1933)
- Bachman, Carl**, M D Univ of Pennsylvania School of Medicine, 3400 Spruce St Philadelphia 4 *Prof and Dir of Obstetrics and Gynecology* (2, 1941)
- Bachmann, George**, M D Emory Univ School of Medicine, Emory University, Ga *Prof Emeritus of Physiology* (1R, 1912)
- Bachrach, William H**, M D, Ph D Univ of Southern California School of Medicine, Dept of Physiology, Los Angeles 7 *Research Assoc* (1, 1949)
- Baer, Erich**, Ph D Banting Inst, Banting and Best Dept of Med Research, 100 College St, Toronto, Ont, Canada *Assoc Prof* (2, 1942)
- Baernstein, Harry D**, Ph D Natl Insts of Health, Bethesda, Md *Sr Biochemist* (2, 1934)
- Baetjer, Anna M**, D Sc Johns Hopkins School of Hygiene and Public Health, 615 N Wolfe St, Baltimore 5, Md *Asst Prof of Environmental Medicine* (1, 1929)
- Baez, Silvio**, M D 525 E 68th St, New York City 32 *Research Assoc* (1, 1950)
- Bahrs, Alice M**, Ph D 2735 Orchard St, Corvallis, Ore (1, 1933)
- Bailey, Cameron Vernon**, C M, M D New York Univ and Hospital 303 E 20th St, New York City *Chn Prof of Medicine* (2, 1920, 5R, 1933)
- Bailey, Orville T**, M D Harvard Univ Med School, 25 Shattuck St, Boston 15, Mass *Asst Prof of Pathology* (4, 1939)
- Bailey, Percival**, M D, Ph D Univ of Illinois College of Medicine, 1853 W Polk St, Chicago 12, Ill *Prof of Neurology and Neurosurgery* (1, 1941)
- Baitsell, George Alfred**, Ph D Yale Univ, Osborn Zoological Lab, 165 Prospect St, New Haven, Conn *Prof of Biology* (1, 1915)
- Baker, A B**, M D Univ of Minnesota Hospital, 19 Millard Hall, Minneapolis *Dir and Prof of Neurology and Neuropathology* (4, 1940)
- Baker, Edgar E**, Ph D Boston Univ School of Medicine, 80 E Concord St, Boston 18, Mass *Assoc Prof of Microbiology* (6, 1950)
- Baker, James A**, D V M, Ph D Cornell Univ New York State Veterinary College, Ithaca *Prof of Bacteriology* (4, 1947)
- Baker, Roger D**, M D Med College of Alabama, Birmingham 5 *Prof of Pathology* (4, 1939)
- Baldes, Edward J**, Ph D 427 Fifth Ave, S W, Rochester, Minn *Asst Prof of Physics, Mayo Foundation, Univ of Minnesota Grad School* (1, 1930)
- Baldwin, Francis Marsh**, Ph D Univ of Southern California, Los Angeles *Prof of Zoology and Dir of Exper Marine Biology* (1, 1919)
- Bale, William F**, Ph D Univ of Rochester School of Medicine and Dentistry, Rochester, N Y *Prof of Radiation Biology* (1, 1943)
- Ball, Eric G**, Ph D Harvard Med School, 25 Shattuck St, Boston 15, Mass *Prof of Biological Chemistry* (2, 1934)
- Ball, Howard A**, M D 233 A St, San Diego, Calif *Pathologist, Paradise Valley and Palomar Memorial Hospitals* (4, 1947)
- Balls, Arnold Kent**, Ph D Enzyme Research Lab, U S Bureau of Agricultural and Industrial Chemistry, Western Regional Research Lab, 800 Buchanan St, Albany 6, Calif *Head Chemist* (2, 1932)
- Bang, Frederik B**, M D Johns Hopkins Hospital, Baltimore, Md *Assoc Prof in Medicine* (4, 1947)
- Banus, Mario Garcia**, D Sc Bright Meadows, Chestertown, Md (1, 1927)
- Bard, Philip**, Ph D, Johns Hopkins Univ School of Medicine, 710 N Washington St, Baltimore, Md *Prof and Dir of Dept of Physiology* (1, 1929)
- Barger, A Clifford**, M D Harvard Med School, Dept of Physiology, 25 Shattuck St, Boston 15, Mass *Associate* (1, 1950)
- Barker, H A**, Ph D Univ of California, 3048 Life Sciences Bldg, Berkeley 4 *Prof of Plant Biochemistry* (2, 1946)
- Barker, S B**, Ph D State Univ of Iowa College of Medicine, Iowa City *Assoc Prof of Physiology* (1, 1938)
- Barlow, O W**, M D, Ph D 3 Warwick Rd, Winchester, N H (3, 1944)
- Barnes, B O**, Ph D Box 967, Station Hospital,

- KAAF, Kingman, Ariz *Prof of Health Education, Univ of Denver* (1, 1932)
- Barnes, LaVerne A, Ph D Naval Med Research Inst, Natl Naval Med Center, Bethesda 14, Md *Head, Bacteriology Facility* (6, 1931)
- Barnes, Richard Henry, Ph D Sharp & Dohme, Glenolden, Pa *Asst Dir of Research* (2, 1941, 5, 1944)
- Barnes, Thomas C, D Sc Hahnemann Med College and Hosp of Philadelphia, Philadelphia, Pa *Assoc Prof of Pharmacology, Electroencephalographer of Hahnemann Hospital* (1, 1942, 3, 1948)
- Barnum, Cyrus P, Jr, Ph D Univ of Minnesota, 210 Millard Hall, Minneapolis 14 *Assoc Prof of Physiological Chemistry* (2, 1946)
- Barott, Herbert G, E E U S Dept of Agriculture, Natl Agricultural Research Center, Beltsville, Md *Biophysicist, Animal Nutrition Div, Bur of Animal Industry* (5R, 1938)
- Barron, Donald H, Ph D Yale Univ School of Medicine, New Haven, Conn *Assoc Prof of Physiology* (1, 1943)
- Barron, E S Guzman, M D Univ of Chicago, Dept of Medicine, Chicago 37, Ill *Assoc Prof of Biochemistry* (2, 1931)
- Bartlett, Paul D, Ph D Edsel B Ford Inst for Med Research, Henry Ford Hospital, Detroit 2, Mich *Sr Assoc in Biochemistry* (2, 1950)
- Bartley, S Howard, Ph D P O Box 763, East Lansing, Mich (1, 1935)
- Bass, Allan D, M D Univ of Syracuse School of Medicine, Syracuse, N Y *Prof of Pharmacology* (3, 1944)
- Batchelder, Esther L, Ph D 8 Devon Rd, Silver Spring, Md *Head of Food and Nutrition Div, Bureau of Human Nutrition and Home Economics* (5, 1933)
- Bateman, John B, Ph D Physical and Chemical Div, Camp Detrick, Frederick, Md (1, 1945)
- Bates, Robert W, Ph D E R Squibb and Sons, Biological Labs, New Brunswick, N J *Head of Endocrine Development Dept* (2, 1936)
- Batson, Herbert C, Ph D Army Med Center, AMDR & GS, Washington, D C *Scientific Dir, Dept of Biological Products* (6, 1949)
- Batterman, Robert C, M D New York Univ - Bellevue Med Center, 477 First Ave, New York City *Asst Prof of Medicine* (3, 1941)
- Bauer, J H, M D Rockefeller Foundation, 20 Rue de la Biome, Paris, (8^e) France (4, 1935)
- Bauer, Walter, M D Massachusetts General Hospital, Boston *Assoc Prof and Tutor in Medicine, Harvard Med School* (1, 1929)
- Bauernfeind, J C, Ph D Hoffmann-LaRoche, Inc, Nutley 10, N J *Chief of Applied Nutrition* (5, 1947)
- Bauman, Louis, M D Columbia Presbyterian Med Center, 180 Fort Washington Ave New York City 32 *Asst Prof of Clin Medicine (retired), Columbia Univ* (2, 1912)
- Baumann, Carl A, Ph D Univ of Wisconsin, Biochemistry Dept, Madison *Prof of Biochemistry* (2, 1938, 5, 1938)
- Baumann, Emil J, Ph D 7 Church Lane, Scarsdale, N Y *Chemist, Montefiore Hospital* (2, 1922)
- Baumberger, J Percy, Sc D Stanford Univ, Physiology Dept, Stanford Univ, Calif *Prof of Physiology* (1, 1921)
- Baxter, James H, M D Natl Insts of Health, Natl Heart Inst, Bethesda, Md (3, 1948)
- Bayne-Jones, Stanhope, M A, M D New York Hospital, Cornell Med Center, 525 E 68th St, New York City 21 *Pres, Joint Admin Board* (4, 1927, 6, 1917)
- Beach, Eliot F, Ph D Metropolitan Life Insurance Co, 1 Madison Ave, New York City 10 *Research Biochemist* (2, 1941, 5, 1942)
- Beadle, Buell W, Ph D 606-A, Essex Circle, U S N O T S, China Lake, Calif (2, 1947)
- Bean, John W, Ph D, M D Univ of Michigan, Ann Arbor *Prof of Physiology* (1, 1932)
- Beard, Howard H, Ph D Terrell's Labs of Clin Medicine, Med Arts Bldg Rm 24, Fort Worth, Tex *Biochemist* (2, 1928)
- Beard, Joseph W, M D Duke Hospital, Durham, N C *Prof of Surgery, Assoc Prof of Virology* (4, 1938, 6, 1940)
- Beatty, Clarissa H, Ph D Univ of Oregon Med School, Portland *Research Fellow* (1, 1949)
- Beazell, James Myler, Ph D, M D 104 S Michigan Ave, Chicago, Ill *Instr in Physiology and Pharmacology, Northwestern Univ School of Medicine* (1, 1939)
- Beck, Claude S, M D Lakeside Hospital, Cleveland, Ohio *Prof of Neurosurgery, Western Reserve Univ, Assoc Surgeon, Lakeside Hospital* (4, 1930)
- Beck, Lyle V, Ph D 5609 Roosevelt St, Bethesda, Md (1, 1941)
- Becker, R Frederick, M S, Ph D Daniel Baugh Inst of Anatomy, 307 S 11th St, Philadelphia, Pa (1, 1949)
- Beckman, Harry, M D Marquette Univ School of Medicine, Milwaukee, Wis *Dir of Dept of Pharmacology* (3, 1937)
- Beecher, Henry K, M D Massachusetts General Hospital, Boston *Anesthetist-in-Chief, Dorr Prof of Research in Anesthesia, Harvard Med School* (3, 1940)
- Beerstecher, Ernest, Jr, Ph D Univ of Texas, Biochemical Inst, Austin *Research Assoc* (2, 1950)
- Behnke, Albert R, M S, M D Com Nav For Ger, Force Med Officer APO 403, c/o Postmaster N Y Capt, MC USA (1 1946)
- Behre, Jeanette Allen, Ph D Columbia Univ

- College of Physicians and Surgeons, Dept of Biochemistry, 630 W 168th St, New York City 32 Associate (2, 1925)
- Behrens, Otto K**, Ph D Lilly Research Labs, Eli Lilly and Co, Indianapolis 6, Ind *Head, Immuno-Chemical Research* (2, 1949)
- Behrmann, Vivian G**, Ph D Wayne Univ, Detroit, Mich *Instr, Grad School, Res Physiologist Henry Ford Hospital* (1, 1948)
- Belding, David L**, M D Boston Univ School of Medicine, Boston, Mass *Prof Emeritus of Bacteriology and Exper Pathology* (4, 1927)
- Belding, Harwood S**, Ph D QMC Climatic Research Lab, Lawrence, Mass *Director* (1, 1945)
- Belkin, Morris**, Ph D Natl Cancer Inst, Bethesda 14, Md *Sr Pharmacologist* (3, 1949)
- Bell, E T**, M D 110 Anatomy Bldg, Univ of Minnesota, Minneapolis *Prof Emeritus of Pathology* (4, 1931)
- Bender, M B**, M D New York Univ College of Medicine, New York City *Assoc Prof of Neurology and Head of Lab of Exper Medicine* (1, 1947)
- Bendich, Aaron**, Ph D Sloan-Kettering Inst for Cancer Research, 444 E 68th St, New York City 21 *Assoc, Protein-Chemistry* (2, 1950)
- Benditt, Earl P**, M D Univ of Chicago Clinics, Dept of Pathology, Chicago 37, Ill *Asst Prof* (4, 1947)
- Benedict, Francis G**, Ph D, M D Machiasport, Maine (1R, 1904, 2R, 1906)
- Benham, Olive Ray**, B S Connecticut State Dept of Health, Bureau of Labs, Hartford *Chief Serologist* (6, 1944)
- Bennett, A Lawrence**, Ph D, M D Univ of Nebraska College of Medicine, Omaha *Prof of Physiology and Pharmacology* (1, 1941)
- Bennett, Granville A**, M D Univ of Illinois College of Medicine, 1853 W Polk St, Chicago *Prof of Pathology* (4, 1931)
- Bennett, Henry S**, M D Univ of Washington School of Medicine, Seattle *Dept of Anatomy* (1, 1946)
- Bennett, Leslie L**, M D Univ of California, Berkeley 4 *Asst Prof of Physiology* (1, 1945)
- Bennett, Mary Adelia**, Ph D Lankenau Hospital Research Inst, 7701 Burholme St Fox Chase, Philadelphia 11, Pa *Assoc Membership, Research Biochemis* (2, 1941)
- Benson, Clara C**, Ph D 160 Dorset St, West, Port Hope, Ontario, Canada *Prof Emeritus of Food Chemistry, Univ of Toronto* (2, 1906)
- Benton, Joseph G**, Ph D, M D New York Univ College of Medicine, 477 First Ave, New York City 16 *Fellow in Medicine, Research Assoc in Physical Medicine* (3, 1949)
- Benzinger, Theodore**, M D, D Sc Naval Med Research Inst, Bethesda 14, Md (1, 1950)
- Berg, Benjamin N**, M D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 *Assoc in Pathology* (4, 1928)
- Berg, Clarence P**, Ph D State Univ of Iowa, Dept of Biochemistry, Chemistry Bldg, Iowa City *Prof of Biochemistry* (2, 1933, 5, 1936)
- Berg, William N**, Ph D 225 W 108th St, New York City *Biochemist* (2, 1906)
- Bergeim, Olaf**, Ph D Univ of Illinois College of Medicine, 1853 W Polk St, Chicago 12 *Prof of Biochemistry* (1, 1916, 2, 1914)
- Berger, F M**, M D 7B-2 Redfield Village, Metuchen, N J *Dir, Research and Development* (3, 1949)
- Bergmann, Werner**, Ph D Yale Univ, Sterling Chemistry Lab, New Haven, Conn *Prof of Chemistry* (2, 1934)
- Berkson, Joseph**, M D, D Sc Mayo Clinic, Rochester, Minn (1, 1933)
- Berliner, Robert W**, M D 229 E 79th St, New York City 21 *Asst Prof of Medicine, Columbia Univ* (1, 1950)
- Bernard, Richard**, Ph D Laval Univ, Dept of Biology, Blvd de l'Entente, Quebec, Canada *Asst Prof of Physiology* (1, 1947)
- Bernheim, Frederick**, Ph D Box 3109, Duke Univ Med School, Durham, N C *Prof of Pharmacology* (2, 1933, 3, 1935)
- Bernthal, Theodore G**, M S, M D Med College of the State of South Carolina, Dept of Physiology, Charleston 16 *Prof of Physiology* (1, 1932)
- Berry, George Packer**, M D Harvard Med School, Boston 15, Mass *Dean and Prof of Bacteriology* (6, 1934)
- Berryman, George H**, Ph D, M D 521 Castleton Ave, Staten Island, N Y *USPHS Marine Hospital* (1, 1949)
- Bessey, Otto A**, Ph D Univ of Illinois College of Medicine, 1853 W Polk St, Chicago 12 *Prof and Head of Dept of Biological Chemistry* (2, 1938, 5, 1943)
- Best, Charles H**, M D, D Sc Univ of Toronto, Toronto, Ontario, Canada *Dir of Banting and Best Dept of Med Research and Dept of Physiology* (1, 1923, 2, 1923)
- Bethell, Frank H**, M D 409 Lenawee Drive, Ann Arbor, Mich *Prof of Internal Medicine and Asst Dir of the Thomas Henry Simpson Memorial Inst* (4, 1936)
- Bethke, Roland M**, Ph D Ohio Agricultural Experiment Station, Wooster *In Charge of Nutritional Investigations* (2, 1928, 5, 1933)
- Beutner, R**, M D, Ph D 5380 Magnolia St, Philadelphia 44, Pa *Prof of Pharmacology Hahnemann Med College* (1R, 1924, 3, 1924)
- Beyer, Karl H**, Ph D, M D Sharp & Dohme, Inc Med Research Div, Glenolden, Pa *Dir of Pharmacological Research* (1, 1942, 3, 1944)
- Bickford, Reginald G**, M D Mayo Clinic, Sec-

- tion on Physiology, Rochester, Minn *Head of Lab of Neurophysiology and Electroencephalography* (1, 1949)
- Bier, Otto, M D Instituto Biologico, Caixa Postal 119A, São Paulo, Brazil *Head, Immunological Dept* (6, 1947)
- Bierman, Howard R, M D, Univ of California School of Medicine, San Francisco 22 *Assoc Clin Prof of Exper Oncology* (3, 1950)
- Bieter, Raymond N, M D, Ph D Univ of Minnesota, Minneapolis *Prof and Head of Dept of Pharmacology* (3, 1930)
- Bills, Charles E, Ph D 3421 Stringtown Rd Evansville 11, Ind *Independent Investigator* (2, 1928, 5, 1935)
- Bing, Franklin C, Ph D 30 W Washington St, Chicago, Ill *Consultant* (2, 1931, 5, 1934)
- Bing, Richard J, M D Johns Hopkins Hospital, Dept of Surgery, Baltimore 5, Md *Assoc Prof of Medicine and Surgery* (1, 1942)
- Binkley, Francis, Ph D Univ of Utah School of Medicine, Salt Lake City *Assoc Prof of Pathology* (2, 1947)
- Binkley, Stephen Bennett, Ph D Univ of Illinois College of Medicine, 1853 W Polk St, Chicago *Assoc Prof of Biochemistry* (2, 1941)
- Bird, Herbert R, Ph D Bureau of Animal Industry, Agricultural Research Center, Beltsville, Md *Sr Biochemist* (5, 1947)
- Bird, Orson D, Ph D Parke, Davis and Co, Research Labs, Detroit 32, Mich *Research Biochemist* (2, 1947)
- Bird, Robert M, M D 214 E 70th St, New York City 21 *Instr in Medicine, Cornell Univ Med College, Physician to Out Patients, New York Hospital* (1, 1950)
- Birren, James E, Ph D 4006 Fordleigh Rd, Baltimore 15, Md *Psychologist, Nail Heart Inst, USPHS* (1, 1950)
- Bisbey, Bertha, Ph D Univ of Missouri, Gwynn Hall, Columbia *Prof of Nutrition* (5, 1933)
- Bischoff, Fritz E, Ph D Cottage Hospital, Santa Barbara, Calif *Dir of Research* (2, 1928, 5, 1933)
- Bishop, George H, Ph D Washington Univ Med School, Euclid and Kingshighway, St Louis, Mo *Prof of Biophysics* (1, 1923)
- Biskind, Gerson R, M D 450 Sutter St, San Francisco, Calif *Pathologist, Mt Zion Hospital, Clin Instr in Pathology, Univ of California Med School* (4, 1944)
- Black, Alex, Ph D Pennsylvania State College, Dept of Animal Nutrition, State College *Prof of Animal Nutrition* (5, 1947)
- Black, Edgar C, Ph D Univ of British Columbia, Dept of Physiology, Vancouver, B C, Canada (1, 1943)
- Black, Simon, Ph D Univ of Chicago, Chicago 37, Ill *Asst Prof of Biochemistry, Dept of Medicine* (2, 1948)
- Blair, Edgar A, Ph D Med Field Service School, Fort Sam Houston, Tex *Lt Col* (1, 1936)
- Blair, Henry A, Ph D Univ of Rochester School of Medicine and Dentistry, Rochester, N Y. *Prof of Physiology and Dir of Dept of Radiation Biology* (1, 1934)
- Blake, Francis G, M D, Sc D Yale Univ School of Medicine, New Haven, Conn *Sterling Prof of Medicine* (4, prior to 1920, 6, 1921)
- Blake, William D, M.D Yale Univ School of Medicine, Lab of Physiology, New Haven, Conn *Instructor* (1, 1950)
- Blanchard, Ernest W, Ph D Schieffelin and Co, 30 Cooper Sq New York City 3 *Dir of Research* (1, 1946)
- Blankenhorn, M A, M.D Univ of Cincinnati, Cincinnati, Ohio *Prof of Medicine* (4, 1932)
- Blatherwick, Norman R, Ph D Metropolitan Life Insurance Co, 1 Madison Ave, New York City *Dir of Biochemical Lab* (1, 1915, 2, 1915, 5, 1934)
- Blau, Nathan F, Ph D Veterans Admin Hospital, 401 S Holyoke Ave, Wichita 8, Kan *Research Biochemist* (2, 1928)
- Blish, Morris J, Ph D Internatl Minerals and Chemical Corp, Box G, Rossford, Ohio *Research Dir* (2, 1944)
- Bliss, Alfred, Ph D Tufts College Med School, Boston, Mass *Assoc Prof of Physiology* (1, 1947)
- Bliss, Chester Ittner, Ph D Conn Agricultural Exper Station, P O Box 1106, New Haven *Biometrician, Lecturer in Biometry, Yale Univ* (3, 1944)
- Bliss, Eleanor A, Sc D Johns Hopkins Hospital, Dept of Preventive Medicine, 615 N Wolfe St, Baltimore, Md *Assoc in Preventive Medicine, Johns Hopkins Univ School of Medicine* (6, 1931)
- Bloch, Konrad, Ph.D Univ of Chicago, Dept of Biochemistry, Chicago 37, Ill *Professor* (2, 1944)
- Block, Richard J, Ph D 15 Cooper Rd, Scarsdale, N Y *Dir Of Research, C M Armstrong Co, Assoc Dept of Physiology and Biochemistry, New York Med College, Flower and Fifth Ave Hospital* (2, 1934, 5, 1933)
- Block, Walter D, Ph D 813 E McCreight Ave, Springfield, Ohio, (2, 1942)
- Blood, Frank R, Ph D Vanderbilt Univ, Dept of Biochemistry, Nashville 4, Tenn *Professor* (1, 1950)
- Bloom, William, M D 1419 E 56th St, Chicago, Ill *Prof of Anatomy, Univ of Chicago* (4, 1930)
- Bloomfield, A L, M D Stanford Univ Hospital, San Francisco, Calif *Prof of Medicine* (3, 1927, 4, 1927)

- Bloor, W R, Ph D Univ of Rochester School of Medicine and Dentistry, Rochester 20, N Y *Prof of Biochemistry* (1R, 1915, 2, 1910)
- Blum, Harold F, Ph D Princeton Univ, Dept of Biology, Princeton, N J *Physiologist, Natl Cancer Inst, and Visiting Lecturer* (1, 1928)
- Blumberg, Harold, Sc D Endo Products, Inc, 84-40 101st St, Richmond Hill 18, N Y *Head of Biological Labs* (5, 1942)
- Blumenstock, Julius, M D Veterans Admin Hospital, Sheridan, Wyo (1, 1925)
- Blumgart, Herrmann L, M D Beth Israel Hospital, 330 Brookline Ave, Boston, Mass *Physician in Chief, Prof of Medicine, Harvard Med School* (1, 1927)
- Blunt, Katharine, Ph D 38 Glenwood Ave, New London, Conn *Pres Emeritus, Connecticut College for Women* (2, 1921)
- Bobb, J Richard R, M D Temple Univ Med School, Dept of Pharmacology, Philadelphia, Pa *Asst Prof* (1, 1949)
- Bock, Joseph C, Ph D 2324 N 46th St, Milwaukee 10, Wis *Prof Emeritus of Biochemistry, Marquette Univ Med School, Biochemist, Milwaukee County Hospital* (2, 1916)
- Bodansky, Aaron, Ph D Hospital for Joint Diseases, 1919 Madison Ave, New York City *Biological Chemist* (2, 1926)
- Bodansky, Oscar, Ph D, M D Memorial Hospital Cancer Center, 444 E 68th St, New York City 21 *Chief, Clin Biochemistry, Assoc Prof of Clin Pharmacology, Cornell Med College* (2, 1937, 3, 1942)
- Bodian, David, Ph D, M D Johns Hopkins Univ, 1901 E Madison St, Baltimore, Md *Assoc Prof of Epidemiology* (6, 1949)
- Bodine, Joseph Hall, Ph D State Univ of Iowa, Iowa City *Prof and Head of Dept of Zoology* (1, 1925)
- Boell, Edgar J, Ph D Yale Univ, Osborn Zoological Lab, New Haven, Conn *Ross G Harrison Prof of Exper Zoology* (1, 1942)
- Boettiger, Edward G, Ph D Univ of Connecticut, Box U-42, Storrs *Asst Prof of Zoology* (1, 1950)
- Boger, William P, M D Univ of Pennsylvania Hospital, Rm 715, Pepper Lab, Philadelphia *Instr in Medicine* (3, 1948)
- Bogert, L Jean, Ph D Hotel Claremont, Berkeley, Calif (2, 1917)
- Bogert, Marston Taylor, Sc D 1158 Fifth Ave, New York City 29 *Prof Emeritus of Organic Chemistry, Columbia Univ, Scientific Consultant to Eians Research and Development Corp* (2, 1925)
- Bohr, David F, M D Univ of Michigan, Physiology Lab, Ann Arbor *Assoc Prof* (1, 1949)
- Bolliger, Adolph, Ph D Univ of Sydney, Gordon Craig Research Lab., Sydney, Australia *Dir of Research* (2, 1928)
- Bollman, J L, M D Mayo Clinic, Rochester, Minn *Chairman of Div of Exper Medicine, Prof of Physiology, Mayo Foundation* (4, 1927)
- Bond, Glenn C, Ph D, M D The Upjohn Co, Research Lab, Kalamazoo, Mich *Asst Dept Head of Bacteriology Research* (6, 1939)
- Bondi, Amedeo Hahnemann Med College, Philadelphia, Pa *Prof and Head of Dept of Bacteriology* (6, 1948)
- Bonner, David M, Ph D Yale Univ, Osborn Botanical Lab, New Haven, Conn *Assoc Prof and Research Assoc* (2, 1948)
- Bonnycastle, Desmond D, M D, Ph D Yale Univ School of Medicine, New Haven, Conn *Asst Prof of Pharmacology* (3, 1947)
- Bonsnes, Roy W, Ph D Cornell Univ Med College, 1300 York Ave, New York City 21 *Assoc Prof of Biochemistry in Obstetrics* (2, 1947)
- Booher, Lela E, Ph D General Mills, Inc, 1081 21st Ave, S E, Minneapolis, Minn *Chief Nutritionist and Dir of Nutrition Lab* (2, 1933, 5, 1933)
- Booker, Walter M, Ph D Howard Univ School of Medicine, Washington, D C *Assoc Prof of Pharmacology* (1, 1948, 3, 1948)
- Boor, Alden K, Ph D Camp Detrick, Basic Science Div, Frederick, Md (2, 1931)
- Boothby, Walter M, M A, M D Univ of Malaya, Dept of Physiology, Singapore *Visiting Prof, Chief Emeritus, Clin Metabolism Sect, Mayo Clinic* (1R, 1915, 2, 1920, 3R, 1923, 4R, 1924)
- Bordley, James, III, M D Mary Imogene Bassett Hospital, Cooperstown, N Y (1, 1938)
- Borek, Ernest, Ph D College of the City of New York, Convent Ave and 140th St, New York City *Asst Prof, Research Assoc in Biochemistry, Columbia Univ* (2, 1947)
- Borison, Herbert Leon, M S, Ph D Univ of Utah, College of Medicine, Dept of Pharmacology, Salt Lake City *Instructor* (1, 1950)
- Boroff, Daniel A, M A, M S % L J Kuhne, 502 Rugby Rd, Brooklyn, N Y (6, 1947)
- Borsook, Henry, M D, Ph D California Inst of Technology, Pasadena 4 *Prof of Biochemistry* (2, 1931)
- Bosshardt, David K, Ph D Sharp & Dohme, Med Research Div, Glenolden, Pa *Research Biochemist* (5, 1947)
- Bostick, Warren L, M D Univ of California Med School, Dept of Pathology, San Francisco 22 *Asst Prof* (4, 1949)
- Bosworth, Alfred Willson, A M, M D R F D 4, Circleville, Ohio *Consulting Chemist* (2, 1936, 5R, 1935)
- Bott, Phyllis A, Ph D Woman's Med College of Pennsylvania, Henry Ave and Abbotsford Rd,

- Philadelphia *Prof and Chairman of Dept of Physiological Chemistry* (2, 1938)
- Boucher, Robert V**, Ph D 303 Frear Labs, State College, Pa *Prof of Agricultural and Biological Chemistry* (5, 1945)
- Bouman, H D**, M D Univ of Wisconsin Med School, Madison *Prof of Physical Medicine* (1, 1943)
- Bourne, Wesley, M D**, M Sc McGill Univ, Montreal, Quebec, Canada *Prof and Chairman, Dept of Anesthesiology* (3, 1936)
- Bourque, Joseph E**, M D, Ph D Univ of Illinois College of Medicine, Dept of Physiology, 1853 W Polk St, Chicago 12 *Asst Prof* (1, 1949)
- Bourquin, Helen**, Ph D 1331 N Tejon St, Colorado Springs, Colo (1, 1925)
- Bowen, William J**, Ph D USPHS, Inst of Exper Biology & Medicine, Bethesda 14, Md *Sr Asst Scientist* (1, 1948)
- Bowman, Donald E**, Ph D 6956 Warwick Rd, Indianapolis, Ind *Assoc Prof of Biochemistry, Indiana Univ School of Medicine* (2, 1944)
- Bowman, Katherine L**, B A 20 Plaza St, Brooklyn 17, N Y *Research Fellow in Allergy, Jewish Hospital, Brooklyn* (6, 1946)
- Boxer, George E**, Ph D 318 Temple Pl, Westfield, N J *Sr Chemist, Research and Development Div, Merck & Co, Inc* (2, 1946)
- Boyd, Eldon M**, M A, M D Queen's Univ, Kingston, Ontario, Canada *Prof and Head of Dept of Pharmacology* (3, 1941)
- Boyd, Linn J**, M D New York Med College and Flower & Fifth Ave Hospitals, 1 E 105th St, New York City 29 *Prof of Pharmacology, Div of Medicine* (3, 1950)
- Boyd, M John**, Ph D Hahnemann Med College, 235 N 15th St, Philadelphia 2, Pa *Prof of Biol Chemistry* (2, 1947)
- Boyd, T E**, Ph D 9 Walworth Ave, Scarsdale, N Y *Assoc Dir of Research, Natl Found for Infantile Paralysis, 120 Broadway, New York City 5* (1, 1924)
- Boyd, William C**, Ph D U S Naval Med Research Unit #3, % American Embassy, Cairo, Egypt (6, 1933)
- Boyden, Allan A** Rutgers Univ, New Brunswick, N J *Prof of Zoology* (6, 1948)
- Boyden, Edward A**, Ph D Univ of Minnesota, Minneapolis 14 *Prof and Chairman of Dept of Anatomy* (1, 1929)
- Boyer, Paul D**, Ph D Univ of Minnesota College of Agriculture, Div of Biochemistry, St Paul 1 *Assoc Prof* (2, 1944)
- Boyle, Paul E**, D M D Univ of Pennsylvania School of Dentistry, 40th and Spruce Sts, Philadelphia 4 *Prof of Oral Pathology* (4, 1939)
- Bozicevich, John**, M A USPHS, Natl Insts of Health, Bethesda, Md *Head, Subsection of Immunology, Trop Diseases Div* (6, 1948)
- Bozler, Emil**, Ph D Ohio State Univ, Columbus *Prof of Physiology* (1, 1932)
- Bradbury, James T**, Sc D Univ of Louisville School of Medicine, 101 W Chestnut St, Louisville 2, Ky (1, 1941)
- Bradley, Harold C**, Ph D 2639 Durant Ave, Berkeley, Calif (1, 1911, 2, 1908)
- Bradley, Stanley E**, M D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 *Assoc Prof of Medicine* (1, 1947)
- Bradley, William B**, Ph D American Inst of Baking, 1046 Elmwood Ave, Wilmette, Ill *Dir of Labs* (1, 1939)
- Bragdon, Joseph Henry**, M D Natl Insts of Health, Exper Biology and Medicine Inst, Pathology Lab, Bethesda 14, Md *Surgeon (R) USPHS* (4, 1950)
- Branch, E Arnold G**, M D Lancaster Hospital, St John, New Brunswick, Canada *Dir of Labs* (4, 1929)
- Brand, Erwin**, Ph D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 *Assoc Prof of Biological Chemistry* (2, 1929)
- Brandes, W W**, M D Roosevelt Hospital, W 59th St, New York City (4, 1931)
- Branham, Sara E**, Ph D, M D, Natl Insts of Health, Bethesda, Md *Sr Bacteriologist* (6, 1926)
- Bramon, Hugh Douglas**, Ph D Ontario Agricultural College, Guelph, Canada *Prof and Head of Dept of Animal Nutrition* (5, 1933)
- Brassfield, Charles R**, Ph D Univ of Michigan, Ann Arbor *Assoc Prof of Physiology* (1, 1937)
- Bratton, Andrew Calvin, Jr**, M D, Ph D Parke Davis and Co, Research Labs, Detroit 32, Mich *Dir of Pharmacological Research* (3, 1941)
- Brauer, Ralph W**, Ph D Louisiana State Univ School of Medicine, New Orleans *Asst Prof of Pharmacology* (3, 1948)
- Braun, Herbert A**, Ph D Fed Security Agency, Food and Drug Admin, Washington, D C *Pharmacologist* (3, 1941)
- Brazier, Mary A B**, Ph D Massachusetts General Hospital, Electroencephalographic Lab, Boston 14 *Research Assoc in Neuropathology, Harvard Med School* (1, 1947)
- Brecher, George**, M D Natl Insts of Health, Pathology and Pharmacology Lab, Bethesda 14, Md (4, 1949)
- Brecher, Gerhard A**, M D, Ph D Western Reserve Univ Med School, Dept of Physiology, Cleveland 6, Ohio *Sr Instructor* (1, 1949)
- Breedis, Charles**, M D Univ of Pennsylvania School of Medicine, Philadelphia *Instr in Pathology* (4, 1948)

- Brewer, Carl R , Ph D Camp Detrick, Frederick, Md *Chief, Bacterial Nutrition Branch, Biological Div , Chemical Corps* (2, 1948)
- Brewer, John H , Ph D Hynson Westcott and Dunning, Baltimore, Md *Dir of Biological Research* (6, 1948)
- Brewer, Nathan R , Ph D Univ of Chicago, Chicago, Ill *Lecturer in Physiology* (1, 1948)
- Bridge, Edward M , M D Univ of Buffalo School of Medicine, 24 High St , Buffalo 9, N Y *Prof of Pharmacology* (2, 1940)
- Briggs, A P , M D Univ of Georgia Med College, Augusta *Prof of Biochemistry* (2, 1923)
- Briggs, David R , Ph D Univ of Minnesota, Div of Agricultural Biochemistry, Univ Farm, St Paul 8 *Prof of Agricultural Biochemistry, Chemist, Minn Agricultural Exper Station* (2, 1946)
- Briggs, George M , Ph D Univ of Minnesota, Univ Farm, St Paul *Asst Prof of Poultry Nutrition* (5, 1947)
- Brink, Frank Jr , Ph D Johns Hopkins Univ , Biological Labs , Baltimore 18, Md (1, 1942)
- Brinkhous, K M , M D Univ of North Carolina School of Medicine, Dept of Pathology, Chapel Hill *Prof of Pathology* (4, 1939)
- Britton, Sydney W , M D Univ of Virginia School of Medicine, Charlottesville *Prof of Physiology* (1, 1925)
- Brobeck, John R , M D , Ph D Yale Univ School of Medicine, New Haven, Conn *Asst Prof of Physiology* (1, 1943)
- Brodie, Bernard B , Ph D Natl Insts of Health, Natl Heart Inst , Bethesda 14, Md *Chief, Section on Chemical Pharmacology* (2, 1940, 3, 1945)
- Brody, Samuel, Ph D Univ of Missouri College of Agriculture and Agricultural Experiment Station, Dairy Building, Columbia *Prof of Dairy Husbandry* (2, 1929, 5, 1933)
- Broh-Kahn, Robert H , M D Bristol Labs , Inc , P O Box 657, Syracuse, N Y (1, 1948)
- Bromuley, Reginald B , Ph D Johns Hopkins School of Medicine, Dept of Physiology, 710 N Washington St , Baltimore 5, Md *Prof of Physiology* (1, 1949)
- Bronfenbrenner, J J , Ph D , D P H Washington Univ School of Medicine, St Louis, Mo *Prof of Bacteriology and Immunology* (4, 1940, 6, 1918)
- Bronk, Detlev W , Ph D , Sc D Johns Hopkins Univ , Baltimore, Md *Pres , Chairman of Natl Research Council* (1, 1927)
- Brookes, Margaret C Hessler, Ph D Univ of Chicago, Chicago, Ill *Asst Prof , Dept of Home Economics* (5, 1935)
- Brookhart, John M , Ph D Univ of Oregon School of Medicine, Dept of Physiology, Portland *Assoc Prof* (1, 1946)
- Brooks, Chandler McCuskey, Ph D State Univ of New York Med Center at New York, College of Medicine, 350 Henry St , Brooklyn 2 *Prof of Physiology and Pharmacology, Exec Officer of Dept* (1, 1934, 3, 1950)
- Brooks, Clyde, Ph D , M D Univ Clinic, 2508 Ponce de Leon Blvd , Coral Gables, Fla *Internal Medicine* (1, 1910, 3, 1912)
- Brooks, Matilda Moldenhauer, Ph D Univ of California, Dept of Physiology, Berkeley *Research Assoc in Biology* (1, 1923)
- Broun, Goronwy Owen, M D 1325 S Grand Blvd , St Louis, Mo *Prof of Internal Medicine, St Louis Univ* (4, 1927)
- Brown, Claude P , M D 1930 Chestnut St , Philadelphia, Pa *Practicing Clin Pathologist* (6, 1913)
- Brown, Dugald E S , Ph D Bermuda Biological Station, St George's W , Bermuda (1, 1932)
- Brown, Edgar D , Pharm D , M D Paynesville, Minn *Retired* (1R, 1907, 3R, 1909)
- Brown, Ernest B , Jr , M S , Ph D Univ of Minnesota, Dept of Physiology, Minneapolis *Asst Prof* (1, 1950)
- Brown, Ethan Allen, L R C P (Eng) , A R C S (London), 75 Bay State Rd , Boston, Mass *Lecturer in Medicine, Tufts College Med School, Physician-in-Chief, Allergy Clinic, Boston Dispensary* (6, 1946)
- Brown, Frank A , Jr , Ph D Northwestern Univ , Zoological Labs , Evanston, Ill *Assoc Prof of Zoology* (1, 1940)
- Brown, George B , Ph D Sloan-Kettering Inst for Cancer Research, 444 E 68th St , New York City 21 *Member, Assoc Prof of Biochemistry, Cornell Univ Med College* (2, 1947)
- Brown, Gordon Campbell, Sc D School of Public Health, Ann Arbor, Mich *Assoc Prof of Epidemiology* (6, 1949)
- Brown, John B , Ph D Ohio State Univ , Columbus *Prof of Physiological Chemistry* (2, 1927, 5, 1934)
- Brown, R A , Ph D Parke Davis and Co , Research Labs , Detroit 32, Mich *Head of Div of Nutritional Research* (5, 1946)
- Brown, Rachel, Ph D 26 Buckingham Drive, Albany, N Y *Sr Biochemist, Div of Labs and Research, N Y State Dept of Health* (6, 1933)
- Brown, Robert V , Ph D Univ of Tennessee, Dept of Pharmacology, Memphis 3 *Assoc Prof* (1, 1945, 3, 1950)
- Browne, J S L , M D , Ph D Royal Victoria Hospital, Univ Clinic, Montreal, Que , Canada *Asst Prof of Medicine, McGill Univ* (1, 1934)
- Brownell, Katharine A , Ph D Ohio State Univ , Dept of Physiology, Columbus *Research Assoc* (1, 1943)
- Brozek, Josef, Ph D Univ of Minnesota, Stadium South Tower, Minneapolis 14 *Assoc Prof , Lab of Physiological Hygiene, School of Public Health* (1, 1947)

- Brues, Austin M**, M D Argonne National Labs, P O Box 5207, Chicago, Ill *Dir of Biology Div, Assoc Prof of Medicine and Instr of Radiobiology and Biophysics, Univ of Chicago* (1, 1940)
- Bruesch, S R**, M D, Ph D Univ of Tennessee, Div of Anatomy, Memphis 3 *Prof of Anatomy* (1, 1949)
- Bruger, Maurice**, M D, M Sc 301 E 20th St, New York City 3 *Assoc Prof of Medicine, New York Univ Post-Grad Med School, Chief, Div of Pathological Chemistry, New York University Hospital* (2, 1935, 5, 1935)
- Bruhn, John M**, Ph D Med College of Alabama, Dept of Physiology, 620 S 20th St, Birmingham 5 *Prof of Physiology and Pharmacology* (1, 1939)
- Bruner, Harry Davis**, M D, Ph D Med Div ORINS, Box 117, Oak Ridge, Tenn *Principal Scientist* (3, 1945)
- Brunschwig, Alexander**, M D Cornell Univ Med College, New York City *Prof of Clin Surgery, Attending Surgeon, Memorial Hosp* (4, 1937)
- Bryan, W Ray**, Ph D 4405 Bywood Lane, Bethesda, Md *Principal Biologist, Natl Cancer Inst* (1, 1934, 4, 1940)
- Buchanan, J William**, Ph D Univ of Southern California, Alan Hancock Foundation, Los Angeles *Prof of Zoology* (1, 1927)
- Buchanan, John M**, Ph D Univ of Pennsylvania School of Medicine, Dept of Physiological Chemistry, Philadelphia *Assoc Prof* (2, 1949)
- Buchbinder, Lean**, Ph D Dept of Health, 125 Worth St, New York City *Sr Bacteriologist in charge of Lab* (6, 1934)
- Buchbinder, William C**, M D 104 S Michigan Ave, Chicago, Ill *Asst Prof of Medicine, Northwestern Univ Med School, Assoc in Medicine, Michael Reese Hospital* (1, 1940)
- Bucher, Gladys R**, Ph D Univ of Illinois, Dept of Biology, Chicago Div, Navy Pier, Chicago 12 (1, 1946)
- Buckner, G Davis**, Ph D Kentucky Agricultural Exper Station, Lexington *In charge of Animal Nutrition* (2, 1920)
- Bucy, Paul C**, M D 4833 S Woodlawn Ave, Chicago, Ill *Prof of Neurology and Neurological Surgery, Univ of Illinois* (1, 1933)
- Buddingh, G John**, M D Louisiana State Univ School of Medicine, New Orleans *Prof of Microbiology* (4, 1940)
- Bueding, Ernest**, M D Western Reserve Univ School of Medicine, Dept of Pharmacology, Cleveland 6, Ohio *Assoc Prof* (2, 1946, 3, 1949)
- Buell, Mary V**, Ph D Washington Univ School of Medicine, Euclid Ave and Kingshighway, St Louis 10, Mo *Research Asst, Dept of Biological Chemistry* (2, 1921)
- Bugher, John C**, M D The Rockefeller Foundation, 49 W 49th St, New York City 21 *Member, Internatl Health Div* (4, 1935)
- Bukantz, Samuel C**, M D Washington Univ School of Medicine, St Louis 10, Mo *Research Asst in Medicine* (6, 1943)
- Bulatao, Emilio**, M D Univ of the Philippines, Manila, P I *Prof of Physiology and Biochemistry* (1, 1924)
- Bull, Henry B**, Ph D Northwestern Univ Med School, 303 E Chicago Ave, Chicago 11, Ill *Prof of Chemistry* (2, 1937)
- Bullock, Theodore H**, Ph D Univ of California, Los Angeles *Assoc Prof of Zoology* (1, 1948)
- Bunde, Carl A**, M D, Ph D Pitman-Moore Co, Indianapolis 6, Ind *Research Dir* (1, 1943)
- Bunney, William Edward**, Ph D E R Squibb and Sons, New Brunswick, N J *Vice Pres, Dir of Manufacturing Labs* (6, 1931)
- Bunting, Charles H**, M D 139 Armory St, Hamden, Conn *Prof Emeritus of Pathology, Univ of Wisconsin, Lecturer in Pathology, Yale Med School* (4, 1913)
- Bunzell, H H**, Ph D Box 44, General Post Office, New York City 1 *Dir of Bunzell Labs* (2, 1908)
- Burchell, Howard B**, M D, Ph D 1506 Durand Court, Rochester, Minn *Instr in Medicine, Mayo Foundation, Consultant in Medicine, Mayo Clinic* (1, 1942)
- Burdick, H O**, Sc D Alfred Univ, Alfred, N Y *Prof of Biology* (1, 1940)
- Burdon, Kenneth L**, Ph D Baylor Univ College of Medicine, Houston, Tex *Prof of Bacteriology, Consultant, USPHS* (6, 1936)
- Burgen, A S V**, M B, MRCP McGill Univ, Dept of Physiology, Montreal, Quebec, Canada *Professor* (1, 1950, 3, 1950)
- Burk, Dean**, Ph D Natl Cancer Inst, Bethesda, Md *Principal Biochemist, USPHS* (2, 1939)
- Burns, Edward L**, M D Mercy Hospital, Toledo, Ohio *Pathologist* (4, 1939)
- Burr, George O**, Ph.D Exper Station H S P A, Honolulu, Hawaii *Head of Dept of Biochemistry and Physiology* (2, 1928, 5, 1933)
- Burris, Robert H**, Ph D Univ of Wisconsin, Dept of Biochemistry, Madison 6 *Prof of Biochemistry* (2, 1946)
- Burrows, William**, Ph D Univ of Chicago, Dept of Bacteriology and Parasitology, Chicago 37, Ill *Prof of Bacteriology* (6, 1947)
- Burton, Alan C**, Ph D Univ of Western Ontario, Dept of Med Research, London, Ontario, Canada *Asst Prof* (1, 1937)
- Burton-Opitz, Russell**, M D, Ph D 218 Bridle Way, Palisade, N J *Attending Cardiologist, Lenox Hill Hospital, Attending Physician, Cumberland Hospital* (1R, 1902, 2, 1906, 3R, 1919)
- Buschke, William H**, M D Manhattan Eye, Ear and Throat Hospital, 210 E 64th St, New York

- City 21 *Research Ophthalmologist, Head of Ayer Foundation Ophthalmic Research Lab* (1, 1947)
- Bush, Milton T, Ph D Vanderbilt Univ School of Medicine, Nashville, Tenn *Assoc Prof of Pharmacology* (3, 1938)
- Butler, G C, Ph D Univ of Toronto, Dept of Biochemistry, Toronto, Ont, Canada *Assoc Prof* (2, 1949)
- Butler, Thomas C, M D Univ of North Carolina School of Med, Chapel Hill *Prof of Pharmacology* (3, 1938)
- Butt, Hugh R, M D Mayo Clinic, 102 Second Ave, S W, Rochester, Minn *Consultant in Medicine, Asst Prof of Medicine, Mayo Foundation* (5, 1942)
- Butts, Joseph S, Ph D Oregon State College, Corvallis *Prof of Biochemistry and Head of Agricultural Chemistry* (2, 1936, 5, 1936)
- Byers, Sanford O, Ph D 2200 Post St, San Francisco 15, Calif *Research Biochemist, Harold Brunn Inst for Cardiovascular Research, Mount Zion Hospital* (1, 1949)
- Cahen, Raymond L, Ph D, M D Maltbie Chemical Co, Research Labs, P O Box 270, Morristown, N J *Dir of Pharmacological Research* (3, 1949)
- Cahill, William M, Ph D, M D 3320 Baldwin St, Los Angeles 31, Calif (2, 1940)
- Cajori, Florian A, Ph D Univ of Colorado Med School, Denver 7 *Asst Prof of Biochemistry* (2, 1922, 5, 1933)
- Caldwell, Mary L, Ph D Columbia Univ, New York City *Prof of Chemistry* (2, 1924, 5, 1933)
- Callison, Elizabeth C, M S U S Dept of Agriculture, Agricultural Research Station, Food and Nutrition Div, Bureau of Human Nutrition, Beltsville Md *Nutrition Physiologist* (5, 1949)
- Calloway, Nathaniel Oglesby, Ph D, M D Univ of Illinois College of Medicine, 1819 W Polk St, Chicago 12 *Asst in Medicine* (3, 1945)
- Calvin, D Bailey, Ph D Univ of Texas Med Branch, Galveston *Prof of Biological Chemistry and Dean of Student and Curricular Affairs* (1, 1934, 2, 1939)
- Calvin, Melvin, Ph D Univ of California, Berkeley 4 *Prof of Chemistry* (2, 1949)
- Campanopetros, John, M D Hellenic Pasteur Inst, 103 Queen Sophie Ave, Athens, Greece *Chief of the Service of Exper Medicine* (6, 1946)
- Camp, Walter J R, M D, Ph D Univ of Illinois College of Medicine, 1853 W Polk St, Chicago, Ill *Prof of Pharmacology and Toxicology, State Toxicologist* (3, 1926)
- Campbell, Berry, Ph D Univ of Minnesota, Minneapolis 14 *Assoc Prof of Anatomy* (1, 1945)
- Campbell, Dan H, Ph D California Inst of Technology, Pasadena *Assoc Prof of Chemistry* (6, 1938)
- Campbell, H Louise, Ph D 900 Windsor Ave, Windsor, Conn (5R, 1933)
- Campbell, J Alexander, Ph D Dept of Natl Health and Welfare, Food and Drug Div, Ottawa, Ont, Canada *Chief, Vitamin and Nutrition Lab* (5, 1950)
- Campbell, James, Ph D Univ of Toronto, Toronto, Ont, Canada *Assoc Prof of Physiology, Lt Comdr, (S B) R C N V R* (1, 1943)
- Campbell, Walter Ruggles, M D Univ of Toronto, 69 Madison Ave, Toronto, Ont, Canada *Assoc Prof of Medicine* (2, 1922)
- Cannan, R Keith, D Sc New York Univ College of Medicine, 477 First Ave, New York City *Prof of Chemistry* (2, 1931)
- Cannon, Paul R, M D, Ph D Univ of Chicago, Chicago, Ill *Prof of Pathology* (4, 1930, 6, 1929)
- Cantarow, Abraham, M D Jefferson Med College, Philadelphia 7, Pa *Prof of Biochemistry* (1, 1932, 3, 1935)
- Cantoni, G L, M D New York Univ College of Medicine, Dept of Pharmacology, New York City *Fellow of the American Cancer Society* (3, 1945)
- Canzanelli, Attilio, M D Tufts College Med School, 136 Harrison Ave, Boston, Mass *Prof of Exper Physiology* (1, 1934)
- Carlson, A J, Ph D, M D Univ of Chicago, Hull Physiological Lab, Chicago, Ill *Prof Emeritus of Physiology* (1, 1904, 5, 1933)
- Carlson, Loren D, Ph D Univ of Washington School of Medicine, Dept of Physiology and Biophysics, Seattle 5 (1, 1945)
- Carmichael, Emmett B, Ph D Med College of Alabama, Dept of Biochemistry, Birmingham 5 *Prof and Head of Dept* (1, 1931, 2, 1946)
- Carmichael, Leonard, Ph D Sc D Tufts College, Medford, Mass *Pres, Dir of Research Lab of Sensory Psychology and Physiology* (1, 1937)
- Carpenter, Frederick H, Ph D Univ of California, Dept of Biochemistry, Berkeley 4 *Asst Prof* (2, 1949)
- Carpenter, Thorne M, Ph D 27 Market St, Foxboro, Mass (1R, 1915, 2, 1909, 5R, 1935)
- Carr, C Jelleff, Ph D Univ of Maryland School of Medicine, Baltimore *Assoc Prof of Pharmacology* (3, 1940)
- Carr, Jesse L, M D Univ of California Med School, Third and Parnassus Aves, San Francisco *Chn Prof of Pathology* (4, 1940)
- Carruthers, Christopher, Ph D Washington Univ School of Medicine, Dept of Anatomy, 4580 Scott Ave, St Louis 10, Mo *Research Assoc* (2, 1948)
- Carter, Charles E, M D Western Reserve Univ School of Medicine, Cleveland 6, Ohio *Dept of Medicine* (2, 1950)
- Carter, Herbert E, Ph D Univ of Illinois, 452

- Noyes Lab , Urbana *Prof of Biochemistry* (2, 1937, 5, 1941)
- Cartland, George F** , Ph D The Upjohn Co , Research Dept , Kalamazoo, Mich *Head of Antibiotics Research* (2, 1936)
- Cary, Charles A** , S B U S Dept of Agriculture, Bureau of Dairy Industry, Dairy Research Lab , Beltsville, Md *Chief of Div of Nutrition and Physiology* (2, 1920)
- Casey, Albert Eugene**, M D 1907 Wellington Rd , Birmingham 9, Ala *Pathologist, Baptist Hospital, Assoc Prof of Pathology, Med College of Alabama* (4, 1933)
- Cash, James Robert**, M D Univ Hospital, Charlottesville, Va *Prof of Pathology, Univ of Virginia* (4, 1924)
- Cassidy, Harold G** , Ph D Yale Univ , Sterling Chemical Lab , New Haven, Conn *Assoc Prof of Chemistry* (2, 1949)
- Castillo, Julio C** , B S Wellcome Research Labs , Tuckahoe, N Y *Pharmacologist* (3, 1950)
- Castle, Edward S** , Ph D Harvard Univ Biological Labs , Divinity Ave , Cambridge, Mass *Assoc Prof of General Physiology* (1, 1934)
- Castle, William B** , M D Boston City Hospital, Boston, Mass *Prof of Medicine, Harvard Med School, Dir of Thorndike Memorial Lab* (4, 1942)
- Catchpole, Hubert Ralph**, Ph D Univ of Illinois College of Medicine, 1853 W Polk St , Chicago 12, Ill *Research Assoc in Pathology* (1, 1941)
- Cathcart, E P** , M D , D Sc Univ of Glasgow, Glasgow, Scotland *Dean of Univ* (5R, 1935)
- Catron, Lloyd**, M D City Hospital, Akron, Ohio *Pathologist* (4, 1939)
- Cattell, McKeen**, Ph D , M D Cornell Univ Med College, 1300 York Ave , New York City *Prof of Pharmacology* (1, 1923, 3, 1924)
- Cavelti, Philip A** , M D 11406 Kingsland St , Los Angeles, Calif (6, 1947)
- Cederquist, Dena C** , Ph D Michigan State College, School of Home Economics, East Lansing *Assoc Prof (Research) of Foods and Nutrition* (5, 1949)
- Ceithaml, Joseph H** , Ph D Univ of Chicago, 947 E 58th St , Chicago 37, Ill *Asst Prof of Biochemistry* (2, 1950)
- Cerecedo, Leopold R** , Ph D Fordham Univ , New York City *Prof of Biochemistry* (2, 1931, 5, 1945)
- Chadwick, Leigh Edward**, Ph D Med Div , Army Chemical Center, Md (1, 1944)
- Chaikoff, I L** , Ph D , M D Univ of California, Berkeley *Assoc Prof of Physiology* (1, 1932)
- Chalkley, Harold W** , Ph D USPHS, Natl Insts of Health, Bethesda, Md *Sr Physiologist* (1, 1932)
- Chamberlain, W Edward**, M D Temple Univ Med School, Philadelphia, Pa *Prof of Radiology* (1, 1948)
- Chambers, Alfred H** , Ph D Univ of Vermont School of Medicine, Dept of Physiology, Burlington (1, 1946)
- Chambers, Leslie Addison**, Ph D Camp Detrick, Frederick, Md *Chief of Physical and Chemical Div* (1, 1940, 6, 1948)
- Chambers, Robert**, Ph D New York Univ , Washington Sq , New York City *Research Prof Emeritus of Biology, N Y U* (1R, 1932)
- Chambers, William H** , Ph D Med Div , Army Chemical Center, Md *Chief of Toxicology Branch* (1, 1924, 5, 1933)
- Chambers, W W** , Ph D Univ of Pennsylvania, School of Medicine, Dept of Anatomy, Philadelphia 4 *Asst Prof* (1, 1950)
- Chance, Britton**, M S , Ph D Univ of Pennsylvania, Johnson Research Found , 612 Maloney Bldg , Philadelphia 4 *Prof and Dir of Johnson Research Foundation* (1, 1950, 2, 1950)
- Chandler, Caroline A** , M D Johns Hopkins School of Hygiene and Public Health, 615 N Wolfe St , Baltimore 5, Md *Asst Prof of Preventive Medicine* (6, 1938)
- Chandler, Joseph P** , Ph D Univ of Michigan Med School, Room G455, Univ Hosp , Ann Arbor *Asst Prof of Biological Chemistry, Biochemist, Univ Hosp* (2, 1944, 5, 1944)
- Chang, Min Cheuh**, Ph D Worcester Foundation, Shrewsbury, Mass *Assoc Fellow* (1, 1946)
- Chanutin, Alfred**, Ph D Box 1862, University Station, Charlottesville, Va *Prof of Biochemistry, Univ of Virginia* (2, 1925)
- Chapanis, Alphonse**, Ph D Johns Hopkins Univ , Baltimore, Md *Asst Prof of Psychology* (1, 1948)
- Chapman, C W** , Ph D Univ of Maryland, Baltimore *Prof of Pharmacology* (3, 1932)
- Chargaff, Erwin**, Ph D Columbia Univ College of Physicians and Surgeons, 630 W 168th St , New York City 32 *Assoc Prof of Biological Chemistry* (2, 1935)
- Charipper, Harry Adolph**, Ph D Washington Square College of Arts and Sciences, 100 Washington Square E , New York City *Prof and Chairman of Dept of Biology* (1, 1941)
- Chase, Aurin M** , Ph D Princeton Univ , Dept of Biology, Princeton, N J *Research Assoc, Asst Prof* (1, 1939)
- Chase, Harold F** , M D Univ of Virginia Hospital, Charlottesville *Dir and Assoc Prof of Anesthesiology* (3, 1944)
- Chase, Merrill W** , Ph D Rockefeller Inst , 66th St and York Ave , New York City *Member of Staff* (6, 1938)
- Chasis, Herbert**, M D , Med Sc D 44 E 67th St , New York City *Asst Prof of Medicine, New York Univ College of Medicine* (1, 1941)
- Chatfield, Charlotte**, B S Food and Agriculture

- Organization of the United Nations, Washington, D C *Nutrition Officer* (5, 1941)
- Chatfield, Paul Oakes, M D Harvard Med School, Dept of Physiology, 25 Shattuck St, Boston 15, Mass *Associate* (1, 1950)
- Chatterjee, Hernendra Nath, M D 9 Romes Mitter Rd, Bhowanipour, Calcutta, India *Professor* (6, 1948)
- Cheever, Francis Sargent, M D Univ of Pittsburgh Grad School of Public Health, Pittsburgh 13, Pa (6, 1950)
- Cheldelin, Vernon H, Ph D Oregon State College, Corvallis *Prof of Chemistry* (2, 1947, 5, 1946)
- Chen, Graham, Sc D, M D Parke Davis and Co, Detroit, Mich (3, 1944)
- Chen, K K, Ph D, M D Lilly Research Labs, Indianapolis, Ind *Dir of Pharmacological Research, Prof of Pharmacology, Indiana Univ School of Medicine* (1, 1929, 3, 1942)
- Cheney, Ralph H, Sc D Brooklyn College, Biology Dept, Bedford Ave and Ave H, Brooklyn 10, N Y *Professor* (3, 1934)
- Chenoweth, Maynard Burton, M D Univ of Michigan Med School, Dept of Pharmacology, Ann Arbor *Assoc Prof of Pharmacology* (3, 1945)
- Chesley, Leon C, Ph D Margaret Hague Maternity Hospital, 88 Clifton Place, Jersey City 4, N J *Chief Chemist* (1, 1949)
- Chesney, Alan M, M D Johns Hopkins Hospital, Baltimore, Md *Dean of Johns Hopkins Med School, Assoc Prof of Medicine* (4, 1925)
- Child, Charles M, Ph D Stanford Univ, Stanford, Calif *Prof Emeritus, of Zoology, Stanford Univ* (1R, 1923)
- Child, George P, Ph D, M D Albany Med College, Albany 3, N Y *Asst Prof of Pharmacology* (3, 1949)
- Chin, Yin-Ch'ang, Ph D 1268 Sixth Ave, San Francisco, Calif *Research Fellow, Univ of California, Asst Prof, Peking Union Med College* (3, 1950)
- Chinn, Herman I, Ph D School of Aviation Medicine, Randolph Field, Tex *Chief, Dept of Pharmacology and Biochemistry* (1, 1950)
- Choucroun, Nine Dept of Public Health and Preventive Medicine, 1300 York Ave, New York City 22 *Research Assoc, Cornell Med College* (6, 1949)
- Chow, Bacon F, Ph D Johns Hopkins Univ School of Hygiene, 615 N Wolfe St, Baltimore 14, Md *Assoc Prof of Biochemistry* (2, 1940, 5, 1948, 6, 1944)
- Christensen, Halvor N, Ph D Tufts College Med School, Boston, Mass *Prof and Head of Dept of Biochemistry* (2, 1947)
- Christensen, L Royal, Ph D New York Univ College of Medicine, Dept of Microbiology, 477 First Ave, New York City *Asst Prof* (6, 1942)
- Christian, Henry A, M D 20 Chapel St, Brookline, Mass *Hersey Prof Emeritus of the Theory and Practice of Physics, Harvard Univ, Physician-in-Chief, Emeritus, Peter Bent Brigham Hospital* (4, 1924)
- Christman, Adam A, Ph D Univ of Michigan Med School, Ann Arbor *Prof of Biological Chemistry* (2, 1929)
- Chu, Wei-chang, M D 546 W 124th St, Apt 53, New York City (3, 1945)
- Clark, Ada R, Ph D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 *Assoc in Bacteriology, Teaching and Research* (6, 1936)
- Clark, Byron B, Ph D Tufts College Med School, 136 Harrison Ave, Boston 15, Mass *Prof of Pharmacology* (3, 1940)
- Clark, Eliot R, M D Univ of Pennsylvania, Philadelphia *Prof of Anatomy* (1R, 1919)
- Clark, Ernest D, Ph D 826 Skinner Bldg, Seattle 1, Wash *Dir of the Labs, Northwest Branch, Nail Cannery's Assoc* (2, 1912)
- Clark, George, Ph D Chicago Med School, Dept of Anatomy, 710 S Wolcott Ave, Chicago 12, Ill *Assoc Prof of Neuroanatomy* (1, 1943)
- Clark, Guy W, Ph D R F D 5330 Wild Horse Valley Rd, Napa, Calif *Technical Dir, Lederle Labs, Div of American Cyanamid Co* (2, 1922)
- Clark, Janet Howell, Ph D Univ of Rochester, Anderson Hall, Rochester, N Y *Dean of the College for Women and Prof in the Div of Biological Sciences* (1, 1922)
- Clark, Paul F, Ph D Univ of Wisconsin Med School, Madison *Prof of Med Microbiology* (4, 1923, 6, 1928)
- Clark, William G, Ph.D 2524 Prosser Ave, Los Angeles, Calif *Clin Assoc Prof of Physiological Chemistry, Univ of Southern California at Los Angeles School of Medicine, Chief, Pharmacology-Physiology Section, General Med Research Service, V A Center* (1, 1942, 3, 1949)
- Clark, William Mansfield, Ph D Johns Hopkins Univ, Baltimore, Md *Prof of Physiological Chemistry* (2, 1920)
- Clarke, Hans Thacher, D Sc Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 *Prof of Biological Chemistry* (2, 1929)
- Clarke, Robert W Route 3, Helmswoods, Elizabethtown, Ky *Physiologist, Med Dept Field Research Lab, Fort Knox* (1, 1936)
- Clausen, Samuel Wolcott, M D Univ of Rochester School of Medicine, Rochester, N Y *Prof of Pediatrics* (2, 1922)
- Cleghorn, Robert Allen, M D, D Sc McGill Univ, Dept of Psychiatry, Montreal, Quebec, Canada (1, 1937)

- Clowes, George Henry Alexander, Ph D Eli Lilly & Co, Indianapolis 6, Ind *Dir of Research Emeritus* (2, 1914, 6, 1919)
- Coburn, Alvin F, M D Rheumatic Fever Research Inst, Chicago, Ill *Director* (6, 1948)
- Code, Charles F, Ph D, M D Mayo Foundation, Rochester, Minn *Prof of Physiology* (1, 1939, 3, 1950)
- Coffey, Julia M, A B Div of Labs and Research, New York State Dept of Health, Albany *Assoc Bacteriologist* (6, 1937)
- Coghill, Robert D, Ph D Abbott Labs, N Chicago, Ill *Dir of Research* (2, 1932)
- Cohen, Barnett, Ph D Johns Hopkins Univ School of Medicine, 710 N Washington St, Baltimore 5, Md *Assoc Prof of Physiological Chemistry* (2, 1935)
- Cohen, Philip P, Ph D, M D Univ of Wisconsin, Service Memorial Inst, Madison *Prof of Physiological Chemistry* (2, 1941)
- Cohen, Saul L, Ph D Univ of Minnesota, 207 Millard Hall, Minneapolis *Asst Prof of Physiological Chemistry* (2, 1948)
- Cohen, Seymour S, Ph D Children's Hospital, 1740 Bainbridge St, Philadelphia, Pa *Assoc Prof of Physiological Chemistry and Pediatrics, Univ of Pennsylvania School of Medicine* (2, 1946)
- Cohen, Sophia M, B S New York State Dept of Health, Div of Labs and Research, Albany *Sr Bacteriologist* (6, 1938)
- Cohn, Alfred E, M D Rockefeller Inst for Med Research, 66th St and York Ave, New York City 21 *Member* (1R, 1911, 3, 1913)
- Cohn, Clarence, M D Michael Reese Hospital, Med Research Inst, Chicago, Ill *Dir of Dept of Biochemistry* (1, 1948)
- Cohn, Edwin J, Ph D 183 Brattle St, Cambridge, Mass *Prof of Biological Chemistry, Harvard Med School, Univ Prof, Harvard Univ* (1, 1919, 2, 1919)
- Cohn, Mildred, Ph D Harvard Univ Med School, Boston 15, Mass *Research Assoc, Biochemistry* (2, 1949)
- Cohn, Waldo E, Ph D Oak Ridge Natl Lab, Biology Div, Oak Ridge, Tenn *Biochemist* (2, 1944)
- Cole, Harold H, Ph D Univ of California, College of Agriculture, Div of Animal Husbandry, Davis *Professor* (1, 1947)
- Cole, Harold N, Ph B, M D 1352 Hanna Bldg, Cleveland, Ohio *Clin Prof of Dermatology and Syphilology, Western Reserve Univ* (3, 1925)
- Cole, Kenneth S, Ph D Univ of Chicago, Inst of Radiobiology and Biophysics, Chicago 37, Ill *Prof of Biophysics* (1, 1934)
- Cole, Rufus, M D, D Sc Mount Kisco, N Y *Member Emeritus, Rockefeller Inst for Med Research* (4R, 1924, 6R, 1917)
- Cole, Versa V, Ph D, M D Indiana Univ School of Medicine, 1040-1232 W Michigan St, Indianapolis *Assoc Prof of Pharmacology* (3, 1941)
- Colfer, Harry F, M D, Ph D 9 Kempshall Ter, Fanwood, N J *Research Physician, Merck & Co, Rahway, Asst Physician, Presbyterian Hospital, N Y C* (1, 1950)
- Collett, Mary Elizabeth, Ph D Western Reserve Univ, Mather College, Cleveland, Ohio *Assoc Prof of Biology* (1, 1921)
- Collier, H Bruce, Ph D Univ of Alberta, Dept of Biochemistry, Edmonton, Alta, Canada *Prof of Biochemistry* (2, 1944)
- Collings, William Doynne, Ph D Michigan State College, Dept of Physiology, East Lansing (1, 1944)
- Collins, Dean A, Ph D, M D Temple Univ School of Medicine, 3400 N Broad St, Philadelphia 40, Pa *Prof of Pharmacology* (1, 1938)
- Collins, Russell J, A M, M D St John, New Brunswick, Canada *Med Superintendent of St John Tuberculosis Hospital* (3, 1915)
- Collip, J B, Ph.D M D Univ of Western Ontario, R R 3, London, Ontario, Canada *Dean of Medicine and Prof of Med Research* (1, 1920, 2, 1920)
- Colowick, Sidney P, Ph D Johns Hopkins Univ, McCollum Pratt Inst, Baltimore 18, Md *Assoc Prof* (2, 1944)
- Coman, Dale R, M D Univ of Pennsylvania School of Medicine, McManes Lab of Pathology, Philadelphia *Prof of Exper Pathology* (4, 1939)
- Comroe, Julius H, Jr, M D Univ of Pennsylvania Grad School of Medicine, Philadelphia *Prof of Physiology and Pharmacology* (1, 1943, 3, 1939)
- Conant, James B, Ph D Harvard Univ, 5 Univ Hall, Cambridge, Mass *President* (2, 1932)
- Concepcion, Isabelo, M D 589 Zamora, Pasay, Rizal, Philippines Faculty of Medicine, Univ Santo Tomas, Manila, Philippines *Prof of Biochemistry and Nutrition* (1, 1919)
- Conklin, Ruth E, Ph D Vassar College, Poughkeepsie, N Y *Prof of Physiology* (1, 1940)
- Conn, Jerome W, M D Univ of Michigan Med School, Ann Arbor *Assoc Prof of Internal Med* (5, 1942)
- Consolazio, William V, B S Office of Naval Research, Navy Dept, Washington 25, D C *Head of Biochemistry Branch* (2, 1949)
- Cook, Donald Hunter, Ph D Univ of Miami, Coral Gables 34, Fla *Prof of Chemistry* (2, 1929)
- Cooke, Robert A, A M, M D 60 E 58th St, New York City *Dir of Dept of Allergy, Roosevelt Hospital* (6, 1920)
- Coolidge, Thomas B, M D, Ph D Univ of Chicago, Abbot Hall, Chicago 37, Ill *Assoc Prof*,

- Dept of Biochemistry and Walter G Zoller Memorial Dental Clinic* (2, 1942)
- Coon, Julius M**, Ph D, M D Univ of Chicago, Chicago 37, Ill *Assoc Prof of Pharmacology, Dir of Toxicity Lab* (3, 1941)
- Coons, Albert H**, M D Harvard Univ, 25 Shattuck St, Boston 15, Mass *Assoc in Bacteriology and Immunology* (6, 1950)
- Coons, Callie Mae**, Ph D U S Dept of Agriculture, Bureau of Human Nutrition and Home Economics, Washington, D C *Asst Chief* (5, 1933)
- Cooper, Merlin L**, M D Children's Hospital Research Foundation, Elland Ave and Bethesda, Cincinnati 29, Ohio *Assoc Prof of Bacteriology, Asst Prof of Pediatrics, Univ of Cincinnati College of Medicine* (6, 1949)
- Cooper, Ruth S** Princeton Univ, Dept of Biology, Princeton, N J (6, 1949)
- Copley, Alfred Lewin**, M D New York Univ, Dept of Biology, Lab of Cellular Physiology, Washington Square, New York City 3 *Research Assoc in Medicine, Asst Clin Prof, New York Med College* (1, 1944)
- Corbin, Kendall B**, M D 919 80th St, S W, Rochester, Minn *Consultant in Neurology, Mayo Clinic, Prof of Neuroanatomy, Mayo Foundation* (1, 1941)
- Corcoran, Arthur Curtis**, C M, M D Cleveland Clinic Foundation, Cleveland 6, Ohio (1, 1940)
- Corey, Edward Lyman**, Ph D Univ of Virginia School of Medicine, Charlottesville *Prof of Physiology* (1, 1931)
- Cori, Carl F**, M D Washington Univ School of Medicine, Kingshighway and Euclid Ave, St Louis 10, Mo *Prof of Biochemistry* (2, 1925, 3, 1934)
- Cori, Gerty T**, M D Washington Univ School of Medicine, St Louis 10, Mo *Prof of Biochemistry* (2, 1927, 3, 1934)
- Corley, Ralph Conner**, Ph D Purdue Univ, Dept of Chemistry, Lafayette, Ind *Prof of Biochemistry* (2, 1927)
- Cornatzer, W E**, Ph D Bowman Gray School of Medicine, Winston-Salem, N C *Asst Prof of Biochemistry* (2, 1950)
- Corper, Harry J**, M D, Ph D 1295 Clermont St, Denver 7, Colo *Dir of Research, Natl Jewish Hospital and Univ of Colorado School of Medicine* (2, 1912)
- Corson, Samuel A**, Ph D Toledo Hospital Inst of Med Research, 2805 Oatis Ave, Toledo 6, Ohio *Chief, Dept of Physiology* (1, 1943)
- Cotts, Gerhard K**, M D 1100 Church St, Lynchburg, Va (3, 1937)
- Co Tui, C** M D 15 E 62nd St, New York City 21 *Dir, Biologic Research, Creadmore Inst for Psychobiologic Studies* (3, 1931)
- Couch, James Russell**, Ph D Texas A & M College, College Station *Prof of Poultry Husbandry, Biochemistry and Nutrition* (5, 1950)
- Cournand, Andre Frederic**, M D Chest Service, Bellevue Hospital, C D Building, 1st Ave at 28th St, New York City *Assoc Prof of Medicine, College of Physicians and Surgeons, Columbia Univ* (1, 1944)
- Cowgill, George Raymond**, Ph D Yale Univ, Nutrition Lab, 333 Cedar St, New Haven 11, Conn *Prof of Nutrition, Dept of Physiological Chemistry* (1, 1923, 2, 1922, 5, 1933)
- Cox, Alvin J, Jr**, M D Stanford Univ School of Medicine, 2398 Sacramento St, San Francisco 15, Calif *Prof of Pathology* (4, 1949)
- Cox, Gerald J**, Ph D Univ of Pittsburgh School of Dentistry, Pittsburgh 13, Pa *Prof of Dental Research* (2, 1930, 5, 1935)
- Cox, Herald R**, Sc D Lederle Labs Div, American Cyanamid Co, Pearl River, N Y *Dir, Viral and Rickettsial Research* (6, 1946)
- Cox, Warren M, Jr**, Ph D Mead Johnson & Co, Evansville, Ind *Dir of Research* (2, 1935, 5, 1945)
- Craig, Francis Northrop**, Ph D Med Div, Army Chemical Center, Md *Chief, Applied Physiology Section* (1, 1946)
- Craig, L C**, Ph D Rockefeller Inst for Med Research, 66th St and York Ave, New York City 21 *Assoc Member* (2, 1938)
- Crampton, E W**, Ph D McGill Univ, Macdonald College, Montreal, Quebec, Canada *Prof of Nutrition* (5, 1940)
- Crandall, Lathan A, Jr**, M D, Ph D Miles Labs, Inc, Elkhart, Ind (1, 1930)
- Cranston, Elizabeth M**, Ph D Univ of Minnesota Med School, Dept of Pharmacology, Minneapolis 14 *Asst Prof* (3, 1946)
- Cravens, W W**, Ph D Univ of Wisconsin, Poultry Dept, Madison *Assoc Prof of Poultry Husbandry* (5, 1947)
- Craver, Bradford N**, Ph D, M D Ciba Pharmaceutical Products, Inc, Lafayette Park, Summit, N J *Sr Pharmacologist* (3, 1946)
- Crawford, Madeleine Field**, Ph D 9 High Rock St, Needham, Mass *Assoc in Physiology, Harvard School of Public Health* (1, 1933)
- Crescitelli, Frederick**, Ph D Univ of California, Dept of Zoology, Los Angeles *Physiologist* (1, 1946)
- Cretcher, Leonard H**, Ph D Univ of Pittsburgh, Mellon Inst of Industrial Research, Pittsburgh Pa *Asst Dir and Head of Dept of Research in Pure Chemistry* (2, 1930)
- Crider, Joseph O**, M D Jefferson Med College, Philadelphia, Pa *Prof of Physiology* (1, 1935)
- Crisler, George R**, Ph D, M D 157 E New England Ave, Winter Park, Fla (1, 1930)
- Crismon, Jefferson Martineau**, M D Stanford Univ, Calif *Assoc Prof of Physiology* (1, 1944)

- Crittenden, Phoebe J**, Ph D Goucher College, Dept of Physiology and Bacteriology, Towson 4, Md *Prof and Chairman of Dept of Physiology* (1, 1937, 3, 1937)
- Crozier, William J**, Ph D Harvard Univ, Biological Labs, Cambridge, Mass *Prof of General Physiology* (1, 1928)
- Csonka, F A**, Ph D U S Dept of Agriculture, Bureau of Human Nutrition and Home Economics, Beltsville, Md *Sr Chemist* (2, 1924)
- Cullen, Stuart C**, M D Univ Hospital, Iowa City, Iowa *Asst Prof of Surgery-Anesthesia* (3, 1944)
- Culler, Elmer A**, Ph D Univ of Rochester, Rochester, N Y *Prof of Psychology and Dir of Lab* (1R, 1936)
- Cunha, Tony Joseph**, Ph D Univ of Florida, Dept of Animal Husbandry, Gainesville *Assoc Prof, Assoc Animal Husbandman* (5, 1950)
- Cunningham, Raymond W**, Ph D Lederle Labs, Inc, Pearl River, N Y *Director, Pharmacology Research* (3, 1941)
- Cunningham, Robert Sydney**, M D, Sc D Albany Med College, Albany, N Y *Prof of Anatomy and Dean* (1, 1923)
- Cureton, Thomas Kirk, Jr**, Ph D Univ of Illinois, School of Physical Education, Urbana *Prof of Physical Education* (1, 1946)
- Curnen, Edward C**, M D Yale Univ School of Medicine, New Haven 11, Conn *Assoc Pediatrician, New Haven Hospital* (6, 1941)
- Cartis, George Morris**, Ph D, M D Ohio State Univ, Kinsman Hall, Columbus *Prof of Surgery, Chairman, Dept of Research Surgery* (1, 1933, 4, 1933)
- Curtis, Howard J**, Ph D Brookhaven Natl Lab, Upton, L I, N Y (1, 1940)
- Cutting, Windsor C**, M D Stanford Univ School of Medicine, San Francisco, Calif *Prof of Pharmacology and Therapeutics* (3, 1939)
- Daft, Floyd Shelton**, Ph D Natl Insts of Health, Bethesda, Md *Acting Director, Exper Biology and Medicine Inst* (2, 1949, 5, 1941)
- Daggs, Ray Gilbert**, Ph D Med Dept, Field Research Lab, Fort Knox, Ky *Dir of Research* (1, 1935, 5, 1933)
- Dakin, Henry D**, D Sc, Ph D Scarborough-on-Hudson, N Y (2, 1906)
- Dalldorf, Gilbert**, M D New York State Dept of Health, Albany *Dir, Div of Labs and Research* (4, 1947)
- Dalton, Albert J**, Ph D Natl Cancer Inst, Bethesda, Md *Principal Cytologist* (4, 1942)
- Dam, Henrik**, Ph D Polytechnic Inst Dept of Biology, Østervoldgade 10 L Copenhagen K Denmark *Professor* (2, 1944, 5, 1943)
- D'Amour, Fred E**, Ph D 2311 S Josephine St, Denver, Colo *Assoc Prof, Dept of Zoology, Univ of Denver* (1, 1934)
- D'Amour, Marie C**, Ph D, M D Olive View Sanatorium, Olive View, Calif (1, 1934)
- D'Angelo, Savino A**, Ph D Jefferson Med College, Dept of Anatomy, 307 S 11th St, Philadelphia, Pa (1, 1947)
- Daniel, Louise J**, Ph D Cornell Univ, Savage Hall, Ithaca, N Y *Asst Prof of Biochemistry* (2, 1949)
- Daniels, Amy L**, Ph D 720 N Van Buren St, Iowa City, Iowa (2, 1919, 5R, 1933)
- Danielson, Irvin S**, Ph D Lederle Labs, Amer Cyanamid Co, 18 Bogert Ave, Pearl River, N Y *Asst Dir, Animal Industry Section* (2, 1937)
- Danowski, T S**, M D Univ of Pittsburgh School of Medicine, Pittsburgh, Pa *Renzieshausen Prof of Research Medicine* (1, 1947)
- Darby, William J**, M D, Ph D Vanderbilt Univ School of Medicine, Div of Nutrition, Nashville, Tenn *Prof of Biochemistry, Asst Prof of Medicine* (2, 1949, 5, 1945)
- Darling, Robert Croly**, M D 157 Glenwood Ave, Leonia, N J Columbia Univ College of Physicians and Surgeons, Dept of Medicine, New York City 32 (1, 1944)
- Darrow, Chester W**, Ph D Inst for Juvenile Research, 907 S Wolcott Ave, Chicago, Ill *Psychophysiologist, Assoc Prof of Criminology, Univ of Illinois College of Medicine* (1, 1937)
- Darrow, Daniel Cady**, M D Yale Univ School of Medicine, New Haven, Conn *Prof of Pediatrics* (2, 1936)
- Daubert, B F**, Ph D Univ of Pittsburgh, 220 Alumni Hall, Dept of Chemistry, Pittsburgh 13, Pa *Research Prof and Research Admin* (2, 1947)
- Davenport, Fred M**, M D, D M Sc 1303 Gardner Ave, Ann Arbor, Mich *Research Assoc* (6, 1950)
- Davenport, Horace Willard**, Ph D Univ of Utah, Dept of Physiology, Salt Lake City 1 (1, 1942)
- Davenport, Virginia D**, Ph D Univ of Utah College of Medicine, Salt Lake City *Research Assoc in Physiology* (1, 1950)
- David, Norman Austin**, M D Univ of Oregon Med School, Portland *Prof of Pharmacology* (3, 1934)
- Davidsohn, Israel**, M D Mount Sinai Hospital, Chicago, Ill *Pathologist and Dir of Labs, Chicago Med School, Prof of Pathology and Chairman of Dept* (4, 1939, 6, 1929)
- Davidson, Charles S**, M D Boston City Hospital, Thorndike Memorial Lib, Boston 18, Mass *Assoc in Medicine, Harvard Med School, Assoc Dir, 2nd and 4th Med Services, Boston City Hospital* (5, 1949)
- Davies, Philip W**, Ph D Johns Hopkins Univ, Biophysics Dept Baltimore 18, Md (1, 1948)

- Davis, Bernard D , M D Cornell Univ Med College, New York City *Research Assoc, Surgeon, USPHS* (6, 1948)
- Davis, George Kelso, Ph D Nutrition Lab , Animal Industry Dept , Agricultural Exper Station, Gainesville, Fla *Nutritional Technologist and Biochemist, Prof of Nutrition, Univ of Florida, Florida Agricultural Exper Station* (5, 1944)
- Davis, Hallowell, M D Central Inst for the Deaf, 818 S Kingshighway, St Louis 10, Mo (1, 1925)
- Davis, Harry A , M D College of Med Evangelists, Dept of Surgery, 2007 Wilshire Blvd , Los Angeles 5, Calif *Clin Prof of Surgery* (4, 1944)
- Davis, John Emerson, Ph D Univ of Arkansas School of Medicine, Little Rock *Prof of Pharmacology and Physiology* (1, 1941, 3, 1941)
- Davis, M Edward, M D Chicago Lying-In Hospital, 5541 Maryland Ave , Chicago 37, Ill *Joseph Bolvar de Lec Prof of Obstetrics, Univ of Chicago* (3, 1950)
- Dawson, Charles R , Ph D Columbia Univ , 411 Havemeyer Hall, New York City 27 *Assoc Prof of Chemistry* (2, 1946)
- Dawson, James Robertson, Jr , M D Univ of Minnesota School of Medicine, Minneapolis 14 *Prof of Pathology* (4, 1940)
- Dawson, Percy M , M D 97 E Portola Ave , Los Altos, Calif *Lecturer in Physiology, Retired, Stanford Univ* (1R, 1900)
- Day, Harry G , D Sc Indiana Univ , Dept of Chemistry, Bloomington *Professor* (2, 1948, 5, 1940)
- Day, Paul L , Ph D Univ of Arkansas School of Medicine, Little Rock *Prof of Biochemistry* (2, 1934, 5, 1933)
- De, N N , M B Indian Inst of Science, P O Malleswaram, Bangalore, India *Asst Prof of Pharmacology* (3, 1948)
- Dearborn, Earl H , Ph D , M D Johns Hopkins Univ School of Medicine, 710 N Washington St Baltimore 5, Md *Asst Prof of Pharmacology and Exper Therapeutics* (3, 1946)
- de Beer, Edwin J , Ph D The Wellcome Research Labs , Tuckahoe, N Y *Acting Dir of Research, Head, Dept of Pharmacology* (3, 1944)
- De Bodo, Richard C , M D 477 First Ave , New York City *Assoc Prof of Pharmacology, New York Univ College of Medicine* (1, 1932, 3, 1931)
- De Boer, Benjamin, Ph D St Louis Univ School of Medicine, 1402 S Grand Blvd , St Louis 4, Mo *Assoc Prof of Pharmacology* (1, 1947, 3, 1948)
- DeEds, Floyd, Ph D 344 Santa Ana Ave , San Francisco, Calif *Principal Pharmacologist, Western Regional Research Lab , Albany, Calif* (2, 1937, 3, 1927)
- Defandorf, James Holmes, Ph D 130 Hesketh St , Chevy Chase, Md *Colonel, Chem Corps , A U S* (3, 1940)
- de Gara, Paul F , M D 200 Pinehurst Ave , New York City *Instr in Pathology, Cornell Univ Med College, Physician, New York Hospital* (6, 1941)
- DeGraff, Arthur C , M D New York Univ College of Medicine, New York City *Prof of Therapeutics* (1, 1950, 3, 1937)
- de Gutierrez-Mahoney, C G , M D St Vincent's Hospital, New York City *Dir , Neurological Div and Neurosurgeon-in-Chief* (1, 1940, 4, 1941)
- Deichmann, William B , Ph D Albany Med College, Albany, N Y *Assoc Prof and Head, Div of Pharmacology* (3, 1941)
- del Pozo, E C , M D Medellin 196, Mexico, D F , Mexico (1, 1943)
- Dempsey, Edward W , Ph D Washington Univ School of Medicine, St Louis 10, Mo *Assoc Prof of Anatomy* (1, 1940)
- Denslow, J S , D O Kirksville College of Osteopathy, Still Memorial Research Trust, Kirksville, Mo *Researcher* (1, 1949)
- Denstedt, Orville F , Ph D McGill Univ , Dept of Biochemistry, Montreal, Que , Canada *Assoc Prof* (2, 1948)
- Derbyshire, Arthur J , Ph D Harper Hospital, EEG Dept , Detroit, Mich (1, 1939)
- de Savitsch, Eugene, M D Suite 24, 1150 Connecticut Ave , Washington, D C *Surgeon (R) USPHS, Consulting Surgeon, Home for Incurables* (4, 1934)
- Dethier, Vincent G , Ph D Johns Hopkins Univ , Dept of Biology, Baltimore 18, Md *Assoc Prof* (1, 1950)
- Dettwiler, Herman A , Ph D Eli Lilly and Co , Indianapolis, Ind *Asst Dir , Biological Div* (6, 1946)
- Deuel, Harry J , Jr , Ph D Univ of Southern California, Los Angeles *Prof of Biochemistry and Dean of Grad School* (1, 1928, 2, 1924, 6, 1933)
- Deulofeu, Venancio D Chem Casilla Correo 2539, Buenos Aires, Argentina *Prof of Organic Chemistry, Univ of Buenos Aires* (2, 1942)
- Deutsch, Harold F , Ph D Univ of Wisconsin, Dept of Physiological Chemistry, Madison 6 *Assoc Prof* (2, 1948)
- Dewey, Virginia C , Ph D Amherst College, Biological Lab , Amherst, Mass *Research Assoc , Dept of Biology* (2, 1949)
- Dey, Frederick L , Ph D , M D Niantic, Conn *Lt (j g) , U S A R* (1, 1945)
- Deyrup, Ingrith J , Ph D Barnard College,

- Columbia Univ, Dept of Zoology, New York City 27 Asst Prof (1, 1949)
- Dickison, H L**, Ph D Bristol Labs, Inc, Bldg 6, Syracuse 1, N Y Dir, *Pharmacological Research* (3, 1946)
- Dieckmann, William J**, M D The Chicago Lying-In Hospital, 5841 Maryland Ave, Chicago 37, Ill Chief of Service, Prof and Chairman of Dept of Obstetrics and Gynecology, Univ of Chicago (3, 1947)
- Dienes, Louis**, M D Massachusetts General Hospital, Boston *Bacteriologist* (6, 1924)
- Dill, David Bruce**, Ph D Med Div, Army Chemical Center, Md *Scientific Div* (1, 1941, 2, 1927, 5, 1936)
- Dille, James M**, Ph D, M D Univ of Washington School of Medicine, Seattle 5 Prof and Exec Officer, Dept of Pharmacology (3, 1939)
- Dillon, Robert T**, Ph D G D Searle and Co, Box 5110, Chicago 80, Ill Head, *Analytical Div* (2, 1934)
- Dingle, John H**, Sc D, M D Western Reserve Univ School of Medicine, Cleveland 6, Ohio Prof of Preventive Medicine (6, 1941)
- Di Palma, Joseph R**, M D Long Island College of Medicine, 350 Henry St, Brooklyn, N Y Assoc in Physiology (1, 1943)
- Dische, Zacharias**, M D Columbia Univ College of Physicians and Surgeons, Dept of Ophthalmology, 630 W 168th St, New York City 32 Asst Prof of Biochemistry (2, 1944)
- Dittmer, Karl**, Ph D Florida State Univ, Dept of Chemistry, Tallahassee Prof and Dept Exec (2, 1950)
- Dixon, Frank James, Jr**, M D Washington Univ, Dept of Pathology, Euclid Ave and Kingshighway, St Louis 10, Mo Instructor (6, 1950)
- Dixon, Harold M**, M D Veterans Admin Regional Office, San Diego, Calif (4, 1936)
- Doan, Charles A**, M D Ohio State Univ College of Medicine, Columbus Dean, Prof of Medicine (4, 1928)
- Dobriner, Konrad**, M D Sloan-Kettering Inst, 444 E 68th St, New York City 21 Member (2, 1946)
- Dochez, A Raymond**, M D Presbyterian Hospital, 620 W 168th St, New York City John E Borne Prof of Medicine and Surgical Research, Columbia Univ (4R, 1917, 6R, 1922)
- Dodd, Matthew C**, Ph D Ohio State Univ, Dept of Bacteriology, Columbus 10 Assoc Prof of Bacteriology and Immunology (6, 1950)
- Dodds, Mary L**, Ph D Pennsylvania State College, State College Prof, *Foods and Nutrition Research* (5, 1948)
- Dohan, F Curtis**, M D 80 Princeton Rd, Chadds Ford, Pa Fellow, George S Cox Med Research Inst, Assoc in Medicine, Univ of Pennsylvania (1, 1941)
- Daisy, Edward A**, Ph D St Louis Univ School of Medicine, St Louis 4, Mo Prof of Biological Chemistry (2, 1920)
- Dolman, C E**, DPH, Ph D Univ of British Columbia, Vancouver, B C, Canada Head of Dept of Bacteriology and Preventive Medicine (6, 1947)
- Dominguez, Rafael**, M D Western Reserve Univ, Cleveland, Ohio Asst Prof of Pathology, Dir, Div of Labs and Research, St Luke's Hospital (1, 1935)
- Donahue, D D**, D Sc 4228 Military Rd, N W, Washington 15, D C (3, 1941)
- Dooley, M S**, M D Syracuse Univ College of Medicine, Syracuse, N Y Prof of Pharmacology (3R, 1923)
- Dorfman, Albert**, PhD, M D Univ of Chicago, Dept of Pediatrics, Chicago, Ill Asst Prof (2, 1948)
- Dorfman, Ralph I**, Ph D Western Reserve Univ School of Medicine, Dept of Biochemistry, Cleveland, Ohio Asst Prof of Biochemistry (2, 1940)
- Doti, Louis Basil**, Ph D St Luke's Hospital, Amsterdam Ave and 113th St, New York City Chemist, Lecturer in Physiology and Biochemistry, New York Med College (1, 1937)
- Doty, J Roy**, Ph D American Dental Assoc, 222 E Superior St, Chicago, Ill Sr Chemist (2, 1941)
- Doudoroff, Michael**, Ph D Univ of California, Dept of Bacteriology, 3519 Life Sciences Bldg, Berkeley 4 Assoc Prof (2, 1946)
- Dougherty, Thomas F**, Ph D Univ of Utah School of Medicine, Dept of Anatomy, Salt Lake City Prof and Chairman of Dept (1, 1950)
- Dounce, Alexander L**, Ph D Strong Memorial Hospital, 260 Crittenden Blvd, Rochester N Y Instr in Biochemistry, Univ of Rochester School of Medicine and Dentistry (2, 1944)
- Doupe, Joseph**, M D Medical College, Dept of Physiology and Med Research, Bannatyne and Emily, Winnipeg, Manitoba, Canada Professor (1, 1949)
- Dow, Philip**, Ph D Medical College of Georgia, Augusta Assoc Prof of Physiology (1, 1939)
- Dow, Robert S**, M D, Ph D Univ of Oregon Med School, Portland, Assoc Prof of Anatomy (1, 1940)
- Downs, Ardrey W**, M D, D Sc Univ of Alberta, Edmonton, Alberta, Canada Prof of Physiology and Pharmacology (1R, 1917)
- Downs, Cora M**, Ph D 1625 Alabama St, Lawrence, Kan Prof of Bacteriology (6, 1929)
- Doyle, William Lewis**, Ph D 930 E 58th St, Chicago 37, Ill Prof of Anatomy, Univ of Chicago (1, 1946)
- Drabkin, David L**, M D Univ of Pennsylvania Grad School of Medicine, Philadelphia 4 Prof

- and Chairman of Dept of Physiological Chemistry (2, 1928, 5, 1934)
- Dragstedt, Carl A**, Ph D, M D Northwestern Univ Med School, 303 E Chicago Ave, Chicago, Ill Prof and Chairman of Dept of Pharmacology (1, 1928, 3, 1932)
- Dragstedt, Lester R**, M D, Ph D Univ of Chicago, Chicago, Ill Prof of Surgery (1, 1920)
- Draize, J H**, Ph D Food and Drug Admin, Div of Pharmacology, Federal Security Agency, Washington, D C Pharmacologist (3, 1940)
- Drake, T G H**, M B Univ of Toronto, Toronto, Canada Jr Demonstrator in Pediatrics, Dept of Medicine, Assoc Dir, Research Lab, Hospital for Sick Children (5, 1936)
- Draper, William B**, M Sc, M D Univ of Colorado School of Medicine, 4200 E 9th Ave, Denver Prof of Physiology and Pharmacology (1, 1947, 3, 1938)
- Dreisbach, Robert H**, Ph D, M D Stanford Univ School of Medicine, San Francisco 15, Calif Assoc Prof of Pharmacology (3, 1945)
- Dreyer, Nicholas Bernard**, M A Univ of Vermont School of Medicine, Burlington Prof of Physiology and Pharmacology (3, 1942)
- Drill, Victor Alexander**, Ph D, M D Wayne Univ College of Medicine, Detroit 26, Mich Prof of Pharmacology (1, 1943, 3, 1946)
- Drinker, Cecil K**, M D P O Box 502, Falmouth, Mass (1, 1915)
- Dripps, Robert D**, M D Univ of Pennsylvania School of Medicine, Philadelphia 4 Prof of Anesthesiology (1, 1947, 3, 1945)
- Driver, Robert L**, B S, M S, Ph D Med College of Alabama, Birmingham 5 (1, 1945, 3, 1947)
- Drury, Douglas R**, M D Univ of Southern California, Los Angeles Prof of Physiology (1, 1932)
- Dubin, Harry E**, Ph D 11 W 42nd St, New York City 18 President, H E Dubin Labs, Inc (2, 1925)
- Dubin, Isadore N**, M D Natl Cancer Inst, Bethesda, Md Special Research Fellow (4, 1947)
- Dubnoff, Jacob W**, Ph D 1201 E California St, Pasadena 4, Calif Sr Research Fellow, California Inst of Technology (2, 1946)
- DuBois, Eugene F**, M D Cornell Univ Med School, 1300 York Ave, New York City Prof and Head of Dept of Physiology and Biophysics, Attending Physician, New York Hospital (1, 1913, 3R, 1921, 5R, 1935)
- Du Bois, Kenneth P**, Ph D Univ of Chicago, Dept of Pharmacology, Chicago 37, Ill Asst Prof (3, 1946)
- Dubos, Rene J**, Ph D, D Sc Rockefeller Inst for Med Research, 60th St and York Ave, New York City Head of Dept of Bacteriology (6, 1938)
- Dugal, L Paul**, Ph D Laval Univ Med School, Research Dept on Acclimatization, Quebec City, Canada Research Prof and Head of Dept, Assoc Dir of Inst of Hygiene (1, 1947)
- Dukes, H H**, D V M, M S Cornell Univ, New York State Veterinary College, Ithaca Prof of Veterinary Physiology (1, 1934)
- Dulaney, Anna D**, Ph D Univ of Tennessee, Pathological Inst, Memphis Assoc Prof of Bacteriology, Med School (6, 1924)
- Dumke, Paul Rudolph**, M D Hospital of the Univ of Pennsylvania, Dept of Anesthesiology, Philadelphia Assoc Prof, Assoc in Clin Pharmacology, Univ of Pennsylvania (3, 1942)
- Dumm, Mary E**, Ph D New York Univ-Belle vue Med Center, 477 First Ave, New York City 16 Adjunct Asst Prof of Chemistry (1, 1950)
- Dunlap, Charles E**, M D Tulane Univ of Louisiana, 1430 Tulane Ave, New Orleans Prof of Pathology (4, 1942)
- Dunn, Max Shaw**, Ph D Univ of California, Los Angeles Prof of Chemistry (2, 1933)
- Dunn, Thelma Brumfield**, M D Natl Cancer Inst, Bethesda, Md Pathologist (4, 1945)
- Durlacher, Stanley H** Med Div, Army Chemical Center, Md Chief, Section on Pathology (1, 1949)
- Durrant, Edwin P**, Ph D Ohio State Univ, Columbus Assoc Prof Emeritus of Physiology (1R, 1928)
- Dury, Abraham**, Ph D Dorn Lab for Med Research, Bradford Clinic, Bennett at Pleasant St, Bradford, Pa Asst Dir of Research (1, 1948)
- Dutcher, James D**, Ph D Squibb Inst for Med Research, New Brunswick, N J Research Assoc, Div of Organic Chemistry (2, 1946)
- Dutcher, R Adams**, D Sc Pennsylvania State College, State College Prof and Head of Dept of Agriculture and Biochemistry (2, 1920, 5, 1933)
- Duval, Charles Warren**, M D Sacred Heart Hospital, Pensacola, Fla Prof Emeritus of Pathology and Bacteriology, Tulane Univ (4, 1913)
- du Vigneaud, Vincent**, Ph D Cornell Univ Med College, 1300 York Ave, New York City 21 Prof of Biochemistry (2, 1929, 5, 1934)
- Dworkin, Simon**, D D S, M D McGill Univ, Biology Building, Montreal, Quebec, Canada Lecturer in Physiology, Faculty of Medicine (1, 1931)
- Dye, J A**, Ph D Cornell Univ, James Law Hall Ithaca, N Y Prof of Physiology (1, 1929)
- Dye, Marie**, Ph D Michigan State College, East Lansing Dean, School of Home Economics (2, 1929, 5, 1933)

- Dyer, Helen M., Ph D Natl Cancer Inst Bethesda, Md *Biochemist* (2, 1936, 5, 1937)
- Dziemian, Arthur J., Ph D Med Div, Army Chemical Center, Md *Physiologist, Biophysics Section* (1, 1948)
- Dziewiatkowski, Dominic D., Ph D Rockefeller Inst for Med Research, Hospital, 66th St and York Ave, New York City 21 *Assoc, Dept of Biochemistry* (2, 1950)
- Eadie, George S., Ph D Duke Univ School of Medicine, Box 3709, Durham, N C *Prof of Physiology and Pharmacology* (1, 1929, 3, 1940)
- Eagle, Edward, Ph D Swift and Co., Research Labs, Chicago 9, Ill *Research Physiologist* (1, 1950)
- Eagle, Harry, M D Natl Insts of Health, Bethesda, Md *Med Dir, USPHS, Chief of Section on Exper Therapeutics, Microbiological Inst* (3, 1946, 4, 1936)
- Eakin, Robert E., Ph D Univ of Texas, Dept of Chemistry, Austin *Assoc Prof of Chemistry* (2, 1948)
- Earle, D P., Jr., M D, Med Sc D New York Univ College of Medicine, 477 First Ave, New York City 16 *Assoc Prof of Medicine* (1, 1947)
- Earle, Wilton R., Ph D Natl Cancer Inst, Bethesda, Md *Cytologist, Head of Tissue Culture Unit* (4, 1940)
- Eaton, Monroe D., M D Harvard Med School, Boston, Mass *Assoc Prof of Bacteriology and Immunology* (6, 1937)
- Eckenhoff, James E., M D Hospital of the Univ of Pennsylvania, Philadelphia *Asst Prof of Anesthesiology, Assoc in Clin Pharmacology, Univ of Pennsylvania* (1, 1948)
- Ecker, E E., Ph D Western Reserve Univ School of Medicine, 2085 Adelbert Rd, Cleveland, Ohio *Prof of Immunology* (4, 1925, 6, 1947)
- Eckstein, Gustav, M D Univ of Cincinnati College of Medicine, Cincinnati, Ohio *Assoc Prof of Physiology* (1, 1948)
- Eckstein, Henry C., Ph D Univ of Michigan, 320 W Med Building, Ann Arbor *Assoc Prof of Biological Chemistry* (2, 1925)
- Eckstein, R W., M A, M D Western Reserve Univ, Dept of Medicine, Cleveland, Ohio *Sr Instr, in charge of Cardiovascular Exper Med Research Lab* (1, 1947)
- Eddy, Bernice E., Ph D Natl Insts of Health, Inst of Microbiology, Bethesda 14, Md *Sr Bacteriologist* (6, 1949)
- Eddy, Nathan B., M D Natl Insts of Health, Bethesda Md *Med Officer* (3, 1929)
- Eddy, Walter H. Ph D Southern Bio Research Lab Florida Southern College Lakeland *Assoc Dir* (2 1913, 5R, 1933)
- Edelmann, Abraham, Ph D Brookhaven Natl Labs, Upton, L I, N Y *Physiologist* (1, 1948)
- Ederstrom, Helge E., Ph D St Louis Univ School of Medicine, Dept of Physiology, 1402 S Grand Blvd, St Louis 4, Mo *Asst Prof* (1, 1949)
- Edholm, O G., M B Natl Inst for Med Research, Hampstead, London, N W 3, England *Head, Dept of Human Physiology* (1, 1948)
- Edsall, Geoffrey, M D Boston Univ School of Medicine, 80 E Concord St, Boston 18, Mass *Prof of Bacteriology* (6, 1943)
- Edsall, John Tileston, M D Harvard Med School, Univ Lab, Boston, Mass *Assoc Prof of Biological Chemistry, Tutor in Biochemical Sciences* (2, 1931)
- Edwards, Dayton J., Ph D Cornell Univ Med College, 1300 York Ave, New York City *Assoc Prof of Physiology, Asst Dean* (1, 1921)
- Edwards, Jesse E., M D Mayo Clinic, Rochester 4, Minn *Asst Prof of Pathologic Anatomy, Mayo Foundation* (4, 1941)
- Edwards, J Graham, Ph D 24 High St, Buffalo, N Y *Assoc Prof of Anatomy, Univ of Buffalo* (1, 1932)
- Edwards, Leslie E., Ph D Med College of Virginia, Dept of Physiology and Pharmacology, Richmond *Asst Prof* (1, 1950)
- Eggerth, Arnold H., Ph D Hoagland Lab, 335 Henry St, Brooklyn, N Y *Assoc Prof of Microbiology and Immunology, State Univ Med Center at New York* (4, 1925)
- Ehrenstein, Maximilian R., Ph D Univ of Pennsylvania Hospital, 806 Maloney Clinic, 36th and Spruce Sts, Philadelphia 4 *Prof of Physiological Chemistry assigned to Medicine, Univ of Pennsylvania School of Medicine* (2, 1942)
- Ehrich, William E., M D Univ of Pennsylvania, Philadelphia *Prof of Pathology, Grad School of Medicine, Chief, Div of Pathology, Philadelphia General Hospital* (4, 1945)
- Eichelberger, Lillian, Ph D Univ of Chicago, Dept of Surgery, Chicago, Ill *Assoc Prof of Biochemistry* (2, 1937)
- Eichna, Ludwig W., M D New York Univ College of Medicine, Dept of Medicine, 477 First Ave, New York City 16 *Assoc Prof of Medicine, Visiting Physician, Bellevue Hospital* (1, 1949)
- Eiler, John J., Ph D Univ of California Med Center, San Francisco 22 *Assoc Prof of Biochemistry and Pharmacology, Univ of California College of Pharmacy* (2, 1948)
- Eisenman, Anna J., Ph D U S Public Health Service Hospital, Lexington Ky *Biological Chemist* (2, 1930)
- Elberg, Sanford S. Ph D Camp Detrick Frederick Md (6 1948)

- Elderfield, Robert C, Ph D Columbia Univ, New York City *Prof of Chemistry* (2, 1934)
- Elftman, Herbert, Ph D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City *Assoc Prof in Anatomy* (1, 1940)
- Ehot, Martha M, M D World Health Organization, Palais des Nations, Geneva, Switzerland *Asst Dir General* (5, 1933)
- Elkinton, J Russell, M D Univ of Pennsylvania Hospital, Dept of Medicine Philadelphia (1, 1947)
- Elliott, Henry W, Ph D Univ of California Med School, San Francisco *Lecturer and Research Assoc in Pharmacology* (3, 1948)
- Elliott, K Allan C, Ph D Montreal Neurological Inst, 3801 Univ St, Montreal, Que, Canada *Asst Prof of Exper Neurology and Biochemistry* (2, 1937)
- Ellis, C H, Ph D Labs of Gordon A Alles, 770 S Arroyo Parkway, Pasadena 5, Calif *Research Assoc in Physiology* (1, 1947)
- Ellis, Fred W, Ph D Univ of North Carolina, Chapel Hill *Assoc Prof of Pharmacology* (3, 1945)
- Ellis, Lillian N, Ph D Adelphi College, Garden City, Long Island, N Y *Instn in Chemistry* (5, 1940)
- Ellis, Max Mapes, Ph D Univ of Missouri Med School, Columbia *Prof of Physiology and Pharmacology* (1, 1923)
- Ellis, N R, M S U S Dept of Agriculture, Bureau of Animal Industry, Beltsville, Md *Asst Chief, Animal Husbandry Div* (2, 1928, 5, 1933)
- Ellis, Sidney, Ph D Temple Univ Med School, Dept of Pharmacology Philadelphia, Pa *Assoc Prof* (3, 1947)
- Elmadjian, Fred, Ph D Worcester State Hospital, Worcester, Mass *Staff Member, Worcester Foundation for Exper Biology, Research Assoc, Physiology Dept, Tufts Med School* (1, 1950)
- Elrod, Ralph P, Ph D 406 Med General Lab, APO 500, % Postmaster, San Francisco, Calif (6, 1947)
- Elser, William J, M D Kent, Conn (6R, 1920)
- Elvehjem, Conrad Arnold, Ph D Univ of Wisconsin, Biochemistry Bldg, Madison *Chairman, Dept of Biochemistry, Dean of Grad School* (2, 1931, 5, 1933)
- Embree, Norris Dean, Ph D Distillation Products, Inc, 755 Ridge Rd W, Rochester 13, N Y *Dir of Research* (2, 1946)
- Emerson, George A, Ph D Univ of Texas, Med Branch, Galveston *Prof of Pharmacology* (3, 1935)
- Emerson, Gladys A, Ph D Merck Inst of Therapeutic Research, Rahway, N J *Nutritionist* (5, 1942)
- Emerson, Oliver H, Ph D U S Dept of Agriculture, Western Regional Research Lab, Albany 6, Calif *Assoc Chemist* (2, 1938)
- Emery, Frederick E, D V M, Ph D Univ of Arkansas School of Medicine, Little Rock *Prof of Physiology and Pharmacology* (1, 1930)
- Emmatt, Emily W, Ph D Natl Insts of Health, Bethesda 14, Md *Cytologist* (3, 1947)
- Enders, John F, Ph D Harvard Univ Med School, Dept of Bacteriology, Boston, Mass *Assoc Prof of Bacteriology and Immunology, Chief, Research Div of Infectious Diseases, Children's Hospital* (6, 1936)
- Endicott, Kenneth M, M D USPHS, Natl Insts of Health, Bethesda 14, Md *Chief of Section on Metabolic and Degenerative Disease* (4, 1947)
- Engel, Frank L, M D Duke Univ School of Medicine, Durham, N C *Asst Prof of Medicine* (1, 1947)
- Engel, Lewis L, Ph D, Massachusetts General Hospital, Huntington Labs, Boston *Research Assoc in Biological Chemistry, Harvard Med School, Tutor in Biological Sciences, Harvard Univ* (2, 1949)
- Engel, Ruben W, Ph D Alabama Polytechnic Inst, Lab of Animal Nutrition, Auburn *Animal Nutritionist* (5, 1948)
- Engle, Earl Theron, Ph D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City *Prof of Anatomy* (1, 1930)
- English, James, Jr, Ph D Yale Univ, Sterling Chemistry Lab, New Haven, Conn *Asst Prof of Chemistry* (2, 1946)
- Enright, John B, Ph D Univ of California, School of Veterinary Medicine, Davis *Super vising Bacteriologist* (6, 1948)
- Epstein, Albert A, M D 1111 Madison Ave, New York City *Physician, Beth Israel Hospital and Hospital for Joint Diseases* (2, 1912)
- Ercoli, N, M D, Ph D Warner Inst for Therapeutic Research, New York City 11 *Dir of Pharmacological Research* (3, 1947)
- Erickson, Cyrus C, M D Univ of Tennessee, Inst of Pathology, Memphis 3 *Prof of Pathology* (4, 1941)
- Erickson, John Otto, Ph D Veterans Admin Center, Radioisotope Lab, Wilshire and Sawtelle Blvds, Los Angeles 25, Calif *Radioisotope Scientist* (6, 1946)
- Erlanger, Joseph, M D, Sc D Washington Univ School of Medicine, St Louis, Mo *Prof Emeritus of Physiology* (1R, 1901)
- Ershoff, Benjamin H, Ph D 1207 S Vermont Ave, Los Angeles 6, Calif *Research Assoc, Dept of Biochemistry and Nutrition, Univ of Southern California, Dir of Research, Emory W Thurston Labs, Los Angeles* (5, 1950)
- Eschenbrenner, Allen B, M D Natl Insts of

- Health, Natl Cancer Inst, Bethesda 14, Md (4, 1946)
- Espe, Dwight L, Ph D U S D A, Office of Exper Stations, Washington 25, D C (1, 1940)
- Essex, Hiram E, Ph D Mayo Foundation, Inst of Exper Medicine, Rochester, Minn *Prof of Physiology* (1, 1932, 3, 1940)
- Ettinger, C H, M D, C M Queen's Univ, Kingston, Ontario, Canada *Prof of Physiology, Asst Dir, Div of Med Research, Natl Research Council, Ottawa* (1, 1943)
- Evans, Earl Alison, Jr, Ph D Univ of Chicago, Dept of Biochemistry, Chicago, Ill *Prof and Chairman of Dept* (2, 1939)
- Evans, Everett Idris, M D, Ph D Med College of Virginia, Richmond *Prof of Surgery, Dir, Lab of Surgical Research* (1, 1935)
- Evans, Gerald Taylor, M D, Ph D Univ of Minnesota Hospitals, Minneapolis *Dir of Lab Service, Assoc Prof of Medicine, Univ of Minnesota* (1, 1942)
- Evans, Herbert M, M D Univ of California, Berkeley *Prof of Anatomy and Dir of Inst of Exper Biology* (1, 1919)
- Evans, Titus C, Ph D State Univ of Iowa, College of Medicine, Iowa City *Research Prof, Head of Radiation Research Lab* (1, 1949)
- Eveleth, D F, Ph D, D V M North Dakota Agricultural College, Fargo *Prof of Veterinary Science, North Dakota Agricultural Exper Station* (2, 1939)
- Everett, Guy M, Ph D Abbott Labs, Research Div, N Chicago, Ill *Sr Pharmacologist* (3, 1950)
- Everett, Mark R, Ph D Univ of Oklahoma School of Medicine, 800 N E 13th St, Oklahoma City 4 *Dean, School of Medicine, Supt of Univ Hospitals* (2, 1929)
- Eversole, Wilburn J, M Sc, Ph D Syracuse Univ, Rm 27, Lyman Hall, Syracuse 10, N Y *Assoc Prof of Zoology* (1, 1950)
- Everson, Gladys, Ph D Iowa State College, Ames *Assoc Prof of Nutrition* (5, 1948)
- Ewing, P L, Ph D Univ of Texas School of Medicine, Galveston *Assoc Prof of Pharmacology* (3, 1938)
- Eyster, John A English, M D Univ of Wisconsin, Madison *Prof of Physiology* (1, 1906, 3R, 1908)
- Fahr, George, M D Univ of Minnesota, 102 Millard Hall, Minneapolis *Prof of Clin Medicine* (1, 1913, 3, 1940)
- Failey, Crawford F, Ph D 416 S 6th St, Terre Haute, Ind *Prof of Biochemistry, Univ of Chicago* (2, 1933)
- Fairhall, Lawrence T, Ph D USPHS, Washington, D C *Scientist Dir* (2, 1924)
- Falk, Carolyn R, B A 40 E 66th St, New York City *Bacteriologist, Bureau of Labs, Dept of Health* (6, 1943)
- Falk, K George, Ph D Lab of Industrial Hygiene, Inc, 254 W 31st St, New York City 1 *Director* (2, 1913)
- Famulener, Lemuel W, Ph D, M D 275 Engle St, Englewood, N J (6R, 1920)
- Farah, Alfred E, M D Univ of Washington School of Medicine, Seattle *Assoc Prof of Pharmacology* (3, 1948)
- Farber, Sidney, M D Harvard Med School, 25 Shattuck St, Boston, Mass *Asst Prof of Pathology* (4, 1934)
- Farmer, Chester J, A.M Northwestern Univ Med School, 303 E Chicago Ave, Chicago, Ill *Prof of Chemistry* (2, 1935)
- Farr, Lee E, M D Brookhaven Natl Lab, Upton, L I, N Y *Chairman, Med Dept, Dir, Dept of Med Sciences, Physician-in-Chief, Brookhaven Natl Lab Hospital* (4, 1941)
- Farrar, George E, Jr, M D Wyeth Inc, 1600 Arch St, Philadelphia 3, Pa *Med Dir* (3, 1947)
- Farrell, James L, Ph D, M D 636 Church St, Evanston, Ill (1, 1938)
- Fassett, David W, M D Eastman Kodak Co, Lab of Industrial Medicine, Kodak Park Works, Rochester, N Y *Dir of Lab* (3, 1942)
- Faulconer, Albert, Jr, M D Mayo Clinic, Rochester, Minn *Consultant in Anesthesiology* (3, 1950)
- Fay, Marion, Ph D Woman's Med College of Pennsylvania, Philadelphia 29 *Dean, Prof of Physiological Chemistry* (2, 1937)
- Featherstone, Robert M, Ph D State Univ of Iowa, College of Medicine, Iowa City *Assoc Prof of Pharmacology* (3, 1947)
- Feigen, George A, Ph D Stanford Univ, Dept of Physiology, Stanford, Calif *Instructor* (1, 1948)
- Feinstein, Robert N, Ph D Univ of Chicago, Toxicity Lab, 930 E 58th St, Chicago 37, Ill *Asst Prof, Dept of Biochemistry* (2, 1950)
- Feldman, Harry A, M D 704 Crawford Ave, Syracuse 10, N Y *Assoc Prof of Medicine* (6, 1943)
- Feldman, William H, D V M, M S, D Sc Mayo Foundation, Rochester, Minn *Prof in the Div of Exper Surgery and Pathology* (4, 1934)
- Fell, Norbert, Ph D 1880 N W 28th St Miami, Fla (6, 1944)
- Feller, A E, M D Western Reserve School of Medicine, Cleveland 6, Ohio *Assoc Prof of Preventive Medicine* (6, 1943)
- Fellows, Edwin J, Ph D Temple Univ School of Medicine, Philadelphia, Pa *Asst Prof of Pharmacology* (3, 1939)
- Felsenfeld, Oscar, M D, D T M & H Heiktoen

- Research Inst , 629 South Wood St , Chicago 12, Ill *Dir of Bacteriology* (6, 1949)
- Felton, Lloyd D**, M D , D Sc USPHS, Natl Insts of Health, Bethesda, Md *Med Dir* (6, 1926)
- Fenn, Wallace Osgood**, Ph D Univ of Rochester School of Medicine and Dentistry, 260 Crittenden Blvd , Rochester, N Y *Prof of Physiology* (1, 1924)
- Fenning, Con**, M D Univ of Utah School of Medicine, Salt Lake City *Prof of Pharmacology and Physiology* (1, 1942)
- Fenton, P F**, Ph.D Brown Univ , Providence, R I *Assoc Prof of Biology* (1, 1947, 5, 1949)
- Ferguson, Frederick P**, Ph D Univ of Maryland School of Medicine, Dept of Physiology, Baltimore 1 (1, 1949)
- Ferguson, James Kenneth Wallace**, M A , M D 76 Kilbarry Rd , Toronto, Ont , Canada *Prof of Pharmacology, Univ of Toronto* (1, 1933, 3, 1941)
- Ferguson, John**, Ph D Creighton Univ School of Medicine, Omaha 2, Neb *Asst Prof of Physiology* (1, 1949)
- Ferguson, John Howard**, M A , M D Univ of North Carolina School of Medicine, Dept of Physiology, Chapel Hill *Prof of Physiology and Acting Prof of Pharmacology* (1, 1933)
- Ferguson, L Kraeer**, M D 133 S 36th St , Philadelphia 4, Pa *Prof of Surgery, Grad School, Univ of Pennsylvania, Surgeon, Doctors' Hospital and Woman's Med College, Philadelphia General Hosp* (4, 1935)
- Ferguson, Ralph L**, M D 606 Porter St , Joplin Mo (4, 1949)
- Ferry, John Douglass**, Ph D Univ of Wisconsin, Madison *Prof of Chemistry* (2, 1941)
- Ferry, Ronald M**, M D 966 Memorial Drive, Cambridge, Mass *Assoc Prof of Biochemistry and Tutor in Biochem Sciences, Harvard Univ , Master of Winthrop House* (2, 1924)
- Fetcher, Edwin S**, Ph D Steamboat Springs, Colo (1, 1944)
- Fetter, Dorothy**, Ph D Brooklyn College, Dept of Hygiene, Brooklyn, N Y *Instr in Physiology* (1, 1944)
- Fevold, Harry L**, Ph D Quartermaster Food and Container Inst , 1849 W Pershing Rd , Chicago 9, Ill *Chief, Food Research Div* (2, 1942)
- Field, John, II**, Ph D Navy Dept Office of Naval Research, Med Sciences Div , Washington, D C *Head of Ecology Branch* (1, 1930)
- Fincke, Margaret L**, Ph D Oregon State College, Corvallis *Prof and Head of Dept of Foods and Nutrition, Dept of Home Economics* (5, 1940)
- Findley, Thomas, Jr**, M D Ochsner Clinic, 3503 Prytanis, New Orleans, La *Head of Dept of Internal Medicine, Asst Prof of Clin Medicine, Tulane Univ School of Medicine* (1, 1938)
- Finerty, John C**, Ph D Univ of Texas Med Branch, Galveston *Assoc Prof of Anatomy* (1, 1947)
- Finland, Maxwell**, M D Boston City Hospital, Boston, Mass *Asst Prof of Medicine, Harvard Med School* (6, 1941)
- Finnegan, J K**, Ph D Med College of Virginia, Richmond 19 *Assoc Prof of Pharmacology* (3, 1947)
- Finor, Warfield Monroe**, M D Johns Hopkins Hospital, Baltimore, Md *Assoc Prof of Surgery, Johns Hopkins Univ* (1, 1932)
- Fischel, Edward E**, M D , D.Med Sc 23 Haven Ave , New York City 32 *Fellow in Medicine, Columbia Univ* (6, 1948)
- Fischer, Ernst**, M D Med College of Virginia, Richmond *Prof of Physiology* (1, 1936)
- Fischer, Hermann O L**, Ph D Univ of California, Virus Lab , Forestry Bldg , Berkeley 4 *Lecturer, Dept of Biochemistry* (2, 1940)
- Fischer, Martin H**, M D Univ of Cincinnati College of Medicine, Eden Ave , Cincinnati 19, Ohio *Prof of Physiology* (1, 1901, 2, 1919)
- Fishberg, Ella H**, M A , M D Beth Israel Hospital, Stuyvesant Park E , New York City *Biochemist* (2, 1931)
- Fisher, Albert Madden**, Ph D Univ of Toronto, Connaught Med Research Labs , Toronto 5, Ont , Canada *Asst Dir* (2, 1944)
- Fisher, Kenneth C**, Ph D Defense Research Northern Lab , Fort Churchill, Manitoba, Canada *Superintendent* (1, 1940)
- Fishman, William H**, Ph D Tufts College Med School, 30 Bennet St , Boston 15, Mass *Research Prof of Biochemistry* (2, 1947)
- Fiske, Cyrus H**, M D Harvard Med School, Boston, Mass *Prof of Biological Chemistry* (2, 1914)
- Fitzgerald, Mabel P**, 54 A George Sq , Edinburgh, Scotland (1R, 1913)
- Fitzhugh, O Garth**, Ph D Federal Security Agency, Food and Drug Admin , Div of Pharmacology, Washington, D C *Chief, Chronic Toxicity Branch* (3, 1940)
- Fleischmann, Walter**, M D , Ph D V A Hospital, Fort Howard, Md *Dr , Sr Grade, Instr in Pediatrics, Johns Hopkins Univ* (1, 1940)
- Fleisher, Moyer S**, M D Jewish Hospital, St Louis, Mo *Research Bacteriologist* (4, 1924, 6, 1932)
- Flexner, Louis B**, M D Carnegie Labs , Carnegie Inst of Washington, Wolfe and Madison Sts , Baltimore 5, Md *Staff member, Dept of Embryology* (1, 1933, 2, 1948)
- Flick, John A** Univ of Pennsylvania School of Medicine, Dept of Bacteriology, Philadelphia 4 *Asst Prof of Bacteriology* (6, 1949)

- Flock, Eunice V**, Ph D Mayo Clinic, Rochester, Minn *Assoc Prof in Exper Medicine, Mayo Foundation, Univ of Minnesota* (2, 1940)
- Florman, Alfred L**, M D Mt Sinai Hospital, New York City *Investigator in Virology* (6, 1942)
- Flosdorf, Earl W**, Ph D Forest Grove, Pa *Dir of Research, F J Stokes Machine Co, Philadelphia* (6, 1941)
- Floyd, Cleveland**, M D, Sc D 246 Marlborough St, Boston, Mass *Chief Examiner, Boston Health Dept* (6, 1916)
- Foa, Piero Pio**, Ph D 710 S Wolcott St, Chicago, Ill *Assoc Prof of Physiology and Pharmacology, Chicago Med School* (1, 1944)
- Folch, Jordi**, M D McLean Hospital, Waverly, Mass *Asst Prof of Biological Chemistry, Harvard Med School, Dir of Scientific Research, McLean Hospital* (2, 1941)
- Folkers, Karl**, Ph D Merck and Co, Inc, Rahway, N J *Dir of Organic and Biochemical Research* (2, 1947)
- Follensby, Edna M**, Ph D 80 E Concord St, Boston, Mass *Research Asst, Evans Memorial, Special Instr in Biology, Simmons College* (6, 1933)
- Follis, Richard H, Jr**, M D Johns Hopkins Univ School of Medicine, Baltimore, Md *Assoc Prof of Pathology* (4, 1942)
- Fontaine, Thomas Davis**, Ph D U S Dept of Agriculture, Bureau of Agricultural and Industrial Chemistry, Beltsville, Md *Biochemist, Head, Div of Biologically Active Compounds* (2, 1946)
- Foot, Nathan Chandler**, M D Cornell Univ Med College, New York City *Prof Emeritus of Surgical Pathology* (4, 1924)
- Forbes, Alexander, A M**, M D Harvard University, Biological Labs, Divinity Ave, Boston, Mass (1, 1910)
- Forbes, Ernest B**, Ph D State College, Pa *Prof Emeritus of Animal Nutrition* (1R, 1917, 5R, 1935)
- Forbes, Henry S**, M D Harvard Med School, Boston, Mass *Assoc in Neuropathology* (1R, 1931)
- Forbes, John C**, Ph D Med College of Virginia, Richmond *Research Prof of Biochemistry* (2, 1937)
- Forbes, William H**, Ph D Harvard Univ Boston, Mass *Asst Dir, Fatigue Lab, Asst Prof of Industrial Physiology* (1, 1943)
- Forman, Carolyn**, M D New York Hospital, Dept of Pediatrics 525 E 68th St New York City 21 *Research Fellow* (4, 1950)
- Forney, Robert B**, Ph D Indiana Univ School of Medicine Indianapolis *Asst Prof of Toxicology* (3, 1950)
- Forsander, C A**, M D Univ College of the West Indies, Dept of Physiology, Kingston, Jamaica, B W I (1, 1950)
- Forster, Francis M**, M D 3800 Reservoir Rd, Washington, D C *Prof and Dir of Dept of Neurology, Georgetown Univ School of Medicine*, (1, 1948)
- Forster, Roy P**, Ph M, Ph D Dartmouth College, Dept of Zoology, Hanover, N H *Professor* (1, 1950)
- Fosdick, Leonard S**, Ph D 311 E Chicago Ave, Chicago, Ill *Prof of Chemistry, Northwestern Univ* (2, 1944)
- Foster, G L**, Ph D Columbia Univ, College of Physicians and Surgeons, 630 W 168th St, New York City *Prof of Biological Chemistry* (2, 1923)
- Foster, Harry E**, M D Fairmont Hotel San Francisco, Calif *Retired Med Dir, Cutter Labs* (6, 1913)
- Foster, Jackson W**, Ph D Univ of Texas, Dept of Bacteriology, Austin 12 *Prof of Bacteriology* (2, 1946)
- Foster, Robert H K**, Ph D, M D St Louis Univ School of Medicine, St Louis, Mo *Prof and Dir of Dept of Pharmacology* (1, 1940, 3, 1944)
- Fothergill, LeRoy D**, M D Camp Detrick, Frederick, Md *Asst Technical Dir* (6, 1936)
- Fowler, Ward S**, M D Univ of Pennsylvania, Grad School of Medicine, Dept of Physiology and Pharmacology, Philadelphia 4 *Asst Prof of Physiology* (1, 1950)
- Fox, Sidney W**, Ph D Iowa State College, Chemistry Dept, Ames *Prof of Chemistry, Prof in charge, Chemistry Section, Iowa Agricultural Exper Station* (2, 1946)
- Fraenkel, Gottfried S**, Ph D Univ of Illinois, Urbana *Prof of Entomology* (5, 1949)
- Fraenkel-Conrat, Heinz**, M D, Ph D U S Dept of Agriculture, Western Regional Research Lab, Albany 6, Calif *Sr Chemist* (2, 1942)
- Francis, Thomas, Jr**, M D Univ of Michigan School of Public Health, Ann Arbor *Henry Sewall Prof of Epidemiology* (4, 1940, 6, 1930)
- Franke, Florent E**, M D 9 Sylvester Ave, Webster Groves, Mo *Asst Prof of Physiology, St Louis Univ School of Medicine* (1, 1934)
- Frankel, Edward M**, Ph D 214 River Rd, Nyack 9, N Y *Consulting Chemist* (2, 1916)
- Fraps, R M**, Ph D U S Dept of Agriculture, Bureau of Animal Husbandry Beltsville, Md *Sr Physiologist* (1, 1947)
- Fraser, Donald T**, M B, D P H Univ of Toronto, Connaught Labs, Toronto 5, Canada *Prof of Hygiene and Preventive Medicine* (6, 1935)
- Frear, Donald E H**, Ph D Pennsylvania State College Dept of Agricultural and Biological

- Chemistry, State College *Prof of Agricultural and Biological Chemistry* (2, 1946)
- Fredette, Victorian Univ of Montreal, Montreal, Que, Canada *Asst Prof of Bacteriology, Assoc Dir, Inst of Microbiology* (6, 1948)
- Free, Alfred H, Ph D Miles Labs, Inc, Research Lab, Elkhart, Ind *Head of Biochemistry Section* (2, 1946, 5, 1944)
- Freeman, Harry, M D Worcester State Hospital, Worcester, Mass *Internist, Research Service* (1, 1939)
- Freeman, Leslie Willard, Ph D, M D Univ of Indiana, Dept of Surgery, Indianapolis *Asst Prof of Surgery, Dir of Surgical Research* (1, 1944)
- Freeman, Norman E, M D Univ of California Med School, Dept of Surgery, San Francisco 22 (1, 1936)
- Freeman, Smith, M D, Ph D Northwestern Univ School of Medicine, 303 E Chicago Ave, Chicago, Ill *Prof and Chairman of Dept of Exper Medicine* (1, 1937)
- French, Cyrus E, Ph D Pennsylvania State College, State College *Assoc Prof of Animal Nutrition* (5, 1948)
- French, C Stacy, Ph D Carnegie Institution of Washington Stanford Univ, Calif *Dir, Div of Plant Biology* (2, 1946)
- Freund, Jules, M D Public Health Research Inst of the City of New York, New York City *Chief of the Div of Applied Immunology* (6, 1924)
- Frey, Charles N, Ph D Standard Brands Inc, 595 Madison Ave at 57th St, New York City 22 *Dir, Scientific Relations* (5, 1948)
- Fried, Josef, Ph D Squibb Inst for Med Research, New Brunswick, N J *Assoc Member, Div of Organic Chemistry* (2, 1948)
- Friedemann, Theodore E, Ph D Northwestern Univ Med School, 303 E Chicago Ave, Chicago, Ill *Assoc Prof of Physiology* (2, 1925)
- Frieden, Edward H, Ph D Harvard Univ, 16 Divinity Ave, Cambridge 38, Mass *Research Fellow, Dept of Biology, Instr, Dept of Biochemistry* (2, 1950)
- Friedenwald, Jonas S, M A, M D 1212 Eutaw Place, Baltimore 17, Md *Assoc Prof of Ophthalmology, Johns Hopkins Hospital* (1, 1947)
- Friedewald, William F, M D Emory Univ School of Medicine, Atlanta, Ga *Prof of Bacteriology, Assoc Prof of Medicine* (4, 1941)
- Friedgood, Harry B, M D Univ of California Los Angeles *Assoc Clin Prof of Medicine, Pres and Chairman, California Inst of Cancer Research* (1, 1936)
- Friedman, Maurice H, Ph D, M D 2040 Belmont Rd, Washington 9, D C (1, 1929)
- Friedman, Meyer, M D Harold Brunn Inst for Cardiovascular Research, Mt Zion Hospital, 2200 Post St, San Francisco, Calif *Director* (1, 1947)
- Friedman, M H F, Ph D Jefferson Med College of Philadelphia, 1025 Walnut St, Philadelphia, Pa *Assoc Prof of Physiology* (1, 1941)
- Friedman, Nathan B, M D Cedars of Lebanon Hospital, Div of Labs, 4833 Fountain Ave, Los Angeles, Calif *Director* (4, 1942)
- Friedman, Sydney M, M D, Ph.D Univ of British Columbia, Vancouver, B C, Canada *Prof of Anatomy* (1, 1947)
- Friedman, Townsend B Children's Memorial Hospital, Chicago, Ill *Attending Allergist* (6, 1948)
- Frost, Douglas Van Anden, Ph D Abbott Labs, N Chicago, Ill *Head of Nutritional Research* (2, 1946, 5, 1947)
- Fruton, J S, Ph D Yale School of Medicine, 333 Cedar St, New Haven, Conn *Prof of Biochemistry* (2, 1938)
- Fugo, Nicholas W, Ph D Univ of Chicago, Dept of Obstetrics and Gynecology, Chicago, Ill (3, 1944)
- Fuhrman, Frederick A, Ph D Stanford Univ, Stanford Univ, Calif *Asst Prof of Physiology* (1, 1946)
- Fulton, George P, Ph D Boston Univ, Dept of Biology, 675 Commonwealth Ave, Boston, Mass *Asst Prof* (1, 1950)
- Fulton, John Farquhar, Ph D, M D Yale Univ School of Medicine, New Haven, Conn *Sterling Prof of Physiology* (1, 1925)
- Funk, Casimir, D Sc, Ph D 186 Riverside Drive, New York City 24 *Emeritus* 1949 (2, 1921)
- Furchgott, Robert F, Ph D Washington Univ School of Medicine, Euclid Ave and Kings highway, St Louis 10, Mo *Asst Prof of Pharmacology* (2, 1948)
- Furth, Jacob, M D Oak Ridge Natl Lab, Oak Ridge, Tenn *Chief of Pathology Section, Div of Biology* (4, 1932)
- Gaddum, John H, ScD, FRS Univ of Edinburgh, Edinburgh, Scotland *Prof of Pharmacology* (3, 1950)
- Gaebler, Oliver H, Ph D, M D Henry Ford Hospital, Detroit, Mich *Head, Dept of Biochemistry, Edsel B Ford Inst for Med Research* (2, 1927)
- Gaffron, Hans, Ph D Univ of Chicago, Dept of Chemistry, 5747 Ellis Ave, Chicago 37, Ill *Assoc Prof of Biochemistry, Member, Inst of Radio-Biology and Biophysics* (2, 1941)
- Gagge, Adolf Pharo, Ph D Office of The Surgeon General, H Q, U S A F, Washington, D C *Chief, Med Research Division, Lt Col, U S A F* (1, 1939)
- Galambos, Robert, Ph D, M D Psycho Acoustic

- Lab, Cambridge, Mass *Research Fellow, Harvard Univ* (1, 1942)
- Galdston, Morton, M D Goldwater Memorial Hospital, New York Univ Research Service, Welfare Island, New York City 17 *Instr in Medicine, Research Assoc* (1, 1950)
- Gall, Edward A, M D Cincinnati General Hospital, Cincinnati, Ohio *Mary M Emery Prof of Pathology, Univ of Cincinnati College of Medicine* (4, 1941)
- Gallagher, Thomas F, Ph D Sloan-Kettering Inst, 444 E 68th St, New York City 21 *Member* (2, 1932)
- Gallup, Willis D, Ph D Oklahoma Agricultural and Mechanical College, Stillwater *Chemist and Prof of Agricultural Chemistry* (2, 1932)
- Gamble, James L, M.D, S M 33 Edgehill Rd, Brookline, Mass *Prof Emeritus of Pediatrics, Harvard Med School* (2, 1922, 5, 1933)
- Gantt, W Horsley, M D Johns Hopkins Hospital, Phipps Psychiatric Clinic, Baltimore, Md *Assoc in Psychiatry* (1, 1935)
- Garbat, Abraham L, M D 103 E 78th St, New York City *Dir, Med Service, Lenox Hill Hospital, Clin Prof of Medicine, New York Univ Med School* (6, 1913)
- Gardner, Ernest, M D Wayne Univ College of Medicine, 1512 St Antoine St, Detroit 26, Mich *Asst Prof of Anatomy* (1, 1949)
- Garner, Raymond L, Ph D Univ of Michigan Med School, 218 W Med Bldg, Ann Arbor *Asst Prof of Biological Chemistry* (2, 1947)
- Garrey, Walter Eugene, Ph D, M D Vanderbilt Univ School of Medicine, Nashville, Tenn *Prof Emeritus of Physiology* (1R, 1910, 2, 1906)
- Gasser, Herbert S, A.M, M D Rockefeller Inst for Med Research, 66th St and York Ave, New York City *Dir of Labs* (1, 1915, 3, 1924)
- Gassner, Frank X, M S, D V M Colorado A and M College, Fort Collins *Prof of Endocrine Research, Colorado State Exper Station* (1, 1947)
- Gates, Olive, M D Harvard Med School, 25 Shattuck St, Boston, Mass *Assoc Pathologist* (4, 1940)
- Gaunt, Robert, Ph D Syracuse Univ, Syracuse, N Y *Prof and Chairman of Dept of Zoology* (1, 1939)
- Gay, Leslie N, M D 1114 St Paul St, Baltimore, Md *Dir of Allergy Clinic, Johns Hopkins Hospital, Assoc Prof of Medicine, Johns Hopkins Univ* (6, 1927)
- Geiger, Ernest, Ph D 3842 Monteith Dr, Los Angeles 43, Calif *Prof of Pharmacology and Toxicology, Univ of Southern California Med School, Los Angeles, Advisor in Nutrition, Van Camp Seafood Co, Terminal Island* (5, 1950)
- Geiling, E M K, M D, Ph D Univ of Chicago, Chicago 37 Ill *Chairman of Dept of Pharmacology* (1, 1933, 2, 1927, 3, 1925)
- Gelfan, Samuel, Ph D Yale Univ School of Medicine, 333 Cedar St, New Haven 11, Conn *Assoc Prof of Physiology, Dir, Aero-Med Research Unit* (1, 1930)
- Gellhorn, Alfred, M D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 *Assoc Prof of Clin Cancer Research, Dept of Medicine* (3, 1946)
- Gellhorn, Ernst, M D, Ph D Univ of Minnesota, Room 116, Med Sciences, Minneapolis *Prof of Neurophysiology* (1, 1930)
- Gemmell, Chalmers L, M D Univ of Virginia Med School, Charlottesville *Prof of Pharmacology* (1, 1928, 2, 1935, 3, 1946)
- Gerard, R W, Ph D, M D Univ of Chicago, Chicago, Ill *Prof of Physiology* (1, 1927)
- Gerheim, Earl B, Ph D Univ of Detroit, Div of Basic Sciences, 630 E Jefferson, Detroit 26, Mich *Asst Prof* (1, 1950, 6, 1950)
- Gersh, Isadore, Ph D Univ of Chicago, Chicago 37, Ill *Assoc Prof of Anatomy* (4, 1947)
- Gerstenberger, Henry John, M D Western Reserve Univ School of Medicine, Cleveland, Ohio *Prof Emeritus of Pediatrics, Dir of Pediatrics, Babies and Children's Hospital* (5R, 1938)
- Gesell, Robert, M D Univ of Michigan, Ann Arbor *Prof of Physiology* (1, 1913)
- Gettler, Alexander O, Ph D New York Univ, 29 Washington Square, New York City *Prof of Chemistry and Toxicology, Toxicologist to Chief Med Examiner's Office* (2, 1916)
- Gey, George Otto, M D Johns Hopkins Univ, Baltimore, Md *Instr in Surgery* (1, 1940)
- Geyer, Robert P, Ph D Harvard School of Public Health, Dept of Nutrition, Boston, Mass *Asst Prof* (5, 1950)
- Gibbard, J, M Sc Dept of Natl Health and Welfare, Ottawa, Ont, Canada *Chief, Lab of Hygiene* (6, 1946)
- Gibbs, Frederick Andrews, M D 723 N Michigan Ave, Suite 610 Chicago, Ill (1, 1935)
- Gibbs, Owen Stanley, M D P O Box 5387, Whitehaven, Memphis 16, Tenn *Dir, Gibbs Med Research Lab* (3, 1930)
- Gibson, Robert Banks, Ph D Univ Hospital, Iowa City, Iowa *Assoc Prof of Biochemistry, State Univ of Iowa* (2, 1906)
- Gies, William John, Ph D Columbia Univ, 632 W 168th St, New York City *Prof of Biological Chemistry* (1R, 1898, 2, 1906, 3R, 1909)
- Gilman, Alfred, Ph D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 *Prof of Pharmacology* (1, 1935, 3, 1934)
- Gilson, Arthur S, Jr, Ph D Washington Univ Med School, St Louis, Mo *Assoc Prof of Physiology* (1, 1927)
- Githens, Thomas S, M D The Cambridge Alden

- Park, Wissahickon and School Lane, Germantown, Philadelphia, Pa (1R, 1915)
- Givens, Maurice H , Ph D Box 5116, Biltmore, N C (1, 1917, 2, 1915)
- Gjessing, Erland C , Ph D Univ of Virginia, Box 1062, Univ Station, Charlottesville Asst Prof of Biochemistry (2, 1948)
- Glaser, O C , Ph D Amherst College, Amherst Mass Prof Emeritus of Biology (1R, 1913)
- Glass, Howard G , Ph D 514 Wesley Ave , Oak Park, Ill (3, 1947)
- Glazko, Anthony J , Ph D Parke, Davis and Co , Research Labs , Detroit 32, Mich Research Biochemist (1, 1942)
- Glick, David, Ph D Univ of Minnesota, 225 Med Sciences Bldg , Minneapolis 14 Prof of Physiological Chemistry (2, 1936)
- Glickman, Nathaniel, MS 8239 Abbot Ave , Miami Beach, Fla (1, 1947)
- Gochenour, William S , Jr , V M D Army Med Center, A M D R G S , Veterinary Div , Washington 12, D C Chief, Dept of Veterinary Bacteriology (6, 1950)
- Goebel, Walther F , Ph D Rockefeller Inst for Med Research, 66th St and York Ave , New York City Member (2, 1929, 6, 1937)
- Goettsch, Marianne, Ph D Univ of Puerto Rico Med School, San Juan Asst Prof of Biochemistry and Nutrition (2, 1933, 5, 1941)
- Goetzl, Franz R , Ph D , M D Permanente Foundation, Dept of Med Research, Oakland 11, Calif Director (1, 1947)
- Gold, Harry, M D Cornell Univ Med College, New York City Prof of Clin Pharmacology, (3, 1927)
- Goldblatt, Harry, M D Cedars of Lebanon Hospital, Los Angeles, Calif Dir , Inst for Med Research (1, 1945, 4, 1927)
- Golden, Alfred, M D Veterans Admin Hosp , Buffalo, N Y Chief of Lab Service, Lecturer in Pathology, Univ of Buffalo Schobl of Medicine (4, 1947)
- Goldfarb, Walter, M D 25 E 86th St , New York City (1, 1938)
- Goldfeder, Anna , D Sc 444 E 52nd St , New York City 22 Sr Biologist in Charge of Cancer Research, Dept of Hospitals, City of New York, Research Assoc , Dept of Biology, New York Univ (1, 1950)
- Goldforb, A J , Ph D College of the City of New York, New York City Prof of Biology (1, 1930)
- Goldie, Horace, M D , D T M 7910 Lynnbrook Drive, Bethesda, Md Research Immunologist, Nail Cancer Inst (6, 1943)
- Goldring, William, M D New York Univ College of Medicine, 477 First Ave , New York City Assoc Prof of Medicine (1, 1939)
- Goldschmidt, Samuel, Ph D Univ of Pennsylvania Med School, Philadelphia Prof of Physiology (1, 1919, 2, 1915)
- Goldsmith, E D , Ph D New York Univ , 477 First Ave , New York City 16 Assoc Prof of Anatomy, Grad School of Arts and Sciences, Assoc Prof of Histology and Research Coordinator, College of Dentistry (1, 1950)
- Goldsmith, Grace A , M D Tulane Univ of Louisiana, New Orleans Asst Prof of Medicine (5, 1943)
- Goldstein, Avram, M D Harvard Med School, Boston, Mass Assoc in Pharmacology (3, 1948)
- Gollan, Frank, M D Antioch College, Fels Research Inst , Fels House, Yellow Springs, Ohio Assoc Prof of Physiology (1, 1950)
- Golub, Orville Joseph, Ph D Bio-Service Labs , Inc , 10717 Venice Blvd , Los Angeles 24, Calif Associate (6, 1944)
- Gomori, George, M D , Ph D Univ of Chicago, Chicago, Ill Prof of Medicine (4, 1948)
- Goodale, Walter T , M D 79 Webster Rd , Weston 93, Mass Research Fellow, Harvard Med School, Asst in Medicine, Peter Bent Brigham Hospital (1, 1949)
- Goodman, Louis Sanford, M S , M D Univ of Utah School of Medicine, Salt Lake City Prof of Pharmacology and Chairman of Dept of Pharmacology (1, 1946, 3, 1937)
- Goodner, Kenneth, Ph D Jefferson Med College, Philadelphia, Pennsylvania Prof of Bacteriology (6, 1932)
- Goodpasture, Ernest William, M D Vanderbilt Univ Med School, Nashville, Tenn Prof of Pathology and Dean (4, 1923)
- Gordon, Albert S , Ph.D New York Univ , Washington Square College of Arts and Sciences, New York City Assoc Prof of Biology (1, 1942)
- Gordon, Francis B , M D Camp Detrick, Chemical Corps, Biology Dept , Frederick, Md Chief of MV Div (4, 1947)
- Gordon, Harry H , M D 4200 E 9th Ave , Denver, Colo Prof of Pediatrics, Univ of Colorado Med School, Pediatrician in Chief, Colorado Gen Hospital (5, 1940)
- Gordon, Irving, M D New York State Dept of Health, Div of Labs and Research, New Scotland Ave , Albany 1 Assoc Med Bacteriologist, Assoc Prof of Medicine and Bacteriology, Albany Med College (6, 1943)
- Gordon, William G , Ph D U S Dept of Agriculture, Eastern Regional Research Lab , Philadelphia 18, Pa Sr Chemist (2, 1939)
- Gortner, Ross Aiken, Jr , Ph D Wesleyan Univ , Shanklin Lab , Middletown, Conn Assoc Prof of Biochemistry (5, 1947)
- Gortner, Willis A , Ph D Pineapple Research Inst of Hawaii, Honolulu, T H Head of Dept of Chemistry (2, 1947)

- Goss, Harold, Ph D Univ of California College of Agriculture, Davis *Prof of Animal Husbandry* (2, 1936, 5, 1933)
- Goth, Andres, M D Southwestern Med College, 2211 Oak Lawn Ave, Dallas 4, Tex *Assoc Prof of Pharmacology* (3, 1947)
- Gottschall, Russell Y, Ph D Michigan Dept of Health, Bureau of Labs, Lansing *Bacteriologist* (6, 1939)
- Goudsmit, Arnoldus, Jr, M D, Ph D 4141 Windsor Rd, Youngstown 7, Ohio (1, 1940)
- Gould, R Gordon, Ph D Presbyterian Hospital, 1753 W Congress St, Chicago 12, Ill *Assoc Attending Biochemist, Assoc Prof of Biological Chemistry, Univ of Illinois College of Medicine* (2, 1949)
- Govier, William M, M D Upjohn Co, Research Div, Kalamazoo 99, Mich *Sr Pharmacologist* (3, 1944)
- Gowdey, Charles W, Ph D Univ of Western Ontario Med School, Dept of Pharmacology, London, Ontario, Canada *Asst Prof* (3, 1950)
- Grabfield, G Philip, M D 27 Forest St, Milton, Mass *Retired* (3, 1923)
- Grady, Hugh G, M D Armed Forces Inst of Pathology, Washington, D C *Scientific Dir, American Registry of Pathology* (4, 1940)
- Graef, Irving, M D New York Univ College of Medicine, New York City *Asst Prof of Clin Medicine* (4, 1941)
- Graham, Claire E, Ph D Wilson Labs, 4221 S Western Ave, Chicago, Ill *Dir of Research* (2, 1948)
- Graham, Clarence H, Ph D Columbia Univ, New York City 27 *Prof of Psychology* (1, 1933)
- Graham, Helen Tredway, Ph D Euclid Ave and Kingshighway, St Louis, Mo *Assoc Prof of Pharmacology, Washington Univ School of Medicine* (1, 1933, 3, 1931)
- Granick, S, Ph D Rockefeller Inst for Med Research, York Ave at 66th St, New York City 21 *Associate, Physical Chemistry* (2, 1949)
- Grant, E Rhoda, Ph D Univ of Illinois Med College, Clinical Science Dept, 1853 West Polk St, Chicago 12, Ill *Researcher in Clin Science* (1, 1949)
- Grant, R Lorimer, Ph D Federal Security Agency, Food and Drug Admin, Div of Pharmacology, Washington 25, D C *Pharmacologist* (2, 1938)
- Grant, Ronald, Ph D Stanford Univ, Dept of Physiology, Stanford, Calif *Asst Prof (Acting)* (1, 1950)
- Grant, Wilson Clark, Ph D Columbia Univ, College of Physicians and Surgeons 630 W 168th St New York City 32 *Asst Prof of Physiology* (1, 1949)
- Grau, Charles R, Ph D Univ of California Div of Poultry Husbandry, Berkeley 4 *Asst Prof of Poultry Husbandry and Asst Poultry Husbandman in Exper Station* (5, 1949)
- Graubard, Mark, Ph D Univ of Minnesota, Dept of Physiology, Minneapolis (1, 1940)
- Grauer, Robert C, M D Allegheny General Hospital, Pittsburgh, Pa *Dir of William H. Singer Memorial Research Lab, Asst Prof of Medicine, School of Medicine, Univ of Pittsburgh* (4, 1941)
- Gray, John S, Ph D, M D Northwestern Univ Med School, 303 E Chicago Ave, Chicago 11, Ill *Prof and Chairman of Dept of Physiology* (1, 1937)
- Gray, M Geneva, Ph D Labs of Arthur D Little Inc, Cambridge, Mass *Dir, Lab of Industrial Toxicity* (3, 1946)
- Gray, Stephen W, Ph D Emory Univ School of Medicine, Emory Univ, Ga *Asst Prof of Anatomy* (1, 1948)
- Graybiel, Ashton, M D U S Naval School of Aviation Medicine and Research, Naval Air Station, Pensacola, Fla *Coordinator of Research* (1, 1948)
- Greaves, J D, Ph D U S Dept of Agriculture, Western Regional Research Lab, 800 Buchanan St, Albany 6, Calif *Biochemist* (2, 1938)
- Greaves, Joseph E, Ph D 3211 S W 10th Ave, Portland 1, Ore (2, 1940)
- Greeley, Paul O, Ph D, M D Univ of Southern California Med School, Univ Park, Los Angeles *Dir, Student Health Service* (1, 1940)
- Green, Arda Alden, M D Cleveland Clinic, Research Div, Euclid and E 93rd St, Cleveland 6, Ohio (2, 1932)
- Green, Daniel M, M D G D Searle Co, Chicago 80, Ill *Dir of Biological Research* (1, 1948, 3, 1942)
- Green, David E, Ph D Univ of Wisconsin, Enzyme Inst, P O Box 2066 Madison 5 *Prof of Enzyme Chemistry* (2, 1941)
- Green, Harold David, M D Wake Forest College, Bowman Gray School of Medicine, Winston-Salem 7, N C *Prof and Dir of Dept of Physiology and Pharmacology* (1, 1936, 3, 1945)
- Green, Robert Holt, Yale Univ School of Medicine, 333 Cedar St, New Haven, Conn *Asst Prof of Medicine* (6, 1949)
- Greenberg, David Morris, Ph D Univ of California, Berkeley 4 *Prof and Chairman, Div of Biochemistry* (2, 1927 5, 1946)
- Greenberg, Louis D, Ph D Univ of California Med Center, 3rd and Parnassus Aves, San Francisco 22 *Assoc Prof of Pathology, Univ of California Med School* (2, 1946)
- Greenberg, Ruven, Ph D Univ of Texas Med Branch Dept of Physiology Galveston *Asst Prof* (1, 1950)
- Greene, Carl Hartlev, Ph D, M D 401 Clinton Ave, Brooklyn 5, N Y *Assoc Prof of Clin*

- Medicine, New York Univ Post-Grad Med School* (1, 1921, 2, 1922, 4, 1924)
- Greene, Harry S N**, M D, C M Yale Univ School of Medicine, Dept of Pathology, New Haven, Conn *Anthony N Brady Prof of Pathology* (4, 1937)
- Greene, James Alexander**, M D Baylor Univ College of Medicine, Buffalo Drive, Houston, Tex *Prof and Chairman of Dept of Internal Medicine and Dean of Clin Faculty* (1, 1939)
- Greene, Ronald R**, M D Northwestern Univ Med School, 303 E Chicago Ave, Chicago, Ill *Instr in Physiology, Instr in Obstetrics and Gynecology* (1, 1941)
- Greengard, Harry**, Ph D, M D Northwestern Univ Med School, 303 E Chicago Ave, Chicago, Ill *Asst Prof of Physiology* (1, 1939)
- Greenstein, Jesse P**, Ph D Natl Cancer Inst, Bethesda, Md *Chief Biochemist* (2, 1935)
- Greenwald, Isidor**, Ph D New York Univ College of Medicine, 477 First Ave, New York City *Prof of Chemistry* (2, 1912, 5, 1933)
- Greenwood, Delbert A**, Ph D Utah State Agricultural College, Box 163, Logan *Prof of Biochemistry* (3, 1950)
- Greep, Roy O**, Ph D Harvard School of Dental Medicine, 25 Shattuck St, Boston 15, Mass *Assoc Prof of Dental Science* (1, 1940)
- Greer, C M**, M S Vanderbilt Univ School of Medicine, Nashville, Tenn *Instr in Chemistry* (3, 1938)
- Gregersen, Magnus I**, Ph D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 *Prof of Physiology* (1, 1933)
- Gregg, Donald Eaton**, Ph D, M D Army Med Dept, Research and Grad School, Army Med Center, Washington 12, D C (1, 1933)
- Gregory, John E**, Hahnemann Med College, Philadelphia, Pa *Prof of Pathology* (6, 1948)
- Gregory, Raymond L**, Ph D, M D Univ of Texas School of Medicine, 1419 24th St, Galveston *Prof of Medicine* (1, 1945)
- Greig, Margaret E**, Ph D Vanderbilt Univ School of Medicine, Nashville 4, Tenn *Assoc Prof in Pharmacology* (3, 1946)
- Greisheimer, Esther M**, Ph D M D Temple Univ Med School, 3400 N Broad St, Philadelphia, Pa *Prof of Physiology* (1, 1925)
- Grenell, Robert G**, Ph D Johns Hopkins Univ, Dept of Biophysics, Baltimore, Md (1, 1945)
- Griffin, A Clark**, Ph D Stanford Univ, Dept of Biochemistry, Stanford, Calif *Asst Prof* (2, 1950)
- Griffin, Angus**, Ph D George Washington Univ School of Medicine, 1335 H St, N W, Washington, D C *Assoc Prof of Bacteriology* (6, 1940)
- Griffith, Fred R, Jr**, Ph D Univ of Buffalo Med School, 24 High St, Buffalo, N Y *Prof of Physiology* (1, 1923, 5, 1933)
- Griffith, Wendell H**, Ph D Univ of Texas Med School, Galveston *Prof and Chairman of Dept of Biochemistry and Nutrition* (2, 1923, 5, 1934)
- Grimson, Keith S**, M D Duke Univ School of Medicine, Durham, N C *Prof of Surgery* (1, 1943, 3, 1949)
- Grindlay, John H**, M D Mayo Clinic, Rochester, Minn (1, 1945)
- Groat, Richard A**, Ph D Wake Forest College, Bowman Gray School of Medicine, Winston-Salem 7, N C *Assoc Prof of Anatomy* (1, 1945)
- Grodins, Fred S**, Ph D, M D Northwestern Univ Med School, 303 E Chicago Ave, Chicago 11, Ill *Assoc Prof of Physiology* (1, 1945)
- Groedel, Franz M**, M D 829 Park Ave, New York City *Research Fellow, Fordham Univ, Biology Dept* (1, 1949)
- Grollman, Arthur**, M D, Ph D Southwestern Med College, 2211 Oak Lawn Ave, Dallas, Tex *Prof of Pharmacology and Medicine* (1, 1928, 3, 1933)
- Gross, Erwin G**, Ph D, M D State Univ of Iowa, Med Labs, Iowa City *Prof of Pharmacology* (1, 1927, 2, 1923, 3, 1927)
- Gross, J**, M D, C M, Ph D Natl Inst for Med Research, The Ridgeway, Mill Hill, London, N W 7, England (1, 1950)
- Gross, Robert E**, M D Children's Hosp, 300 Longwood Ave, Boston 15, Mass *Surgeon in-Chief, Ladd Prof of Children's Surgery, Harvard Med School* (4, 1940)
- Grossman, Morton Irvin**, Ph D, M D Univ of Illinois College of Medicine, Chicago 12 *Assoc Prof of Physiology* (1, 1946)
- Groupé, Vincent**, Ph D Rutgers Univ, Dept of Microbiology, New Jersey Agricultural Experiment Station, New Brunswick *Assoc Prof in Animal Diseases* (6, 1946)
- Grubbs, Robert C**, M Sc, M D Ohio State Univ, Columbus *Assoc Prof of Physiology* (1, 1950)
- Gruber, Charles M.**, M D, Ph D Jefferson Med College, 1025 Walnut St, Philadelphia, Pa *Prof of Pharmacology*, (1, 1914, 3, 1919)
- Gruber, Charles M, Jr**, M D Jefferson Med College Hospital, Philadelphia, Pa *Assoc Prof of Pharmacology* (3, 1948)
- Gruhzit, Oswald M**, M D Parke, Davis and Co, Research Labs, Detroit, Mich *Research in Pathology and Pharmacology* (4, 1928)
- Grundfest, Harry**, Ph D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 *Assoc in Neurology* (1, 1932)
- Gudernatsch, F**, Ph D 41 Fifth Ave, New York City 3 (1, 1930)
- Guerra, Francisco**, M Sc, M D Facultad de Medicina, Univ Nacional de Mexico, Mexico, D F *Prof of Pharmacology* (3, 1947)

- Guerrant, N B , Ph D Pennsylvania State College, State College *Prof of Biological Chemistry* (2, 1934, 5, 1933)
- Guest, George Martin, M S , M D Children's Hospital, Cincinnati 29, Ohio *Prof of Pediatrics, Univ of Cincinnati College of Medicine and Grad School* (2, 1933)
- Guest, Maurice Mason, Ph D Wayne Univ College of Medicine, Detroit 26, Mich *Assoc Prof of Physiology* (1, 1946)
- Gulick, Addison, Ph D 308 Westmount Ave , Columbia, Mo *Prof of Physiological Chemistry, Univ of Missouri* (1, 1915, 5, 1933)
- Gunn, Francis D , M D , Ph D Univ of Utah School of Medicine, Salt Lake City *Prof and Head of Dept of Pathology* (4, 1938)
- Gunsalus, Irwin C , Ph D Univ of Illinois, 362 Noyes Lab , Urbana *Prof of Bacteriology* (2, 1946)
- Gurin, Samuel, Ph D Univ of Pennsylvania School of Medicine, Philadelphia *Prof of Physiological Chemistry* (2, 1938)
- Gustavson, R G , Ph D Univ of Nebraska, Lincoln *Chancellor* (2, 1927)
- Gustus, Edwin L , Ph D Bjorksten Research Lab , 13791 Ave O, Chicago 33, Ill *Vice President* (2, 1934)
- Guterman, Henry S , M D Michael Reese Hospital, Chicago 16, Ill *Asst Dir, Dept of Metabolic and Endocrine Research* (1, 1949)
- Guthrie, Charles Claude, M D , Ph D Univ of Pittsburgh Med School, Pittsburgh, Pa *Prof of Physiology and Pharmacology* (1, 1905, 3, 1909)
- Gutman, Alexander B , M D Columbia Research Service, Welfare Island, New York City 17 *Director* (2, 1947)
- Guttman, Rita M , Ph D Brooklyn College, Brooklyn, N Y *Asst Prof of Physiology* (1, 1946)
- Guyton, Arthur C , M D Univ of Mississippi School of Medicine, Dept of Physiology, University *Chairman, Dept of Physiology* (1, 1949)
- Gyorgy, Paul, M D Univ of Pennsylvania School of Medicine, 3400 Spruce St Philadelphia 4 *Prof of Nutrition* (2, 1938, 5, 1939)
- Haag, Harvey B , M D Med College of Virginia, Richmond *Prof of Pharmacology* (3, 1934)
- Haag, J R , Ph D Oregon Agricultural Exper Station, Corvallis *Chemist* (2, 1947, 5, 1941)
- Haas, Erwin, Ph D Cedars of Lebanon Hospital, Inst for Med Research, 4751 Fountain Ave , Los Angeles 27, Calif *Asst Dir* (2, 1946)
- Haberman, Sol, Ph D Wm Buchanan Blood, Plasma and Serum Center, Baylor Hospital, Dallas, Tex *Chief of Bacteriology and Serology Services* (6, 1944)
- Hadidian, Zareh, Ph D Tufts College Med School, Boston, Mass (1, 1945)
- Hadley, Philip B , Ph D Western Pennsylvania Hospital, Inst of Pathology, Pittsburgh *Chief of Bacteriological Service and Research Bacteriologist* (4R, 1927)
- Hafkenschiel, Joseph H , Jr , M D 1458 Hampstead Rd , Philadelphia 31, Pa *Instr in Medicine, Univ of Pennsylvania School of Medicine* (1, 1950)
- Hafkesbring, H Roberta, Ph D Woman's Med College of Pennsylvania, East Falls, Philadelphia *Prof of Physiology* (1, 1931)
- Haggard, Howard W , M D 4 Hillhouse Ave , New Haven, Conn *Dir of Lab of Applied Physiology, Yale Univ* (1, 1919, 2, 1920)
- Hahn, Paul F , Ph D Meharry Med College, Nashville, Tenn *Dir of Cancer Research Labs , Prof of Exper Oncology* (1, 1946, 4, 1939)
- Haig, Charles, Ph D New York Med College, Fifth Ave at 105th St , New York City *Assoc Prof of Physiology and Biochemistry* (1, 1942)
- Haimovici, Henry, M D , 1148 Fifth Ave , New York City 28 *Assoc Attending Surgeon, Montefiore Hospital* (1, 1950)
- Haist, Reginald E , M D , Ph D Univ of Toronto, Toronto, Ontario, Canada *Prof of Physiology* (1, 1943)
- Halbert, Seymour P , M D Columbia Univ College of Physicians and Surgeons, Dept of Ophthalmology, 630 W 168th St , New York City 32 (6, 1947)
- Haldi, John, Ph D Emory Univ School of Dentistry, Emory University, Ga *Prof of Physiology* (1, 1928)
- Hale, Wm M , M D Brookhaven Natl Lab , Upton, L I , N Y *Head, Div of Bacteriology and Virology, Bacteriologist-in-Chief, Brookhaven Natl Lab Hosp* (4, 1941, 6, 1935)
- Hale, Worth, M D Antrim, N H *Retired* (1R, 1908, 3R, 1908)
- Haley, Thomas J , Ph D 12007 Thermo St , Los Angeles 34, Calif *Chief, Div of Pharmacology and Toxicology, A E Project, Med School, Univ of California* (3, 1949)
- Hall, Charles E , Ph D Univ of Texas, Med Branch, Dept of Physiology, Galveston *Asst Prof* (1, 1949)
- Hall, F G , Ph D Duke Univ School of Medicine, Dept of Physiology and Pharmacology , Durham, N C (1, 1937)
- Hall, George Edward, M D , Ph D Univ of Western Ontario Ottawa Ave and Waterloo St , London, Ontario, Canada *Dean of the Faculty of Medicine* (1, 1938)
- Hall, Victor E , M A , M D Stanford Univ , Stanford University, Calif *Prof of Physiology* (1, 1934)
- Hall, W Knowlton, Ph D Med College of Georgia, Univ Place, Augusta *Assoc Prof of Biochemistry* (2, 1948)

- Hallenbeck, George Aaron**, Ph D , M D Mayo Clinic, Rochester, Minn *Research Assoc* (1, 1946)
- Halliday, Nellie**, Ph D Univ of California School of Medicine, Room 307, Hooper Fndn , San Francisco (5, 1933)
- Halpert, Béla**, M D Veterans Admin Hospital, 2002 Holcombe Blvd , Houston 4, Texas *Chief, Lab Service, Assoc Prof of Pathology, Baylor Univ College of Medicine* (4, 1936)
- Halsey, John T**, M D P O Box 264, Waveland, Miss *Prof Emeritus of Pharmacology, Tulane Univ* (3R, 1929)
- Halstead, Ward C**, Ph D , Univ of Chicago, Dept of Medicine, Chicago, Ill *Assoc Prof of Exper Psychology, Div of Psychiatry* (1, 1942)
- Ham, Arthur W**, M B Univ of Toronto, Toronto 5, Ontario, Canada *Prof of Anatomy in charge of Histology* (4, 1939)
- Hambourger, Walter E**, Ph D , M D G D Searle and Co , P O Box 5110, Chicago, Ill *Chief Pharmacologist* (3, 1934)
- Hamilton, Bengt L K**, M D U S Marine Hospital, Staten Island 4, N Y *Med Dir , USPHS* (2, 1925)
- Hamilton, James B**, Ph D Long Island College of Medicine, Dept of Anatomy, 350 Henry St , Brooklyn 2, N Y (1, 1938)
- Hamilton, Paul B**, M D , Ph D Alfred I duPont Inst , Nemours Foundation, Rockland Rd , Wilmington 99, Del *Chief of Biochemistry* (2, 1946)
- Hamilton, Tom R**, M S , M D Kansas Univ Med Center, Kansas City 3 *Prof of Microbiology and Pathology* (4, 1950)
- Hamilton, Tom S**, Ph D Univ of Illinois, 1513 W Clark St , Champaign *Prof and Chief in Animal Nutrition* (2, 1937, 5, 1938)
- Hamilton, W F**, Ph D Medical College of Georgia, Augusta *Prof of Physiology* (1, 1924)
- Hammitt, Frederick S**, Ph D 493 Commercial St , Provincetown, Mass *Sr Member Emeritus, Lakenau Hospital Research Inst* (1R, 1920, 2, 1917)
- Hammon, William McD**, M D , D P H Univ of Pittsburgh, Pittsburgh, Pa *Prof of Epidemiology, Grad School of Public Health* (4, 1944, 6, 1946)
- Hampel, C W**, Ph D New York Univ -Bellevue Medical Center, New York City *Assoc Prof of Physiology* (1, 1936)
- Hamre, Dorothy**, Ph D Squibb Inst for Med Research, New Brunswick, N J *Research Assoc* (6, 1948)
- Hand, David B**, Ph D New York State Agricultural Exper Station, Geneva *Head, Div of Food Science and Technology and Prof of Biochemistry* (2, 1947)
- Handler, Philip**, Ph D Duke Univ School of Medicine, Durham, N C *Prof of Biochemistry and Nutrition* (2, 1944, 5, 1946)
- Handley, Carroll A**, Ph D Baylor Univ College of Medicine, 1200 M D Anderson Blvd , Houston 5, Tex *Prof and Chairman of Dept of Pharmacology* (3, 1942)
- Haney, Hance F**, Ph D , M D Univ of Oregon Med School, Portland *Asst Prof of Medicine* (1, 1939)
- Hanger, Franklin**, M D Columbia Univ College of Physicians and Surgeons, 630 W 168th St , New York City 32 *Assoc Prof of Medicine* (6, 1930)
- Hanke, Martin E**, Ph D Univ of Chicago, Chicago, Ill *Assoc Prof of Biochemistry* (2, 1925)
- Hanke, Milton Theo**, Ph D 7550 S Green St , Chicago, Ill *Research Consultant, Biochemistry and Nutrition* (2, 1919)
- Hanks, John H**, Ph D Harvard Univ Med School, Boston, Mass *Dept of Bacteriology, Bacteriologist of the Leonard Wood Memorial* (6, 1935)
- Hansen, Arild E**, M D Univ of Texas Med School, Galveston *Prof and Chairman of Dept of Pediatrics, Dir of Univ of Texas Child Health Program* (4, 1941, 5, 1942)
- Hanzlik, Paul J**, M D 303 Franklin St , San Mateo, Calif *Prof Emeritus of Pharmacology, Stanford Univ* (1R, 1912, 3R, 1912)
- Hardy, James Daniel**, Ph D Russell Sage Inst of Pathology, 525 E 68th St , New York City *Research Assoc* (1, 1939)
- Hardy, Mary**, D Sc The Brearley School, 610 E 83rd St , New York City *Teacher of Science* (1, 1933)
- Hare, Kendrick**, Ph D Children's Hospital, 219 Bryant St , Buffalo 9, N Y (1, 1938)
- Harford, Carl G**, M D Washington Univ School of Medicine, St Louis, Mo *Asst Prof of Medicine and Preventive Medicine* (6, 1948)
- Harger, R N**, Ph D Indiana Univ School of Medicine, Indianapolis *Prof of Biochemistry and Toxicology, Chairman, Dept of Biochemistry and Pharmacology* (2, 1938, 3, 1949)
- Harkins, Henry N**, Ph D , M D Univ of Washington School of Medicine, Seattle 5 (1, 1942)
- Harmon, Paul M**, Ph D Indiana Univ , Bloomington *Prof of Physiology* (1, 1930)
- Harne, O G** Univ of Maryland School of Medicine, Baltimore *Assoc Prof of Anatomy, Asst to the Dean* (1, 1935)
- Harned, Ben King**, Ph D Lederle Labs , Pearl River, N Y *Assoc Dir , Pharmacology Research* (2, 1931, 3, 1941)
- Harris, Albert H**, M D N Y State Dept of Health, Div of Labs and Research, New Scotland Ave , Albany 1 *Asst Dir for Local Labs* (6, 1937)

- Harris, Albert Sidney, Ph D Baylor Univ College of Medicine, Houston 5, Texas (1, 1939)
- Harris, Milton, Ph D Harris Research Labs, 1246 Taylor St, N W, Washington 11, D C President (2, 1939)
- Harris, Paul N, M D Eli Lilly and Company, Indianapolis, Ind *Pathologist, Div of Pharmacology* (3, 1948)
- Harris, Philip L, Ph D Distillation Products, Inc, 755 Ridge Rd W, Rochester 13, N Y Head of Biochemistry Research Dept, Assoc in Physiology, Univ of Rochester School of Medicine and Dentistry (2, 1946, 5, 1945)
- Harris, Robert S, Ph D Massachusetts Inst of Technology, Cambridge Prof of Nutritional Biochemistry (5, 1941)
- Harris, S C, Ph D Northwestern Univ Dental School, Dept of Pharmacology, 311 E Chicago Ave, Chicago 12, Ill Assoc Prof and Chairman of Dept (1, 1947)
- Harris, T N, M D Children's Hospital of Philadelphia, Philadelphia, Pa Assoc in Pediatrics, Univ of Pennsylvania (6, 1946)
- Harris, William H, M D Tulane Univ School of Medicine, New Orleans, La Assoc Prof of Pathology (4, 1925)
- Harrison, Frank, Ph D Univ of Tennessee College of Medicine, Memphis Prof and Chief, Div of Anatomy (1, 1941)
- Harrison, James A, Ph D Temple Univ, Philadelphia 22, Pa Prof of Biology (6, 1946)
- Harrison, Ross Granville, M D, Ph D Osborn Zoological Lab, New Haven, Conn Sterling Prof Emeritus of Biology, Yale Univ (1, 1906)
- Harrison, R Wendell, M D, Ph D Ellis Ave at 58th St, Chicago 37, Ill Prof of Bacteriology, Dean of Faculties and Vice Pres, Univ of Chicago (6, 1934)
- Harrow, Benjamin, Ph D College of the City of New York, Convent Ave and 139th St, New York City Prof of Chemistry (2, 1927)
- Hart, E B, B S Univ of Wisconsin Agricultural College, Madison Prof of Biochemistry (2, 1910, 5, 1933)
- Hart, E Ross, Ph D Med Div, Army Chemical Center, Md Toxicologist (3, 1944)
- Hart, William M, Ph D Temple Med School, Broad and Ontario Sts, Philadelphia 40, Pa Asst Prof of Physiological Chemistry (1, 1945)
- Hartline, H K, M D Johns Hopkins Univ, Dept of Biophysics, Baltimore 18, Md (1, 1929)
- Hartman, Carl G, Ph D Ortho Research Foundation, Raritan, N J Dir of Physiology (1, 1921)
- Hartman, Frank Alexander, Ph D Ohio State Univ, Dept of Physiology, Columbus Research Prof of Physiology (1, 1916)
- Hartman, F W, M D Henry Ford Hospital, Detroit, Mich Pathologist (4 1927)
- Hartmann, Alexis F, M D 500 S Kingshighway, St Louis, Mo Prof of Pediatrics, Washington Univ School of Medicine (2, 1932)
- Hartroft, Walter Stanley, M D, Ph D Univ of Toronto, Banting Inst, Toronto 5, Ontario, Canada Assoc Prof (4, 1950)
- Harvey, A McGhee, M D Johns Hopkins Hospital, Baltimore 5, Md Physician-in-Chief, Prof of Medicine, Johns Hopkins Univ Med School (1, 1946, 3, 1946)
- Harvey, E Newton, Ph D Princeton Univ, Guyot Hall, Princeton, N J Henry Fairfield Osborn Prof of Biology (1, 1914, 2, 1916)
- Hass, George, M D Presbyterian Hospital of Chicago, Chicago, Ill Prof and Chairman of Dept of Pathology, Univ of Illinois College of Medicine (4, 1939)
- Hassid, William Z, Ph D, Univ of California, Berkeley Prof of Plant Biochemistry (2, 1946)
- Hastings, A Baird, Ph D Harvard Med School, 25 Shattuck St, Boston 15, Mass Hamilton Kuhn Prof of Biological Chemistry (1, 1927, 2, 1921, 5, 1940)
- Hathaway, Millicent L, Ph D Agricultural Research Administration, Bureau of Human Nutrition and Home Economics, Food and Nutrition Div, Washington 25, D C (5, 1945)
- Hauck, Hazel M, Ph D Cornell Univ, Ithaca, N Y Prof of Home Economics (5, 1941)
- Hauge, Siegfried M, Ph D Purdue Univ Agricultural Exper Station, Lafayette, Ind Research Assoc in Biochemistry (5, 1933)
- Haurowitz, Felix, M D, D Sc Indiana Univ, Bloomington Prof of Chemistry (2, 1949, 6, 1948)
- Haury, Victor G, M D Box 206, Wellsville, Kansas Private Practice in Medicine (3, 1939)
- Haven, Frances L, Ph D Univ of Rochester School of Medicine and Dentistry, 260 Crittenden Blvd, Rochester, N Y Assoc in Biochemistry (2, 1941)
- Hawk, Philip B, Ph D Food, Drug and Cosmetic Consultants, 4818 33rd St, Long Island City, N Y Pres of Food Research Labs, Inc (1, 1903, 2, 1906)
- Hawkins, J E, Jr, Ph D Merck Inst for Therapeutic Research, Rahway, N J Physiologist (1, 1943)
- Hawkins, William Bruce, M D Univ of Rochester School of Medicine and Dentistry, 260 Crittenden Blvd, Rochester, N Y Assoc Prof of Pathology (4, 1933)
- Hawley, Estelle E, Ph D Univ of Rochester School of Medicine and Dentistry, Rochester, N Y Research Fellow in Pediatrics (5, 1935)
- Hawn, Clinton van Zandt, M D Marv Imogene Bassett Hospital, Cooperstown, N Y Pathologist, Dir of Otsego County Labs (4, 1948)

- Hay, Eleanor Clarke, Ph D 7 Greenhill Rd , Madison, N J (1, 1945)
- Hayman, J M , Jr , M D Lakeside Hospital, Cleveland, Ohio *Prof of Medicine, Western Reserve Univ* (1, 1928, 3, 1932)
- Haynes, Florence W , Ph D Harvard Med School, 25 Shattuck St , Boston, Mass *Research Assoc in Medicine* (1, 1937)
- Hays, Edwin Everett, Ph D Armour Labs , 1425 W 42nd St , Chicago 9, Ill *Dir of Biochemical Research* (2, 1946)
- Hays, Harry W , Ph D Wayne Univ College of Medicine, 1512 St Antoine St , Detroit 26, Mich *Assoc Prof of Pharmacology* (3, 1949)
- Haywood, Charlotte, Ph D Mount Holyoke College, South Hadley, Mass *Prof of Physiology* (1, 1939)
- Hazen, Elizabeth L , Ph D New York State Dept of Health Labs , 339 E 25th St , New York City *Sr Bacteriologist* (6, 1931)
- Hazleton, Lloyd W , Ph D Box 333, Falls Church, Va *Dir , Hazleton Labs* (3, 1944)
- Hechter, Oscar M , Ph D Worcester Foundation for Exper Biology, 222 Maple Ave , Shrewsbury, Mass (1, 1945)
- Hegnauer, Albert H , Ph D Boston Univ School of Medicine Boston, Mass *Assoc Prof of Physiology* (1, 1937)
- Hegsted David Mark, Ph D Harvard School of Public Health, Boston, Mass *Prof of Nutrition* (5, 1944)
- Heidelberger, Charles, Ph D Univ of Wisconsin, McArdle Memorial Lab , Madison 6 *Asst Prof , Dept of Oncology* (2, 1950)
- Heidelberger, Michael, Ph D Columbia Univ , 630 W 168th St , New York City 32 *Prof of Immunochemistry, Chemist to the Presbyterian Hospital* (2, 1927, 6, 1935)
- Heilbrunn, Lewis Victor, Ph D Univ of Pennsylvania, Philadelphia *Prof of Zoology* (1, 1930)
- Heim, J William, Ph D 1 Yale Ave , Dayton 6, Ohio *Aero Med Lab , Army Air Forces, Wright Field* (1, 1936)
- Heinbecker, Peter, M D Washington Univ Med School, St Louis, Mo *Assoc Prof of Clin Surgery* (1, 1930)
- Heinle, Robert W , M D Western Reserve Univ , Cleveland, Ohio *Asst Prof of Medicine* (5, 1948)
- Helff, O M , Ph D New York Univ , University Heights, New York City *Assoc Prof of Biology* (1, 1932)
- Hellbaum, Arthur A , Ph D , M D Univ of Oklahoma School of Medicine, Oklahoma City *Prof of Pharmacology* (1, 1937, 3, 1945)
- Hellebrandt, Frances Anna, M D Med College of Virginia, Richmond *Prof of Physical Medicine* (1, 1933)
- Heller, Carl G , M D , Ph D Univ of Oregon Med School, Portland 1 *Assoc Prof of Physiology and Medicine* (1, 1945)
- Heller, Victor G , Ph D Oklahoma A & M College, Stillwater *Head of Dept of Agricultural Chemistry Research* (2, 1935, 5, 1935)
- Hellerman, Leslie, Ph D Johns Hopkins Univ School of Medicine, 710 N Washington St , Baltimore 5, Md *Assoc Prof of Physiological Chemistry* (2, 1935)
- Helmer, Oscar Marvin, Ph D Indianapolis General Hospital, Indianapolis, Ind *Head of Dept of Physiological Chemistry* (2, 1935)
- Hemingway, Allan, Ph D 241 Cecil St , S E , Minneapolis, Minn *Assoc Prof of Physiology, Univ of Minnesota* (1, 1933)
- Henderson, Francis G , M D 740 S Alabama St , Indianapolis 2, Ind *Pharmacologist, Eli Lilly & Co* (3, 1950)
- Henderson, LaVell M , Ph D Univ of Illinois, Urbana *Asst Prof of Chemistry* (2, 1949)
- Hendley, Charles D , Ph D Ohio State Univ , Hamilton Hall, Columbus 10 *Asst Prof of Physiology* (1, 1949)
- Hendrix, Byron M , Ph D Univ of Texas School of Medicine, Galveston *Prof of Biochemistry* (2, 1920)
- Hendrix, James Paisley, M A , M D Duke Hospital, Durham, N C *Asst Prof of Medicine (in charge of Therapeutics), Assoc in Physiology and Pharmacology, Duke Univ School of Medicine* (3, 1942)
- Hendry, Jessie L , M A Div of Labs and Research, New York State Dept of Health, New Scotland Ave , Albany *Sr Bacteriologist* (6, 1938)
- Henle, Werner, M D 1740 Bainbridge St , Philadelphia 46, Pa *Assoc Prof of Virology in Pediatrics* (6, 1938)
- Henry, James P , M A , M Sc Aero-Med Lab , Wright Field, Dayton, Ohio *On leave of absence from the Univ of Southern California, Los Angeles* (1, 1947)
- Henschel, Austin F , Ph D Univ of Minnesota, Minneapolis *Asst Prof of Physiological Hygiene* (1, 1944)
- Hepburn, Joseph Samuel, Ph D , M D Chem D 2045 N Franklin St , Philadelphia 22, Pa *Prof of Chemistry and Research Assoc in Gastroenterology, Registrar, Hahnemann Med College and Hospital* (2, 1915)
- Hepler, Opal E , Ph D , M D Northwestern Univ Med School, 303 E Chicago Ave , Chicago, Ill *Assoc Prof of Pathology* (4, 1939)
- Heppel, Leon A , Ph D , M D Natl Insts of Health, Bethesda 14, Md *Surgeon, USPHS* (2, 1949)
- Herbst, Robert M , Ph D Kedzie Chemical Lab , Michigan State College, East Lansing *Prof of Chemistry* (2, 1938)

- Herget, Carl M , Ph D Med Div , Army Chemical Center, Md *Chief, Biophysics Section* (1, 1948)
- Herrick, C Judson, Ph D 236 Morningside Dr , Grand Rapids 6, Mich *Prof Emeritus of Neurology, Univ of Chicago, Chicago, Ill* (1R, 1907)
- Herrick, Julia F , Ph D Mayo Foundation, Rochester, Minn *Assoc Prof of Exper Medicine* (1, 1933)
- Herrin, Raymond C , Ph D , M D Univ of Wisconsin Med School, Madison *Prof of Physiology* (1, 1932)
- Herrington, Lovic P , Ph D 290 Congress Ave , New Haven, Conn *Assoc Dir , John B Pierce Lab of Hygiene, Research Assoc Prof , Dept of Public Health, Yale Med School* (1, 1942)
- Herriott, Roger M , Ph D Johns Hopkins School of Hygiene and Public Health, 615 N Wolfe St , Baltimore 5, Md *Prof of Biochemistry* (2, 1940)
- Herrmann, George R , Ph D , M D Univ of Texas Med Branch, Galveston *Prof of Medicine* (4, 1925)
- Herrmann, Julian B , Ph B , M D 1185 Park Ave , New York City (3, 1941)
- Herrmann, Louis George, M D Cincinnati General Hospital, Cincinnati 29, Ohio *Attending Surgeon, Assoc Prof of Surgery, Univ of Cincinnati College of Medicine* (4, 1933)
- Hertz, Roy, Ph D , M D Natl Insts of Health, Bethesda 14, Md *Public Health Surgeon (R), Div of Physiology* (1, 1945)
- Hertzman, Alrick B , Ph D St Louis Univ School of Medicine, St Louis, Mo *Prof of Physiology and Dir of Dept* (1, 1925)
- Herwick, Robert P , Ph D , M D American Home Products Corp , 22 E 40th St , New York City *Vice Pres , Whitehall Div* (3, 1938)
- Hess, Charles L , M S , M D 308 Davidson Bldg , Bay City, Mich (1, 1916)
- Hess, Walter C , Ph D Georgetown Med School, 39th St and Reservoir Rd , N W , Washington 7, D C *Prof of Biological Chemistry* (2, 1935)
- Hetherington, Albert W , Ph D School of Aviation Medicine, Randolph Field, Texas (1, 1943)
- Hewetson, Jean Hawks, M D 115 E York St , Biglerville, Pa (5, 1944)
- Hewitt, Earl Albon, Ph D Iowa State College, Ames *Assoc Prof of Veterinary Physiology* (1, 1932)
- Hewston, Elizabeth M , M A 1121 24th St , N W , Apt 300, Washington 7, D C *Chemist, Bureau of Human Nutrition and Home Economics* (5, 1950)
- Heyroth, Francis F , M D , Ph D Univ of Cincinnati, Cincinnati, Ohio *Assoc Prof of Ind Toxicology and Asst Dir , Kettering Lab , Assoc Prof of Biological Chemistry, College of Medicine* (2, 1935)
- Hiatt, Edwin P , Ph D Univ of North Carolina School of Medicine, Chapel Hill *Assoc Prof of Physiology* (1, 1942)
- Hickman, Kenneth C D , Ph D 56 Thackeray Rd , Rochester 10, N Y *Consultant, Arthur D Little, Inc* (2, 1944)
- Hier, Stanley W , Ph D Wilson Labs , 4221 S Western Ave , Chicago 9, Ill *Tech Asst to General Manager* (2, 1950)
- Hiestand, William A , Ph D Purdue Univ , Dept of Biology, Lafayette, Ind *Prof of Physiology* (1, 1947)
- Higgins, George M , Ph D Mayo Clinic, Rochester, Minn *Prof of Exper Biology, Mayo Foundation* (5, 1948)
- Higgins, Harold Leonard, M D 322 Franklin St , Newton, Mass (1, 1914, 5, 1933)
- Highman, Benjamin, M D Natl Insts of Health, Bethesda, Md *Sr Surgeon, Lab of Pathology and Pharmacology* (4, 1947)
- Hill, Edgar S , Ph D Washington Univ College of Dentistry, St Louis, Mo *Assoc Prof of Biological Chemistry and Physiology* (2, 1936)
- Hill, Robert M , Ph D Univ of Colorado Med Center, 4200 E 9th Ave , Denver *Prof of Biochemistry* (2, 1933)
- Hill, Samuel E , Ph D 18 Collins Ave , Troy, N Y *Research Worker, The Behr-Manning Corp* (1, 1934)
- Hilleman, Maurice, Ph D Army Med Center, AMDR & GS, Dept of Virus and Rickettsial Diseases, Washington 12, D C *Med Bacteriologist* (6, 1949)
- Hiller, Alma, Ph D Presbyterian Hospital, 1753 W Congress St, Chicago 12, Ill *Assoc , Rush Dept of Biochemistry* (2, 1929)
- Himwich, Harold E , M D Fallston, Md *Chief, Clin Research Branch, Med Div , Army Chemical Center, Md* (1, 1925)
- Himwich, William A , Ph D Johns Hopkins Univ , Welch Med Library, Baltimore, Md *Med Bibliographer* (1, 1947)
- Hine, Charles H , Ph D , M D Univ of California Med School, Pharmacology and Toxicology Dept , Med Center, San Francisco 22 *Asst Prof* (3, 1947)
- Hines, Harry M , Ph D State Univ of Iowa, Iowa City *Prof of Physiology* (1, 1928)
- Hines, Marion, Ph D Emory Univ Dept of Anatomy, Emory University, Ga *Prof of Exper Anatomy* (1, 1932)
- Hinrichs, Marie, Ph D , M D Southern Illinois Normal Univ , Carbondale *Prof of Physiology, Dir of Student Health Service* (1, 1928)
- Hinsey, Joseph C , Ph D Cornell Univ Med College, 1300 York Ave , New York City *Prof of Anatomy and Dean of the Med College* (1, 1929)
- Hirschmann, Hans, M D , Ph D Lakeside Hospital, Cleveland, Ohio *Asst Prof of Biochem-*

- istry, Dept of Medicine, Western Reserve Univ (2, 1946)
- Hirszfeld, Ludwik**, M D Univ of Wroclaw, Wroclaw, Ul Chaubinskiego 4, Poland *Prof of Microbiology, Dir of Med Microbiology* (6, 1946)
- Hisaw, Frederick L**, Ph D Harvard Univ Biological Labs, Cambridge, Mass *Prof of Zoology* (1, 1932)
- Hitchcock, David I**, Ph D Yale Univ, 333 Cedar St, New Haven 11, Conn *Assoc Prof of Physiology* (2, 1930)
- Hitchcock, Fred A**, Ph D Ohio State Univ, Columbus *Prof of Physiology* (1, 1927, 5, 1933)
- Hitchcock, Philip**, Ph D, M D Med College of Alabama, Dept of Physiology and Pharmacology, Birmingham 5 *Assoc Prof of Pharmacology* (3, 1946)
- Hitchings, George H**, Ph D 50 Primrose Ave, Tuckahoe 7, N Y, *Chief Biochemist, Wellcome Research Labs* (2, 1942)
- Hjort, Axel M**, M D, Ph D P O Box 281, Fern Way, Scarsdale, N Y *Private Research Lab and Practice of Medicine* (2, 1925)
- Hoagland, Hudson**, Ph D 222 Maple Ave, Shrewsbury, Mass *Exec Dir, Worcester Foundation for Exper Biology, Neurophysiologist, Worcester State Hospital* (1, 1932)
- Hobby, Gladys L**, Ph D 11 Bartlett St, Brooklyn 6, N Y *Research Bacteriologist in charge of Biological Control, Chas Pfizer & Co* (6, 1946)
- Hober, Rudolf** 5307 Larchwood Ave, Philadelphia 43, Pa *Visiting Prof of Physiology, Univ of Pennsylvania Med School* (1R, 1936)
- Hoberman, Henry D**, Ph D, M D Yale Univ School of Medicine, Dept of Physiological Chemistry, 333 Cedar St, New Haven 11, Conn *Asst Prof of Physiological Chemistry* (2, 1949)
- Hockett, Robert C**, Ph D Sugar Research Foundation, Inc, 52 Wall St, New York City 5 *Scientific Dir* (2, 1950)
- Hodes, Robert**, Ph D Tulane Univ School of Medicine, Dept of Psychiatry, New Orleans 12, La *Prof of Exper Neurology and Neurophysiology* (1, 1941)
- Hodge, Harold Carpenter**, Ph D Univ of Rochester School of Medicine and Dentistry, 260 Crittenden Blvd, Rochester 7, N Y *Prof of Pharmacology and Toxicology* (2, 1937, 3, 1948)
- Hoefel, Paul F A**, Ph D, M D Neurological Inst of New York, 710 W 168th St, New York City 32 *Assoc Prof of Neurology* (1, 1945)
- Hoff, Ebbe Curtis**, Ph D Med College of Virginia, Richmond 19 *Assoc Prof* (1, 1933)
- Hoff, Hebbel E**, Ph D Baylor Univ College of Medicine, Dept of Physiology, 1200 M D Anderson Blvd, Houston 5, Tex *B F Hambleton Prof and Chairman of the Dept* (1, 1933)
- Hoffman, William Samuel**, Ph D, M D Hektoen Inst for Med Research, 629 S Wood St, Chicago 12, Ill *Dir of Biochemistry, Cook County Hospital* (2, 1935)
- Hofmann, Klaus**, Ph D Univ of Pittsburgh, Dept of Chemistry, Pittsburgh, Pa *Research Prof* (2, 1947)
- Hogan, Albert G**, Ph D Univ of Missouri, 105 Schweitzer Hall, Columbia *Prof of Animal Nutrition* (2, 1916, 5, 1933)
- Hogness, Thorfin R**, Ph D Univ of Chicago, Chicago Ill *Dir, Inst of Radiobiology and Biophysics* (2, 1941)
- Holck, Harald G O**, Ph D Univ of Nebraska, College of Pharmacy, Lincoln *Prof of Pharmacology* (1, 1935, 3, 1938)
- Hollaender, Alexander**, Ph D Oak Ridge Natl Lab, Oak Ridge, Tenn (1, 1947)
- Hollander, Franklin**, Ph D Mount Sinai Hospital, Fifth Ave and 100th St, New York City *Assoc in Physiology, Head, Gastro-enterology Research Lab* (1, 1942, 2, 1932)
- Hollinshead, W Henry**, Ph D Mayo Clinic, Rochester, Minn *Head of Section on Anatomy, Prof of Anatomy, Mayo Foundation* (1, 1949)
- Holm, August**, Sc D E R Squibb & Sons, New Brunswick, N J *Research Assoc* (6, 1946)
- Holman, Ralph Theodore**, Ph D A and M College of Texas, College Station *Assoc Prof of Biochemistry and Nutrition* (2, 1948)
- Holman, Russell Lowell**, M D Louisiana State Univ School of Medicine, New Orleans *Prof of Pathology* (4, 1940)
- Holmes, Arthur Dunham**, Ph D Univ of Massachusetts, Amherst *Research Prof of Chemistry* (2, 1931, 5R, 1933)
- Holmes, Joseph H**, M D, D Med Sc Univ of Colorado School of Medicine, 4200 E Ninth Ave, Denver 7 *Assoc Prof of Medicine* (1, 1947)
- Holmes, Julia O**, Ph D 79 Old Town Rd, Amherst, Mass (2, 1942, 5, 1936)
- Holt, Joseph Paynter**, M D, Ph D Standard Oil Co, Room 2400, 30 Rockefeller Plaza, New York City 20 *Dir of Med Research* (1, 1942)
- Holt, L Emmett, Jr**, M D 477 First Ave, New York City 16 *Prof of Pediatrics, New York Univ College of Medicine* (2, 1930, 5, 1946)
- Hoobler, Icie Macy**, Ph D 660 Frederick St, Detroit 2, Mich *Dir, Research Lab Children's Fund of Michigan* (2, 1925, 5, 1933)
- Hoobler, Sibley W**, M D Univ of Michigan Hospital, Dept of Medicine and Surgery, Ann Arbor *Asst Prof of Medicine* (1, 1949)
- Hooker, Davenport**, Ph D Univ of Pittsburgh School of Medicine, Pittsburgh, Pa *Prof of Anatomy* (1, 1920)
- Hooker, Sanford B**, A M, M D 80 E Concord St, Boston, Mass *Member Evans Memorial* (6, 1918)

- Hoover, Sam R , Ph D 7815 Linden Rd , Philadelphia 18, Pa *Sr Chemist Eastern Regional Research Lab , U S Dept of Agriculture* (2, 1946)
- Hoppe, James O , Ph D Sterling-Winthrop Research Inst , Rensselaer, N Y *Research Assoc in Pharmacology* (3, 1950)
- Hoppert, C A , Ph D Michigan State College, Box 626, East Lansing *Prof of Biological Chemistry* (5, 1935)
- Hopps, Howard C , M D Univ of Oklahoma School of Medicine, 801 E 13th St , Oklahoma City *Prof and Chairman of Dept of Pathology* (4, 1947, 6, 1946)
- Horecker, Bernard L , Ph D Natl Insts of Health, Bethesda 14, Md *Biochemist* (2, 1947)
- Horn, Millard J , Ph D 1811 Varnum St , N E , Washington 18, D C *U S Dept of Agriculture, Chemist* (2, 1949)
- Horowitz, Norman H , Ph D California Inst of Technology, Pasadena *Assoc Prof of Biology* (2, 1946)
- Horsfall, Frank L , Jr , M D C.M Rockefeller Inst for Med Research, 66th St and York Ave , New York City *Member, Physician to the Hospital of the Rockefeller Inst for Med Research* (6, 1937)
- Horton, Richard G , Ph D Med Div , Army Chemical Center, Md *Acting Chief, Toxicology Section* (1, 1948)
- Horvath, Steven M , Ph D State Univ of Iowa School of Medicine, Dept of Physiology, Iowa City (1, 1943)
- Horwitt, M K , Ph D Biochemical Research Lab , Elgin State Hospital, Elgin, Ill *Dir , Asst Prof , Biological Chemistry, Univ of Illinois College of Medicine* (2, 1941, 5 1949)
- Hoskins, R G , Ph D , M D 86 Varick Rd , Waban 68, Mass (1R, 1911)
- Hotchkiss, Rollin D , Ph D Rockefeller Inst for Med Research, 66th St and York Ave , New York City *Assoc Member* (2, 1941)
- Hou, H C , M D % Union Embroidery Co , 22 Wyndham St , Hong Kong, China (5, 1949)
- Houck, C Riley, Ph D Univ of Tennessee Med School, Memphis 3 *Asst Prof in Physiology* (1, 1947)
- Hove, Edwin L , Ph D Alabama Polytechnic Inst , Lab of Animal Nutrition, Auburn *Research Biochemist* (5, 1946)
- Howard, Evelyn, Ph D Johns Hopkins School of Medicine, Baltimore, Md *Asst Prof of Physiology* (1, 1933)
- Howard, John Eager, A B , M D Johns Hopkins Hospital, Baltimore 5, Md *Asst Prof of Medicine* (1, 1946)
- Howard, Marion E , M D 38 Trumbull St , New Haven, Conn *Assoc Clin Prof of Medicine, Yale School of Medicine Assoc Physician New Haven Hospital and New Haven Dispensary* (4, 1939, 6, 1937)
- Howe, Howard A , Ph D Johns Hopkins Univ , 1901 East Madison St , Baltimore 5, Md *Adjunct Prof of Epidemiology* (6, 1949)
- Howe, Paul E , Ph D U S Dept of Agriculture, Bureau of Animal Ind , Washington 25, D C , *Nutrition Consultant* (1, 1913, 2, 1909, 5, 1933)
- Howell, Katherine M , M D 6830 S Merrill Ave , Chicago, Ill *Head of Depts of Bacteriology and Serology* (6, 1940)
- Howell, Stacey F , Ph D Veterans Admin Hospital, Perry Point, Md *Biochemist, Clin Lab* (2, 1940)
- Howland, Joe W , M D , Ph D Strong Memorial Hospital, 260 Crittenden Blvd , Rochester 7, N Y *Asst Prof of Radiation Biology, Univ of Rochester, Chief, Div of Med Services* (1, 1949)
- Hubbard, Roger Sanford, Ph D Univ of Buffalo School of Medicine, 24 High St , Buffalo 2, N Y *Prof of Pharmacology* (1, 1922, 2, 1920)
- Hubbell, Rebecca B , Ph D Connecticut Agricultural Exper Station, New Haven *Asst Biochemist* (2, 1937, 5, 1935)
- Hudack, Stephen Sylvester, M D 180 Fort Washington Ave , New York City *Assoc Prof of Orthopedic Surgery, Columbia Univ* (4, 1933)
- Huddleston, Ora L , M D , Ph D Kabat-Kaiser Inst , 1815 Ocean Front, Santa Monica, Calif *Med Dir* (1, 1936)
- Hueper, Wilhelm C , M D Natl Cancer Inst , Bethesda, Md *Chief, Cancerigenic Studies Section, Chief, Cancerigenic Research Labs* (4, 1940)
- Huf, Ernst G , Ph D , M D 3500 Carolina Ave , Richmond, Va *Assoc Prof of Physiology, Med College of Virginia* (1, 1949)
- Huff, Jesse W , Ph D Sharp & Dohme, Inc , Glenolden, Pa *Dir , Biochemical Research* (2, 1949)
- Huffman, C F , Ph D Michigan State College, East Lansing *Research Prof and Prof in Dairy Husbandry* (5, 1937)
- Huffman, Max N , Ph D Southwestern Med College, 2211 Oak Lawn Ave , Dallas, Tex *Research Prof of Biochemistry* (2, 1947)
- Huggins, Charles Brenton, M D Univ of Chicago, Chicago, Ill *Prof of Surgery* (1, 1932)
- Huggins, Joseph, M D 111 N 49th St , Philadelphia, Pa *Asst Prof of Exper Neurology, Grad School of Medicine, Univ of Pennsylvania, Dir of Lab , Pennsylvania Hospital for Mental Diseases* (1, 1936)
- Huggins, Russell A , Ph D Baylor Univ College of Medicine, 1200 M D Anderson Blvd , Houston 5, Tex *Assoc Prof of Pharmacology* (3, 1949)
- Hughes, Hettie B , Ph D Christ Hospital, Cincinnati 19, Ohio *Research Assoc* (2, 1916)
- Hughes, Josiah Simpson, Ph D Kansas State

- College, Manhattan *Prof of Chemistry* (2, 1931, 5, 1939)
- Hughes, Thomas P , Ph D Johns Hopkins Univ School of Public Health, Baltimore, Md *Member Field Staff, Rockefeller Foundation, New York City* (6, 1934)
- Hulpieu, Harold R , Ph D Indiana Univ School of Medicine, Indianapolis *Prof of Pharmacology* (3, 1939)
- Humphreys, Eleanor M , M D Univ of Chicago, Dept of Pathology, Chicago 37, Ill *Professor* (4, 1950)
- Humphries, James C , Ph D Univ of Kentucky, College of Arts and Science, Lexington *Assoc Prof of Bacteriology* (6, 1950)
- Hundley, James M , M D Natl Insts of Health, Exper Biology and Medicine Inst , Bethesda 14, Md *Chief, Section on Nutrition* (5, 1950)
- Hunscher, Helen A , Ph D Western Reserve Univ , 2023 Adelbert Rd , Cleveland 6, Ohio *Head of Dept of Home Economics* (5, 1934)
- Hunt, Carlton C , M D Wilmer Inst , Johns Hopkins Hospital, Baltimore, Md *Sr Fellow, Natl Research Council* (1, 1950)
- Hunter, Andrew, C B E , M B Hospital for Sick Children, Toronto, Ontario, Canada *Consulting Biochemist, Prof Emeritus of Pathological Chemistry, Univ of Toronto* (2, 1908)
- Hunter, Francis Edmund, Jr , Ph D Washington Univ Med School, St Louis 10, Mo *Asst Prof of Pharmacology* (2, 1946)
- Hunter, George, M A , D Sc 108 Walton Way, Aylesbury, Bucks, England (2, 1924)
- Hunter, Jesse E , Ph D Allied Mills, Inc , 7500 S Adams St , Peoria, Ill *Dir of Research* (5, 1936)
- Hunter, John, Ph D 61 Connaught Ave , Newtonbrook, Ontario, Canada (1, 1948)
- Hussey, Raymond, M D American Med Association, 535 N Dearborn, Chicago 10, Ill *Scientific Dir , Council on Industrial Health* (4, 1927)
- Hutchens, John O , Ph D Univ of Chicago, Dept of Physiology, Chicago 37, Ill *Assoc Prof and Chairman of Dept of Physiology, Dir of Toxicity Lab* (1, 1947)
- Hutchings, Brian L , Ph D Lederle Labs Div , American Cyanamid Co , Pearl River, N Y *Head, Dept of Biochemistry* (2, 1949)
- Hyman, Chester, Ph D Univ of Southern California School of Medicine, Los Angeles *Asst Prof of Physiology* (1, 1948)
- Ingalls, Mabel S , Ph D 1075 Park Ave , New York City 28 *Consultant, World Health Organization, Lake Success* (6, 1940)
- Ingle, Dwight J , Ph D Upjohn Co , Research Labs , Kalamazoo, Mich *Sr Research Scientist* (1, 1939)
- Ingraham, Raymond Clifford, Ph D Univ of Illinois College of Medicine, 1853 W Polk St , Chicago *Asst Prof in Physiology* (1, 1938)
- Ingram, W R , Ph D State Univ of Iowa College of Medicine, Iowa City *Prof and Head of Dept of Anatomy* (1, 1936)
- Irish, Don D , Ph D Dow Chemical Co , Midland, Mich *Dir , Biochemical Research Lab* (3, 1948)
- Irvin, J Logan, Ph D Univ of North Carolina, School of Medicine, Chapel Hill *Assoc Prof Biological Chemistry and Nutrition* (2, 1942)
- Irving, George Washington, Jr , Ph D U S Dept of Agriculture, Bureau of Agricultural and Industrial Chemistry, South Bldg , Washington 25, D C *Asst Chief* (2, 1946)
- Irving, Laurence, Ph D USPHS, P O Box 960, Anchorage, Alaska *Biologist, Alaska Health and Sanitation Activities* (1, 1927)
- Irwin, M R , Ph D Univ of Wisconsin, Madison *Prof of Genetics* (6, 1936)
- Isaacs, Raphael, M A , M D 104 S Michigan Ave , Suite 630, Chicago 3, Ill *Attending Physician, Dept of Hematology, Michael Reese Hospital* (4, 1928)
- Isbell, Harris, M D USPHS Hospital, Lexington, Kv *Dir , Research Div* (3, 1949)
- Isenberger, R M , M A , M D Univ of Kansas School of Medicine, Kansas City *Prof of Pharmacology* (3, 1937)
- Ivy, A C , Ph.D , M D Univ of Illinois, Chicago Professional Colleges, Chicago, Ill *Vice-Pres , Distinguished Service Prof of Physiology* (1, 1919, 5, 1933)
- Iwamoto, Harry K , Ph D Univ of Buffalo, Schools of Medicine and Pharmacy, Dept of Pharmacology, Buffalo 14, N Y (3, 1948)
- Izquierdo, J Joaquin, M D Natl School of Medicine, Mexico City *Prof of Physiology in Natl School of Medicine and the Escuela Medico Militar of Mexico* (1, 1928)
- Jackson, Dennis Emerson, Ph D , M D 144 Louis Ave , Cincinnati 20, Ohio *Prof Emeritus of Pharmacology, Univ of Cincinnati College of Medicine* (1R, 1910, 3R, 1912)
- Jackson, Elizabeth B , A B Army Med Center—AMDR & GS, Dept of Virus and Rickettsial Diseases, Washington 12, D C *Bacteriologist* (6, 1949)
- Jackson, Eugene L , Ph D 1322 W Broad St , Richmond 20, Va *Med Dir , A H Robins Co* (3, 1942)
- Jackson, Richard W , Ph D U S Dept of Agriculture, Northern Regional Research Lab , 825 N University St , Peoria 5, Ill *Head of Fermentation Div* (2, 1930, 5, 1933)
- Jacobs, Merkel Henry, Ph D Univ of Pennsylvania, Philadelphia *Prof of General Physiology* (1, 1919)
- Jacobs, Walter A , Ph D Rockefeller Inst for Med Research, 66th St and York Ave , New

- York City 21 *Member Emeritus* (2, 1908, 3, 1913)
- Jacobsen, Erik**, M D Medicinalco Ltd, Copenhagen S, Denmark *Research Dir* (3, 1950)
- Jacobson, Edmund**, Ph D, M D Lab for Clin Physiology, 55 E Washington St, Chicago, Ill (1, 1929)
- Jaffe, Henry L**, M D Hospital for Joint Diseases, 1919 Madison Ave, New York City *Dir of Labs* (4, 1925)
- Jahn, Theodore Louis**, Ph D Univ of California, Los Angeles 24 *Chairman, Dept of Zoology* (1, 1944)
- Jailer, Joseph W**, Ph D Columbia Univ College of Physicians and Surgeons, New York City *Instr in Medicine* (1, 1948)
- Jamieson, Walter A**, Sc D Green Braes, R.R. 2, Indianapolis, Ind *Dir, Biological Div, Eli Lilly & Co* (6, 1927)
- Jandorf, Bernard J**, Ph D Med Div, Army Chemical Center, Md *Asst Chief, Biochemistry Section* (2, 1946)
- Janes, Ralph G**, Ph D State Univ of Iowa College of Medicine, Iowa City *Assoc Prof of Anatomy* (1, 1948)
- Jansen, Eugene F**, B A Enzyme Research Lab, Western Regional Research Lab, Albany 6, Calif *Sr Chemist, Bureau of Agricultural and Industrial Chemistry, U S Dept of Agriculture* (2, 1947)
- Jaques, L B**, Ph D Univ of Saskatchewan, Saskatoon, Canada *Prof of Physiology* (1, 1943)
- Jasper, Herbert H**, Ph D, D s Sci Montreal Neurological Inst, 3801 University St, Montreal, Quebec, Canada *Lecturer in Neuro-electrography and Dir of Dept of Electrophysiology* (1, 1940)
- Jawetz, Ernest**, Ph D, M D Univ of California Med Center, 3rd and Parnassus Aves, San Francisco 22 *Assoc Prof of Bacteriology, Lecturer in Medicine and Pediatrics* (6, 1950)
- Jeans, P C**, M D Childrens' Hospital, State Univ of Iowa Iowa City *Prof of Pediatrics* (5, 1937)
- Jensen, Hans F**, Ph D Med Dept Field Research Lab, Fort Knox, Ky *Chief Biochemist* (2, 1929)
- Jobling, James W**, M D Suwanee Hotel, St Petersburg, Fla *Prof Emeritus of Pathology, Columbia Univ* (4R, 1913)
- Jochim, Kenneth E**, Ph D Univ of Kansas, Dept of Physiology, Lawrence (1, 1942)
- Johlin, J M**, Ph D Vanderbilt Univ School of Medicine, Nashville, Tenn *Assoc Prof of Biochemistry* (2, 1928)
- Johnson, B Connor**, Ph D Univ of Illinois, Div of Animal Nutrition, 554 Davenport Hall, Urbana *Assoc Prof* (2, 1947, 5, 1947)
- Johnson, Frank H**, Ph D Univ of Utah, Dept of Chemistry, Salt Lake City 1 (1, 1942)
- Johnson, Joseph L**, Ph D, M D Howard Univ School of Medicine, Washington, D C *Prof and Head of Dept of Physiology* (1, 1934)
- Johnson, J Raymond**, Ph D Univ of Ottawa, Faculty of Medicine, Ottawa, Ontario, Canada (1, 1938)
- Johnson, Marvin J**, Ph D Univ of Wisconsin, Biochemistry Bldg, Madison *Prof of Biochemistry* (2, 1941)
- Johnson, Robert E**, M D, Ph D Univ of Illinois College of Liberal Arts and Sciences, Dept of Physiology, Urbana *Head of Dept* (1, 1944, 2, 1939, 5, 1946)
- Johnson, S R**, Ph D Missouri Farmers Assoc Milling Co, Springfield *Dir of Nutrition* (5, 1947)
- Johnson, Victor**, Ph D, M D Mayo Foundation, Rochester, Minn (1, 1933)
- Johnston, Charles G**, M S, M D Wayne Univ College of Medicine, Detroit, Mich *Prof of Surgery* (1, 1933)
- Johnston, Frances A**, Ph D Cornell Univ, Ithaca, N Y *Asst Prof of Foods and Nutrition* (5, 1948)
- Johnston, Margaret W**, Ph D B-452, Univ Hospital, Ann Arbor, Mich *Research Assoc in Internal Medicine* (2, 1930, 5, 1938)
- Jolliffe, Norman**, M D 39 E 75th St, New York City (1, 1932)
- Jones, D Breese**, Ph D 2901 18th St, N W, Washington 9, D C *Retired* (2, 1920, 5R, 1935)
- Jones, Hardin B**, Ph D Univ of California, Div of Med Physics, Berkeley 4 *Asst Prof of Med Physics and Physiology, Asst Dir of Donner Lab* (1, 1949)
- Jones, James H**, Ph D Univ of Pennsylvania School of Veterinary Medicine, Philadelphia 4 *Prof of Physiological Chemistry* (2, 1928, 5, 1933)
- Jones, Kenneth K**, Ph D Northwestern Univ Med School, 303 E Chicago Ave, Chicago, Ill *Assoc Prof of Physiology and Pharmacology* (1, 1936)
- Jones, Ralph, Jr**, M D Univ of Pennsylvania Med School, Philadelphia 4 *Assoc in Medicine* (3, 1950)
- Joseph, Norman R**, Ph D Univ of Illinois, 1853 W Polk St Chicago 12 *Asst Prof of Chemistry* (2, 1947)
- Joslin, Elliott P**, M A, M D, 81 Bay State Rd, Boston, Mass *Dir, George F Baker Clinic* (5R, 1933)
- Jukes, Thomas Hughes**, Ph D Lederle Labs, Pearl River, N Y *Head, Dept of Nutrition and Physiology Research* (2, 1935, 5, 1935)
- Jung, Frederic Theodore**, Ph D, M D American

- Med Assoc, Chicago, Ill Asst Secretary (1, 1930)
- Jungeblut, Claus W, M D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 Prof of Bacteriology (4, 1929, 6, 1926)
- Kabat, Elvin A, Ph D Columbia Univ College of Physicians and Surgeons, 710 W 168th St, New York City 32 Assoc Prof of Bacteriology (2, 1940, 6, 1943)
- Kabat, Herman, Ph D, M D 2633 16th St, N W, Washington, D C Consultant in Neurology, Health Dept District of Columbia (1, 1941)
- Kahn, Reuben L, Sc D, Univ of Michigan Hospital, Ann Arbor Assoc Prof of Serology of Syphilis and Chief of Serology Lab, (4, 1934, 6, 1919)
- Kalckar, Herman M, M D, Ph D Univ of Copenhagen, Inst f Cell Physiology, 28 Juliane Maries Vej, Copenhagen, Denmark Dir of Inst, Research Prof, Inst f Cell Physiology (2, 1942)
- Kalmitsky, George, Ph D State Univ of Iowa, Iowa City Assoc Prof of Biochemistry (2, 1948)
- Kalter, Seymour Sanford, Ph D Syracuse Univ, College of Medicine, Dept of Bacteriology and Parasitology, Syracuse, N Y Asst Prof, Assoc Bacteriologist, Syracuse Health Dept (6, 1950)
- Kamen, Martin D, Ph D Washington Univ Med School, 510 S Kingshighway, St Louis 10, Mo Assoc Prof of Chemistry (2, 1946)
- Kamm, Oliver, Ph D 365 Lakeshore Drive, Detroit 30, Mich Research Consultant, Parke, Davis and Co (2, 1928)
- Kaplan, Nathan O, Ph D Johns Hopkins Univ, McCallum-Pratt Inst, Baltimore 18, Md Asst Prof of Biology (2, 1949)
- Karel, Leonard, Ph D Natl Insts of Health, Div of Research and Fellowships, Bethesda 14, Md Exec Sec, Hematology and Pharmacology Study Sections (3, 1947)
- Karlson, Alfred G, Ph D, D V M Mayo Foundation, Rochester, Minn Assoc in Exper Pathology (4, 1950)
- Karpovich, Peter V, M D, M P E Springfield College, Springfield, Mass Prof of Physiology (1, 1942)
- Karshan, Maxwell, Ph D Columbia Univ, Dept of Biological Chemistry, 630 W 168th St, New York City 32 Assoc Prof of Biochemistry (2, 1939)
- Karsner, Howard T, M D Dept of the Navy, Bureau of Medicine and Surgery, Research Div, Washington 25, D C Med Research Advisor (4, 1913)
- Katz, Louis Nelson, M A, M D 2900 Ellis Ave, Chicago, Ill Dir of Cardiovascular Research, Michael Reese Hospital, Professorial Lecturer in Physiology, Univ of Chicago (1, 1924)
- Katzman, Philip A, Ph D St Louis Univ School of Medicine, 1402 S Grand Blvd, St Louis 4, Mo Assoc Prof of Biochemistry (2, 1935)
- Kaulbersz, Jerzy, Ph D M D Collegium Medica, Grzegorzeczka 16, Cracow, Poland Prof of Physiology (1, 1944)
- Kaunitz, Hans, M D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 Research Assoc in Pathology (4, 1947)
- Kazal, Louis Anthony, Ph D Sharp & Dohme, Inc, Med Research Div, Glenolden, Pa Research Biochemist (2, 1947)
- Keeton, Robert W, M S, M D Univ of Illinois College of Medicine, 1853 W Polk St, Chicago Prof and Head of Dept of Medicine (1, 1916, 3, 1924)
- Kehoe, Robert A, M D Univ of Cincinnati College of Medicine, Kettering Lab of Applied Physiology, Eden Ave, Cincinnati, Ohio Research Prof of Physiology (1, 1940)
- Keith, Norman M, M D Mayo Clinic, Rochester, Minn Consulting Physician, Div of Medicine, Mayo Clinic, Prof of Medicine, Mayo Foundation, Univ of Minnesota (1, 1920, 3, 1932, 4, 1924)
- Keith, T B, Ph D Dept of Animal Husbandry, Univ of Idaho, Moscow, Idaho (5, 1941)
- Kellaway, Peter E, Ph D Baylor Univ College of Medicine, Houston, Texas Assoc Prof of Neuropsychiatry and Physiology, Dir of Lab of Clin Electrophysiology (1, 1947)
- Keller, Allen D, Ph D Field Research Lab, Fort Knox, Ky Med Dept (1, 1931)
- Kellner, Aaron, M D Cornell Univ Med College, Dept of Pathology, 1300 York Ave, New York City 21 Asst Prof of Pathology, Dir of Central Labs, New York Hospital and Cornell Med Center (4, 1949)
- Kelser, Raymond A, D V M, Ph D The Wyndon, Apt A-105, Wynnwood, Pa Prof of Bacteriology and Dean of Faculty, School of Veterinary Medicine, Univ of Pennsylvania, Philadelphia (4, 1932)
- Kelsey, F Ellis, Ph D Univ of Chicago, Chicago, Ill Assoc Prof of Pharmacology (3, 1941)
- Kelsey, Frances Kathleen O, Ph D Univ of Chicago, Chicago, Ill Assoc Prof of Pharmacology (3, 1941)
- Kemmerer, A R, Ph D Univ of Arizona, Tucson Head of Dept of Human Nutrition (5, 1946)
- Kempner, Walter, M D Duke Univ School of Medicine, Durham, N C Asst Prof of Medicine (1, 1940)
- Kendall, Edward C, Ph D, 627 Eighth Ave, S W, Rochester, Minn Prof of Biochemistry, Mayo Foundation, Univ of Minnesota (1, 1916, 2, 1913, 4, prior to 1920)

- Kendall, Forrest E**, Ph D 240-06-53rd Ave, Douglaston, Long Island, N Y Asst Prof of Biochemistry, Research Service, Columbia Div, Goldwater Memorial Hospital Welfare Island (6, 1943)
- Kennard, Margaret A**, M D Univ of Oregon Med School, Dept of Surgery, Portland Assoc Prof of Exper Surgery (1, 1934)
- Kennedy, Cornelia**, Ph D 2700 W Robbins St, Minneapolis 10, Minn Prof Emeritus of Agricultural Biochemistry, Univ of Minnesota (2, 1924, 5R, 1934)
- Kensler, Charles J**, Ph D Cornell Univ Med College, 1300 York Ave, New York City 21 Instr, Dept of Pharmacology, St Research Fellow, Natl Research Council (3, 1949)
- Kent, John F**, A M Army Med Center, Washington 12, D C Scientific Div, Dept of Serology, Army Med Dept Research and Grad School, (6, 1947)
- Kenton, Harold B**, Ph D New England Deaconess Hospital, Boston, Mass Bacteriologist and Div of the Blood Bank (6, 1934)
- Kenyon, Allan T**, M D Univ of Chicago, Div of Biological Sciences, 950 E 59th St, Chicago, Ill Asst Prof of Medicine (3, 1940)
- Keresztesy, John C**, Ph D Natl Insts of Health, Bethesda 14, Md Scientist Officer, Div of Physiology (2, 1941, 5, 1945)
- Kerr, Stanley E**, Ph D American Univ of Beirut, Beirut, Lebanon Prof of Biological Chemistry (2, 1937)
- Kerr, Wm J**, M D Univ of California Hospital, 3rd and Parnassus Aves, San Francisco Physician-in-Chief, Prof of Medicine, Univ of California (3, 1930)
- Kesten, Homer D**, M D Columbia Univ College of Physicians and Surgeons, New York City 32 Assoc Prof of Pathology (4, 1931)
- Keston, Albert S**, Ph D New York Univ College of Medicine, 477 First Ave, New York City Asst Prof, Dept of Biochemistry (2, 1950)
- Kety, Seymour S**, M D Univ of Pennsylvania Grad School of Medicine, Dept of Physiology and Pharmacology, Philadelphia 4 Prof of Clin Physiology (1, 1949, 3, 1945)
- Keys, Ancel**, Ph D Univ of Minnesota, Stadium Gate 27, Minneapolis 14 Prof and Dir of Lab of Physiological Hygiene (1, 1939, 2, 1936)
- Kibrick, Andre C**, Ph D Bronx Hospital, 169th St and Fulton Ave, New York City 56 Head, Dept of Chemistry (2, 1950)
- Kidd, John G**, M D Cornell Univ Med College, 1300 York Ave, New York City 32 Prof of Pathology, Pathologist, New York Hospital (4, 1937, 6, 1937)
- Kidder, George W**, Ph D Amherst College, Amherst, Mass Stone Prof of Biology (2, 1949)
- Kies, Marian W**, Ph D Agricultural Research Center, North Bldg, Beltsville, Md Biochemist, Bureau of Agricultural and Industrial Chemistry, (2, 1948)
- Kik, M C**, Ph D Univ of Arkansas College of Agriculture, Fayetteville Assoc Prof of Agricultural Chemistry (5, 1942)
- Kilborn, Leslie G**, M D, Ph D 47 Warren Rd, Toronto, Ontario, Canada At present West China Union Univ, Chengtu, Sze, China (1, 1928)
- Kilham, Lawrence**, M D, Natl Insts of Health, Bethesda 14, Md Surgeon, USPHS (6, 1950)
- Killian, John Allen**, Ph D Killian Research Labs, Inc, 49 W 45th St, New York City (2, 1921)
- Kimura, Kazuo K**, Ph D St Louis Univ School of Medicine, Dept of Pharmacology, St Louis 4, Mo Instructor (3, 1950)
- Kinard, F W**, Ph D, M D Med College of the State of South Carolina, Charleston 16 Assoc Prof of Physiology (1, 1947)
- King, Barry G**, Ph D Dept of Commerce, Civil Aeronautics Admin, Med Service, Safety Regulations, Washington, D C Chief, Aeromed Design and Material Div (1, 1938)
- King, Charles Edwin**, Ph D Vanderbilt Univ, Nashville, Tenn Assoc Prof of Physiology (1, 1916)
- King, Charles Glen**, Ph D Nutrition Foundation, Inc, Chrysler Building, New York City Scientific Dir, Prof of Chemistry, Columbia Univ 2, 1931, 5, 1933)
- King, Henry Eugene**, Ph D Tulane Univ School of Medicine, Dept of Psychiatry and Neurology, 1430 Tulane Ave, New Orleans 12, La Asst Prof, Visiting Scientist, Charity Hospital of Louisiana (1, 1950)
- King, Jesse**, Ph D Towson, Md (1R, 1914)
- King, Joseph T**, M D, Ph D Univ of Minnesota Med School, 314 Millard Hall, Minneapolis Assoc Prof of Physiology (1, 1931)
- Kinney, Thomas D**, M D Cleveland City Hospital, 3395 Scranton Rd, Cleveland, Ohio Pathologist-in-Chief, Prof of Pathology, Western Reserve Univ (4, 1950)
- Kirch, Ernst R**, Ph D Univ of Illinois College of Pharmacy, 808 S Wood St, Chicago 12, Ill Assoc Prof of Chemistry (2, 1948)
- Kirchhof, Anton C**, M S, M D 758 S View Rd, Oswego, Ore Anesthesiologist, Providence, Veterans, and Matson Memorial Hospitals (3, 1947)
- Kirk, John E**, M D Washington Univ School of Medicine, 5600 Arsenal St, St Louis 9 Mo Dir of Research, Div of Gerontology, Assoc Prof of Medicine (2, 1950)
- Kirk, Paul L**, Ph D Univ of California, Berkeley Prof of Biochemistry (2, 1933)
- Kirschbaum, Arthur**, M D, Ph D Univ of Min-

- nesota Med School, Minneapolis Assoc Prof of Anatomy (4, 1948)
- Kisch, Bruno, M D Yeshiva Univ, New York City Prof of Biochemistry (1, 1943)
- Kleiber, M., D Sc Univ of California, Davis Prof of Animal Husbandry (1, 1943, 5, 1933)
- Klein, J Raymond, Ph D Brookhaven Natl Lab, Upton, L I, N Y Sr Biochemist, Dept of Biology (2, 1941)
- Kleiner, Israel Simon, Ph D New York Med College and Flower and Fifth Ave Hospitals, New York City 29 Prof of Biochemistry, Dir, Dept of Physiology and Biochemistry (1, 1911, 2, 1912, 3R, 1912, 5R, 1933)
- Kleitman, Nathaniel, Ph D Univ of Chicago, Chicago, Ill Prof of Physiology (1, 1923)
- Klemperer, Friedrich Wilhelm, M D Trudeau Foundation, Trudeau, N Y Head of Dept of Biochemistry (2, 1941)
- Kletzien, Seymour W, Ph D 330 S Ninth St, Philadelphia, Pa Nutrition Research Clinic, Philadelphia Living-In and Pennsylvania Hospitals Biochemist (5, 1933)
- Kline, O L, Ph D Federal Security Agency, Food and Drug Admin, Washington, D C Biochemist (5, 1936)
- Kline, Raymond F, M S Route 1, Box 69, Fairborn, Ohio (1, 1946)
- Klotz, Irving M, Ph D Northwestern Univ, Evanston, Ill Prof of Chemistry (2, 1947)
- Kluver, Heinrich, Ph D Univ of Chicago, Chicago, Ill Prof of Exper Psychology (1, 1935)
- Knight, C Arthur, Ph D Univ of California, Virus Lab, Berkeley 4 Assoc Prof (2, 1946)
- Knisely, Melvin H, Ph D Med College of South Carolina, Charleston Chairman of Dept of Anatomy (1, 1949)
- Knoefel, Peter K, M A, M D Univ of Louisville, 101 W Chestnut St, Louisville, Ky Prof and Chairman of Dept of Pharmacology (3, 1934)
- Knowlton, Frank P, M D Syracuse Univ College of Medicine, Syracuse, N Y Prof Emeritus of Physiology (1R, 1911)
- Knowlton, G Clinton, Ph D Emory Univ, Room 101, Physiology Bldg, Emory University, Ga (1, 1938)
- Knox, W Eugene, M D 3026 S California Ave, Chicago 8, Ill Research Assoc, Rheumatic Fever Research Inst, Northwestern Univ Med School (2, 1948)
- Knudson, Arthur, Ph D Albany Med College, New Scotland Ave, Albany, N Y Prof of Biochemistry and Assoc Dean (2, 1919, 5, 1936)
- Knutti, Ralph Eddy, M D Children's Hospital, Los Angeles, Calif Dir of Labs, Asst Prof of Pathology, Univ of Southern California (4, 1933)
- Kober, Philip A, B S Sherman Labs, Detroit, Mich Dir of Research (2, 1912)
- Kobrak, Heinrich G, Ph D Univ of Chicago, Chicago, Ill Asst Prof of Surgery (1, 1948)
- Kochakian, Charles D, Ph D Univ of Rochester School of Medicine and Dentistry, Rochester 20, N Y Assoc Prof of Physiology (1, 1942, 2, 1948)
- Kocher, Rudolph Alfred, M D Box 936, Carmel, Calif Dir of Velje Metabolic Clinic (2, 1915)
- Kocholaty, Walter F, Ph D Field Research Lab, Med Dept, Fort Knox, Ky Biochemist, Dept of Biochemistry (2, 1950)
- Koehler, Alfred E, M D, Ph D 317 W Pueblo St, Santa Barbara, Calif Physician, Sansum Clinic, Santa Barbara Cottage Hospital (2, 1924)
- Koehn, Carl J, Ph D Surgeon General's Office, Preventive Medicine Div, Washington, D C Chief, Nutrition Branch (5, 1949)
- Koehne, Martha, Ph D 235 15th Ave, Apt 22, Columbus, Ohio Nutritionist, Ohio State Dept of Health (5, 1933)
- Koelle, George B, Ph D, M D Columbia Univ College of Physicians and Surgeons, Dept of Pharmacology, 630 W 168th St, New York City 32 Asst Prof (3, 1947)
- Koepf, George F, M D 537 Delaware Ave, Buffalo 2, N Y Assoc in Physiology, Univ of Buffalo (1, 1942)
- Koerber, Walter L, Ph D E R Squibb and Sons, New Brunswick, N J Asst Dept Head (6, 1943)
- Kohlstaedt, Kenneth G, M D Lilly Lab for Clinical Research, Indianapolis General Hospital, Indianapolis 7, Ind Director (1, 1947)
- Kohn, Henry I, Ph D, M D Univ of California Hospital, Radiology Dept, San Francisco Sr Surgeon, USPHS (1, 1940)
- Kolmer, John A, M S, M D, D P H 2101 Pine St, Philadelphia, Pa Prof of Medicine, Temple Univ, Dir, Research Inst of Cutaneous Medicine (6, 1913)
- Komarov, Simon A, M D, Ph D S S Fels Fund, Med Research Lab, 255 S 17th St, Philadelphia, Pa Dir of Dept of Biochemistry, Temple Univ School of Medicine (1, 1933)
- Kopeloff, Nicholas, Ph D New York State Psychiatric Inst, 722 W 168th St, New York City Principal Research Bacteriologist, New York State Psychiatric Inst and Hospital (6, 1937)
- Koppanyi, Theodore, Ph D Georgetown Univ, Washington, D C Prof of Pharmacology (1, 1924, 3, 1935)
- Koprowski, Hilary, M D Lederle Labs Div, American Cyanamid Co, Pearl River, N Y Asst Dir, Viral and Rickettsial Research (6, 1946)
- Kornberg, Arthur, M D Natl Insts of Health, Bethesda 14, Md Sr Surgeon (2, 1949)

- Korr, Irwin M , Ph D Kirksville College of Osteopathy and Surgery, Kirksville, Mo *Prof of Physiology* (1, 1939)
- Koshland, Marian Elliott, Ph D Harvard Med School, Dept of Bacteriology and Immunology, Boston, Mass (6, 1950)
- Kosman, A J , Ph D Northwestern Univ Med School, Dept of Physiology, 303 E Chicago Ave, Chicago 11, Ill *Asst Prof* (1, 1949)
- Koster, Rudolf, Ph D Tulane Univ, Dept of Pharmacology, Station 20, New Orleans, La *Asst Prof* (3, 1949)
- Kottke, Frederic J , Ph D , M D Univ of Minnesota, Minneapolis *Baruch Fellow in Physical Medicine* (1, 1947)
- Kozelka, Frank L , Ph D Univ of Wisconsin, Dept of Pharmacology and Toxicology, Madison *Assoc Prof of Toxicology* (3, 1939)
- Kraatz, Charles P , Ph D Jefferson Med College, 1025 Walnut St , Philadelphia, Pa *Assoc Prof of Pharmacology* (3, 1950)
- Krahl, Maurice E , Ph D Washington Univ School of Medicine, St Louis 10, Mo *Assoc Prof of Biological Chemistry* (2, 1939, 3, 1949)
- Krakower, Cecil Alexander, M D Univ of Illinois College of Medicine, 1853 West Polk St , Chicago *Prof of Pathology* (4, 1945)
- Kramer, Benjamin, A M , M D 60 Plaza St , Brooklyn 17, N Y *Pediatrician-in-Chief, Brooklyn Jewish Hospital, Prof of Clin Pediatrics, Long Island College Med School* (1, 1915, 2, 1914)
- Kramer, Martha, Ph D Kansas State College, Manhattan *Asst Dean, School of Home Economics* (5, 1933)
- Kramer, S D , M D , Ph D 92 Washington Sq East, Salem, Mass *Virologist* (6, 1944)
- Krampritz, Lester O , Ph D Western Reserve Univ , 2109 Adelbert Rd , Cleveland 6, Ohio *Head, Dept of Microbiology* (2, 1946, 6, 1949)
- Krantz, John C , Jr , Ph D Univ of Maryland Med School, Baltimore *Prof of Pharmacology* (3, 1937)
- Kratzer, F H , Ph D Univ of California, Div of Poultry Husbandry, Davis *Asst Prof* (5, 1949)
- Krauss, William E , Ph D Ohio Agricultural Exper Station, Wooster *Assoc Dir* (2, 1932, 5, 1933)
- Kraybill, Henry R , Ph D 5720 Woodlawn Ave , Chicago 37, Ill *Professorial Lecturer, Dept of Biochemistry, Univ of Chicago, Dir , Dept of Scientific Research, American Meat Inst Foundation* (2, 1942)
- Krayer, Otto, M D Harvard Med School, 25 Shattuck St , Boston, Mass *Assoc Prof and Head of Dept of Comparative Pharmacology* (3, 1938)
- Kreezer, George L , Ph D Washington Univ , St Louis 10, Mo *Assoc Prof of Psychology* (1, 1948)
- Krehl, Willard A , Ph D Yale Univ , 333 Cedar St , New Haven, Conn *Asst Prof of Nutrition* (2, 1947, 5, 1949)
- Krop, Stephen, Ph D Warner Inst for Therapeutic Research, Dept of Pharmacology, 113 W 18th St , New York City *Dir of Dept of Pharmacology and Chemotherapy* (1, 1949, 3, 1944)
- Krueger, Albert Paul, M D Univ of California, Berkeley *Prof and Chairman, Dept of Bacteriology, Scientific Dir , Naval Biological Lab* (4, 1930, 6, 1937)
- Krueger, Hugo, Ph D Oregon State College, Dept of Zoology, Corvallis *Prof of Physiology* (1, 1931, 3, 1935)
- Krumbhaar, Edward B , M D , Ph D Univ of Pennsylvania Med School, Philadelphia *Prof Emeritus of Pathology* (1R, 1914, 4, prior to 1920)
- Kruse, Harry Dayton, M D , Sc D Milbank Memorial Fund, 40 Wall St , New York City (2, 1933, 5, 1950)
- Kruse, Theophile K , Ph D Univ of Pittsburgh Med School, Pittsburgh, Pa *Prof of Physiology and Pharmacology* (1, 1919, 3, 1920)
- Kubicek, William G , Ph D Univ of Minnesota Hospitals, Div of Physical Medicine, Minneapolis *Assoc Prof* (1, 1947)
- Kubie, Lawrence S , M D 7½ E 81st St , New York City 28 *Clin Prof of Psychiatry and Mental Hygiene, Yale Univ School of Medicine, Faculty, N Y Psychoanalytic Inst* (4, 1928)
- Kuffler, Stephen W , M D Johns Hopkins Med School, Wilmer Inst , Baltimore 5, Md *Assoc Prof of Physiological Optics* (1, 1949)
- Kuhn, Harry A , M S 3915 Fulton St N W , Washington, D C *Consultant* (3, 1927)
- Kuhn, L Roland, Ph D Army Med Center, AMDR GS , Washington 12, D C *Chief, Dept of Bacteriology* (6, 1939)
- Kuiken, Kenneth A , Ph D A & M College of Texas, College Station *Assoc Prof , Dept of Biochemistry and Nutrition* (2, 1950)
- Kuizenga, Marvin H , Ph D Upjohn Company, Kalamazoo, Mich *Head of Dept of Pharmacology-Endocrinology* (2, 1947)
- Kun, Ernest, M D Tulane Univ , Dept of Medicine, 1430 Tulane Ave , New Orleans 12, La *Asst Prof* (3, 1949)
- Kunde, Margarete M , Ph D , M D 30 N Michigan Ave , Suite 1308, Chicago, Ill *Instr in Medicine, Northwestern Univ Med School, Clin Asst in Endocrinology, Cook County Hospital* (1, 1924)
- Kuntz, Moses, Ph D Rockefeller Inst for Med Research, 66th St and York Ave , New York City 21 *Member* (2, 1947)
- Kupperman, Herbert S , M D , Ph D New York Univ College of Medicine, Dept of Thera-

- peutics 477 First Ave, New York City *Research Assoc* (3, 1950)
- Kurtz, Alton C, Ph D Univ of Oklahoma Med School, Dept of Biochemistry, Oklahoma City *Assoc Prof* (2, 1942)
- Kuiper, Adrian C, Ph D Wayne Univ College of Medicine, Detroit 26, Mich *Asst Prof of Physiological Chemistry* (2, 1946)
- Kydd, David M, M D Yale Univ School of Medicine, New Haven, Conn *Assoc Prof of Medicine* (5, 1934)
- Kyes, Preston, Sc D, M D North Jay, Maine *Prof Emeritus* (6R, 1918)
- Kyker, Granvil C, Ph D Univ of Puerto Rico, School of Medicine, San Juan *Prof and Head, Dept of Biochemistry and Nutrition* (2, 1947)
- LaBelle, Annette, B A, 271 Alta Vista Dr, Tuckahoe 7, N Y (1, 1948)
- Lackey, Robert W, Ph D Southwestern Med College, Dept of Physiology and Pharmacology, Dallas 4, Texas *Prof of Physiology* (1, 1947)
- Lacorte, Jose G, M D Instituto Oswaldo Cruz, Rio de Janeiro, Brazil, S A *Dir, Virus Section, Oswaldo Cruz Inst* (6, 1946)
- Lacy, G R, M D Univ of Pittsburgh, Pittsburgh, Pa *Prof of Bacteriology and Immunology* (4, 1927)
- Lalich, Joseph J, M D Univ of Wisconsin, 426 N Charter St, Madison 6 *Assoc Prof of Pathology* (4, 1946)
- Lamb, Alvin R, Ph D Hawaiian Sugar Planters' Assoc, Exper Station, Honolulu *Research Assoc* (2, 1923, 5, 1934)
- Lambert, Edward H, Ph D, M D Mayo Foundation, Rochester, Minn *Asst Prof of Physiology* (1, 1945)
- Lambert, Robert A, M D Bricks Springs, Greensboro, Ala *Consultant, Pan American Sanitary Bureau, Regional Office of World Health Organization* (4, 1922)
- Lambertsen, Christian J, M D Univ of Pennsylvania School of Medicine, Philadelphia, Pa *Asst Prof of Pharmacology*, (3, 1948)
- Lampen, J Oliver, Ph D Western Reserve Univ School of Medicine, Cleveland 6, Ohio *Assoc Prof of Microbiology* (2, 1947)
- Lamport, Harold, M D Yale Univ School of Medicine New Haven, Conn *Assoc Prof of Physiology* (1, 1943)
- Lamson, Paul Dudley, M D Vanderbilt Univ Med School, Nashville, Tenn *Prof of Pharmacology* (1, 1921, 3, 1915)
- Lancefield, Rebecca C, Ph D 66th St and York Ave, New York City 21 *Assoc Member, Rockefeller Inst for Med Research* (6, 1933)
- Landis, Carney, Ph D Columbia Univ, Psychiatric Inst, 722 W 168th St, New York City *Principal Research Psychologist and Prof of Psychology* (1, 1939)
- Landis, Eugene Markley, Ph D, M D Harvard Med School, Dept of Physiology, 25 Shattuck St, Boston, Mass *George Higginson Prof of Physiology* (1, 1928)
- Landowne, Milton, M D 6101 Stewart Ave, Baltimore 9, Md *Assoc Chief, Cardiovascular Disease and Gerontology Section, Baltimore City Hospital* (1, 1947)
- Lands, Alonzo, M, Ph D Sterling-Winthrop Research Inst, Rensselaer, N Y *Head, Pharmacology Section* (1, 1942, 3, 1947)
- Lange, Carl, M D 371 Morris St, Albany, N Y *Assoc Bacteriologist, Divs of Labs and Public Health, N Y State Dept of Health* (6, 1938)
- Langley, Wilson D, Ph D Univ of Buffalo Med School, 24 High St, Buffalo, N Y *Prof of Biochemistry* (2, 1937)
- Langworthy, Orthello R, M A, M D Johns Hopkins Hospital, Baltimore, Md *Assoc Prof of Psychiatry, Johns Hopkins Univ* (1, 1928)
- Lanni, Frank, Ph D Duke Univ School of Medicine, Durham, N C *Research Assoc* (6, 1949)
- Lardy, Henry A, Ph D Inst for Enzyme Research, Univ of Wisconsin, Madison 6 *Prof of Biochemistry* (2, 1946)
- Larrabee, Martin G, Ph D Johns Hopkins Univ, Biophysics Dept, Baltimore 18, Md *Assoc Prof* (1, 1940)
- Larsen, Eleanor M, Ph D Univ of Wisconsin, Dept of Physiology, S M I, Madison 6 *Instr in Physiology* (1, 1949)
- Larson, Carl L, M D Natl Insts of Health, Div of Infectious Diseases, Bethesda, Md *Surgeon, USPHS* (6, 1948)
- Larson, Edward, Ph D Med Research, Unit of the Veterans Hospital, Coral Gables, Fla *Prof of Physiology and Pharmacology* (1, 1929, 3, 1937)
- Larson, Hardy W, Ph D Metropolitan Life Insurance Co, Biochemical Lab, 1 Madison Ave, New York City *Research Chemist* (2, 1937)
- Larson, Paul S, Ph D Med College of Virginia, Richmond *Research Prof of Pharmacology* (1, 1939, 3, 1947)
- Lashley, K S, Ph D Yerkes Labs, Orange Park, Fla *Research Prof of Neuropsychology, Harvard Univ, Dir, Yerkes Labs of Primate Biology, Inc* (1, 1923)
- Laskowski, M, Ph D Marquette Univ Med School, Milwaukee 3, Wis *Assoc Prof of Biochemistry* (2, 1944)
- Last, Jules H, Ph D, M D Northwestern Univ Med School, Chicago, Ill *Asst Prof Exper Medicine* (3, 1948)
- Lauffer, Max A, Jr, Ph D Univ of Pittsburgh, Pittsburgh 13, Pa *Research Prof and Head of Dept of Biophysics* (2, 1946)

- Laug, E P**, Ph D Food and Drug Admin, Div of Pharmacology, 12th and C Sts S W, Washington 25, D C *Pharmacologist* (2, 1938, 3, 1947)
- Laurens, Henry D**, Ph D, M D Box 157, Flatrock, N C (1R, 1913)
- Lauson, Henry D**, Ph D, M D, New York Hospital, Dept of Pediatrics, 525 E 68th St, New York City 21 *Associate* (1, 1946)
- Lavine, T F**, Ph D Lankenau Hospital Research Inst, Philadelphia, Pa *Research Chemist* (2, 1938)
- Lawrence, W Sherwood**, M D 906 Hazel St, Gridley, Calif *Physician and Surgeon* (3, 1944)
- Lawson, Hampden**, M D, Ph D Univ of Louisville, Louisville, Ky *Prof of Physiology* (1, 1933)
- Lawton, Alfred H**, M D, Ph D Veterans Admin, Bureau of Medicine and Surgery, Washington, D C *Acting Asst Chief Med Dir* (1, 1949, 3, 1948)
- Leake, Chauncey D**, Ph D Univ of Texas Med Branch, Galveston, *Vice-Pres of Univ of Texas in Charge of Med Program* (1, 1923, 3, 1924)
- Leathem, James H**, Ph D Rutgers Univ, New Brunswick, N J *Prof of Zoology* (1, 1945)
- Leathes, John Beresford**, M A, M B Westfield Ware Lane, Lyme Regis, Dorset, England *Emeritus* (2, 1909)
- Lederer, Ludwig George**, Ph D, M D Pennsylvania Central Airlines, Natl Airport, Washington, D C, *Med Dir* (1, 1940)
- Lee, Douglas H K**, M D Johns Hopkins Univ, I Bowman School of Geography, Baltimore 18, Md *Prof of Physiological Climatology, Lecturer in Physiological Hygiene* (1, 1949)
- Lee, Milton O**, Ph D 2101 Constitution Ave, Washington 25, D C *Exec Sec and Managing Editor, American Physiological Society, Federation Sec* (1, 1927, 5, 1933)
- Lee, Richard E**, Ph D, M D New York Hospital, Dept of Medicine, New York City 21 *Fellow in Medicine, Cornell Univ College of Medicine* (1, 1950)
- Leese, Chester E**, Ph D George Washington Univ School of Medicine, Washington, D C *Assoc Prof of Physiology* (1, 1934)
- LeFevre, Paul G**, Ph D Univ of Vermont College of Medicine, Dept of Physiology and Biophysics, Burlington *Asst Prof* (1, 1950)
- Lehman, Arnold J**, Ph D, M D Food and Drug Admin, Washington 25, D C *Chief, Div of Pharmacology* (3, 1937)
- Lehman, Robert A**, Ph D Campbell Pharmaceutical Co, 254 W 31st St, New York City 1 *Dir of Research* (3, 1942)
- Lehninger, Albert L**, Ph D Univ of Chicago Med School, 950 E 59th St, Chicago 37, Ill *Assoc Prof of Biochemistry in Depts of Biochemistry and Surgery* (2, 1946)
- Lehr, David**, M D New York Med College, Flower and Fifth Ave Hospitals, Fifth Ave at 105th St, New York City 29 *Assoc Prof in Pharmacology and Medicine* (3, 1947)
- Leichsenring, Jane M**, Ph D Univ of Minnesota, Univ Farm, St Paul *Prof of Nutrition* (5, 1948)
- Leimdorfer, Alfred**, M D Univ of Illinois College of Medicine, Dept of Psychiatry, Chicago 12 *Assoc Prof* (1, 1947)
- Lein, Allen**, Ph D Northwestern Univ Med School, Dept of Physiology, Chicago 11, Ill *Asst Prof* (1, 1946)
- Lenhart, Carl H**, M D Lakeside Hospital, 2065 Adelbert Rd, Cleveland, Ohio *Oliver H Payne Prof of Surgery, Western Reserve Univ* (1, 1921)
- Lennette, Edwin H**, Ph D, M D California State Dept of Public Health, Berkeley *Dir, Viral and Rickettsial Disease Lab, Lecturer in Virology, School of Public Health, Univ of California* (4, 1941, 6, 1947)
- Leonard, Clifford Shattuck**, Ph D 286 Whitefield St, Guilford, Conn *Technical Assoc, Chemical-Biological Coordination Center, Natl Research Council* (3, 1927)
- Leonards, Jack Ralph**, Ph D Western Reserve Univ Med School, Cleveland, Ohio *Asst Prof of Biochemistry* (2, 1948)
- Le Page, G A**, Ph D Univ of Wisconsin, McArdle Memorial Lab for Cancer Research, Madison 6 *Assoc Prof of Oncology* (2, 1949)
- Lepkovsky, Samuel**, Ph D Univ of California, Poultry Div, Berkeley 4 *Prof of Poultry Husbandry* (2, 1933, 5, 1933)
- L'Esperance, Elise L**, M D 2 East 61st St, New York City *Dir, Strong Cancer Precaution Clinic, Memorial Hospital, and New York Infirmary* (6, 1920)
- Leverton, Ruth M**, Ph D Univ of Nebraska, Dept of Home Economics, Lincoln *Assoc Prof of Human Nutrition Research* (5, 1942)
- Levin, Louis**, Ph D Columbia Univ, College of Physicians and Surgeons, 630 W 168th St, New York City 32 *Asst Prof of Anatomy* (2, 1939)
- Levine, Harold**, Ph D 941 E Sylvan Ave, Milwaukee 11, Wis (2, 1933, 5, 1933)
- Levine, Milton**, Ph D Kabat Kuser Inst 2600 Alameda St, Vallejo Calif *Dir of Research* (6, 1942)
- Levine, Philip**, M D Ortho Research Foundation, Raritan, N J *Dir, Biologic Div* (6, 1925)
- Levine, Rachmiel**, M D Michael Reese Hospital, Chicago, Ill *Acting Dir, Dept of Metabolic Research, Professorial Lecturer in Physiology, Univ of Chicago* (1, 1942)

- Levine, Samuel Z, M D, New York Hospital, 525 E 68th St, New York City *Prof of Pediatrics, Cornell Univ Med College, Pediatrician-in Chief, New York Hospital* (5, 1933)
- Levine, Victor Emanuel, Ph D, M D Creighton Univ School of Medicine, Omaha, Nebr *Prof of Biological Chemistry and Nutrition* (2, 1936)
- Levinson, Samuel A, Ph D, M D Univ of Illinois College of Medicine, 808 S Wood St, Chicago *Prof of Pathology, Dir of Labs, Research and Educational Hospital* (4, 1938)
- Levison, Louis A, M D 421 Michigan St, Toledo, Ohio *Physician to Toledo and St Vincent Hospitals* (6, 1916)
- Levy, Milton, Ph D New York Univ College of Medicine, 477 First Ave, New York City *Assoc Prof of Chemistry* (2, 1933)
- Levy, Robert L, M D 730 Park Ave, New York City *Prof of Clin Medicine, Columbia Univ College of Physicians and Surgeons* (3, 1915)
- Lewey, F H, M D 3400 Spruce St, Philadelphia 4, Pa *Prof of Neuroanatomy, Univ of Pennsylvania Grad School of Medicine, Assoc in Neuro-pathology and Neurosurgery, Med School* (1, 1937)
- Lewis, George T, Ph D Emory Univ School of Dentistry, Dept of Biochemistry, Emory Univ, Ga *Professor* (2, 1949)
- Lewis, Gladys Kinsman, Ph D 401 S Lafayette St, Denver 9, Colo *Nutrition Consultant, Colorado State Dept of Health* (5, 1944)
- Lewis, Howard Bishop, Ph D Univ of Michigan Med School, Ann Arbor *John Jacob Abel Univ Prof of Biological Chemistry* (1, 1925, 2, 1913, 5, 1933)
- Lewis, James C, Ph D U S Dept of Agriculture, Western Regional Research Lab, Albany 6, Calif *Biochemist* (2, 1946)
- Lewis, Jessica H, M D Univ of North Carolina School of Medicine, Dept of Physiology, Chapel Hill *Research Assoc* (1, 1949)
- Lewis, John R, Ph D Sterling-Winthrop Research Inst, Rensselaer, N Y *Research Assoc in Pharmacology* (3, 1950)
- Lewis, Julian Herman, M D 4750 Champlain Ave, Chicago, Ill *Assoc Prof of Pathology, Univ of Chicago, Member, Otho S A Sprague Memorial Inst* (4, 1924)
- Lewis, Lena A, Ph D Cleveland Clinic, Euclid Ave and E 93rd St, Cleveland 6, Ohio *Research Staff* (1, 1946)
- Lewis, Robert C, Ph D Univ of Colorado, 4200 E 9th Ave, Denver *Prof of Biochemistry, Dean, School of Medicine* (2, 1931, 5, 1933)
- Lewis, Warren H, M D Wistar Inst of Anatomy and Biology, Philadelphia, Pa *Member* (1R, 1919)
- Ley, Herbert Leonard, Jr, M D Army Med Center, AMDR & GS, Dept Virus and Rickettsial Diseases, Washington 12, D C *Assistant* (6, 1949)
- Li, Choh Hao, Ph D Univ of California, 4596 Life Sciences Bldg, Berkeley *Prof of Biochemistry* (2, 1944)
- Li, Richard C, M D c/o Dr T S Yu, 392 MaDang Rd, Shanghai 25, China *Guest in Dept of Pharmacology, Natl Med College of Shanghai* (3, 1941)
- Libby, Raymond L, Ph D Veterans Admin, Wilshire and Sawtelle Blvds, Los Angeles 25, Calif *Assoc Prof of Radio Biology, Univ of California Med School, Chief of Isotope Section, Veterans Hospital, Los Angeles* (6, 1938)
- Liberson, W T, M D Inst of Living, Hartford, Conn *Neurophysiologist* (1, 1948)
- Libet, Benjamin, Ph D Univ of California Med Center, Dept of Anatomy, San Francisco *Asst Prof of Physiology, Med School* (1, 1942)
- Licklider, J C R, Ph D Massachusetts Inst of Technology, Acoustics Lab Cambridge (1, 1938)
- Liddell, Howard S, Ph D Cornell Univ, Ithaca, N Y *Prof of Psychology* (1, 1925)
- Lieb, Charles C, M D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City *Hosack Prof of Pharmacology* (1R, 1936, 3R, 1915)
- Lieberman, Arnold L, M D, Ph D 328 No Country Club Rd, Tucson, Ariz (1, 1934)
- Lieberman, Seymour, Ph D Columbia Univ, College of Physicians and Surgeons, 630 W 168th St, New York City 32 *Asst Prof of Biochemistry assigned to Obstetrics and Gynecology* (2, 1948)
- Lifson, Nathan, M D, Ph D 6129 Wentworth Ave, S, Minneapolis, Minn *Prof of Physiology, Univ of Minnesota Med School* (1, 1944)
- Lightbody, Howard D, Ph D QM Food and Container Inst for the Armed Forces, 1849 W Pershing Rd, Chicago 9, Ill *Dir of Food Labs* (2, 1936)
- Ligon, Edgar Williams, Jr, Ph D Production and Marketing Admin, Insecticide Div, Agricultural Research Center, Beltsville, Md *Pharmacologist* (3, 1948)
- Lilienthal, Joseph L, Jr, M D Johns Hopkins Hospital, Baltimore 5, Md *Assoc Prof of Medicine* (1, 1945)
- Lillie, Ralph Stayner, Ph D Physiological Labs, Univ of Chicago, Chicago 37, Ill *Prof Emeritus of Physiology* (1R, 1905, 2, 1913)
- Lillie, R D, M D Pathology and Pharmacology Lab, E B M I, Natl Insts of Health, Bethesda, Md *Chief, Med Dir, USPHS* (4, 1941)
- Lilly, John C, M D 3400 Spruce St, Philadelphia 4, Pa *Asst Prof in Biophysics, E R Johnson Foundation for Med Physics, Univ of Pennsylvania, School of Medicine* (1, 1950)

- Lim, Robert Kho-Seng**, M B , Ph D , D Sc
Creighton Univ School of Medicine, Omaha 2,
Nebr *Prof of Physiology* (1, 1923)
- Lindeman, V F**, Ph D Syracuse Univ , 16 Lyman
Hall, Syracuse, N Y *Prof of Zoology*, (1, 1949)
- Lindsay, Stuart, M D** Univ of California Med
School, Univ Hospital, San Francisco 22
Assoc Prof and Pathologist (4, 1949)
- Lindsley, Donald B**, Ph D Northwestern Univ ,
Evanston, Ill *Prof of Psychology* (1, 1937)
- Linegar, Charles R**, Ph D E R Squibb and Sons,
Research and Development Labs , New Brun-
swick, N J *Dir , Pharmacological Development*
Div , (3, 1938)
- Lineweaver, Hans**, Ph D U S Dept of Agricul-
ture, Western Regional Research Lab , Albany
6, Calif *Head of Poultry Products Div* (2, 1941)
- Link, Karl Paul**, Ph D Univ of Wisconsin, Bio-
chemistry Bldg , Madison *Prof of Biochemistry*
(2, 1931)
- Lintz, William**, M D 36 Plaza St , Brooklyn, N Y
Late Prof of Immunology and Bacteriology and
Clin Prof of Medicine, Long Island College of
Medicine (6, 1920)
- Lipman, Mrs Miriam O**, A M Presbyterian Hos-
pital, Dept of Medicine, 620 W 168th St , New
York City *Research Asst , Edward Daniels*
Faulkner Arthritis Clinic (6, 1931)
- Lipmann, Fritz**, M D , Ph D Massachusetts Gen-
eral Hospital, Biochemical Research Lab , Bos-
ton 14 *Head, Prof of Biological Chemistry*, Har-
vard Med School (2, 1941)
- Lippincott, Stuart W**, M D Univ of Washington
School of Medicine, Seattle *Chairman, Dept of*
Pathology (4, 1947)
- Lippman, Richard W**, M D 4751 Fountain Ave ,
Los Angeles 27, Calif *Research Assoc , Inst for*
Med Research, Cedars of Lebanon Hospital (1,
1950)
- Lipton, Morris A**, Ph D , M D Univ of Chicago,
Dept of Medicine, 950 E 59th St , Chicago 37,
Ill *Fellow, Psychiatry* (2, 1946)
- Lisco, Hermann**, M D Argonne Natl Lab , Chi-
cago 80, Ill *Dir , Med Div , Asst Prof of Pathol-*
ogy, Univ of Chicago (4, 1947)
- Litchfield, John T , Jr**, M D American Cyanamid
Co , 1937 W Main St Stamford, Conn *Phar-*
macologist (3, 1940)
- Little, James Maxwell**, Ph D Bowman Gray
School of Medicine of Wake Forest College,
Winston-Salem, N C *Prof of Pharmacology*,
Assoc Prof of Physiology (1, 1942, 3, 1947)
- Little, Robert C**, M D Univ of Tennessee, Dept
of Physiology, Memphis *Asst Prof* (1, 1950)
- Livingston, Alfred E**, Ph D Temple Univ School
of Medicine, Philadelphia, Pa *Prof of Pharma-*
cology (1, 1917, 3, 1920)
- Livingston, Robert B**, M D 333 Cedar St , New
Haven, Conn *Instr , Lab of Physiology* (1,
1949)
- Lloyd, David P C**, Ph D Rockefeller Inst for
Med Research, 66th St and York Ave , New
York City 21 *Assoc Member* (1, 1939)
- Locke, Arthur P**, Ph D Zonite Products Corp ,
New Brunswick, N J *Chief Research Chemist*
(6, 1926)
- Lodholz, Edward**, M D Univ of Pennsylvania,
Med Labs , Philadelphia *Prof Emeritus of*
Physiology, Grad School of Medicine (1, 1913)
- Loeb, Leo**, M D Washington Univ Med School,
St Louis, Mo *Prof Emeritus of Pathology* (1R,
1907, 4, 1913)
- Loebel, Robert O**, M D 205 East 78th St , New
York City (1R, 1928)
- Loefer, John B**, Ph D Foundation of Applied Re-
search, P O Box 2296, San Antonio 6, Tex
Chief Biologist (1, 1949)
- Loew, Earl R**, Ph D Boston Univ School of Medi-
cine, 80 E Concord St , Boston 18, Mass *Prof*
of Physiology (1, 1940, 3, 1946)
- Loewe, W S**, M D Univ of Utah School of Medi-
cine, Dept of Pharmacology, Salt Lake City 1
(3, 1936)
- Logan, Milan A**, Ph D Univ of Cincinnati Col-
lege of Medicine, Eden and Bethesda, Cincinnati
19, Ohio *Prof of Biological Chemistry* (2, 1936)
- Long, C N H**, D Sc , M D Yale Univ , 333 Cedar
St , New Haven 11, Conn *Dean, School of Medi-*
cine and Sterling Prof of Physiological Chemistry
(1, 1935, 2, 1927)
- Long, Esmond R**, M D 7th and Lombard Sts ,
Philadelphia, Pa *Dir , Henry Phipps Inst , Prof*
of Pathology, Univ of Pennsylvania (4, 1930)
- Long, Perrin Hamilton**, M D Johns Hopkins
Univ , 615 N Wolfe St , Baltimore, Md *Prof of*
Preventive Medicine (3, 1940)
- Longcope, Warfield T**, M D Cornhill Farm, Lee,
Mass (3R, 1921, 4, 1913, 6R, 1923)
- Longenecker, Herbert Eugene**, Ph D Univ of
Pittsburgh, Pittsburgh 13, Pa *Dean, Grad*
School, Prof of Biochemistry (2, 1940, 5, 1945)
- Longwell, Bernard B**, Ph D Univ of Colorado
School of Medicine, 4200 East 9th Ave , Denver
7, Colo *Prof of Biochemistry* (2, 1946)
- Loofbourrow, G N**, Ph D Univ of Kansas, Dept
of Physiology, Lawrence *Asst Prof* (1, 1950)
- Looms, Ted A** Ph D , M D Univ of Washington
School of Medicine, Seattle *Assoc Prof of*
Pharmacology (3, 1948)
- Looney, Joseph M**, M D 75 Park St , West Rox-
bury, Mass *Chief of Labs , V A Regional*
Office, Boston (2, 1922)
- Loosli, Clayton Garr**, M D , Ph D Univ of Chi-
cago, Chicago, Ill *Prof of Preventive Medicine*
(4, 1940)
- Loosli, J K**, Ph D Cornell Univ , Animal Nutri-
tion Lab , Ithaca, N Y *Assoc Prof of Animal*

- Nutrition and Assoc Animal Nutritionist in Exper Station* (5, 1944)
- Lorber, Victor**, M D , Ph D Western Reserve Univ School of Medicine, Dept of Biochemistry, Cleveland, Ohio *Assoc Prof* (1, 1944)
- Lorente de N6, Rafael**, M D Rockefeller Inst for Med Research, 66th St and York Ave , New York City *Member* (1, 1937)
- Lorenz, Egon**, Ph D Natl Cancer Inst , Bethesda, Md *Biophysicist* (4, 1942)
- Loring, H S**, Ph D Stanford Univ , Dept of Chemistry Stanford Univ , Calif *Prof of Biochemistry* (2, 1938)
- Lotspeich, William D**, M D Oxford Univ , Dept of Biochemistry, Oxford, England *Research Fellow in Biochemistry* (1, 1948)
- Louis, Lawrence H**, D Sc Univ Hospital, Dept of Internal Medicine, Ann Arbor, Mich *Asst Prof of Biological Chemistry, Univ of Michigan* (2, 1949)
- Loveless, Mary H**, M D New York Hospital, 525 I 68th St , New York City *Research Assoc , Cornell Med School, Physician to Out-Patients, New York Hospital* (6, 1941)
- Lowell, Francis C**, M D 65 East Newton St , Boston, Mass *Assoc Prof of Medicine, Boston Univ School of Medicine* (6, 1942)
- Lowenbach, Hans**, M D Duke Univ Med School, Durham, N C *Assoc Prof of Neuropsychiatry and Physiology* (1, 1946)
- Lowry, Oliver H**, M D , Ph D Washington Univ School of Medicine, 4580 Scott Ave , St Louis 10, Mo *Prof and Head of Dept of Pharmacology* (2, 1942, 3, 1950)
- Lu, Go**, M D Univ of Maryland Med School, Dept of Pharmacology, Baltimore 1 *Fellow* (3, 1949)
- Lubinski, Herbert**, M D Jewish General Hospital, 3755 St Catherine Rd , Montreal, Quebec, Canada *Bacteriologist and Serologist* (6, 1941)
- Lucas, Colin C**, Ph D Univ of Toronto, Banting and Best Dept of Med Research, Toronto, Ontario, Canada *Professor* (2, 1946)
- Lucas, George H W**, Ph D Univ of Toronto, Toronto, Ontario, Canada *Prof of Pharmacology* (2, 1925, 3, 1928)
- Luck, James Murray**, Ph D Stanford Univ , Stanford Univ , Calif *Prof of Biochemistry* (2, 1925)
- Lucké, Balduin**, M D , D P H 141 Montgomery Ave , Bala-Cynwyd, Pa *Prof of Pathology, Univ of Pennsylvania Med School, Philadelphia* (4, 1924)
- Luckhardt, Arno Benedict**, Ph D , M D Univ of Chicago, Chicago, Ill *The Dr Wm Beaumont Distinguished Service Prof of Physiology* (1, 1911)
- Ludewig, Stephan**, Ph D Univ of Virginia School of Medicine, Charlottesville *Assoc Prof of Biochemistry* (2, 1941)
- Luduena, Froilan P**, Ph D , M D Sterling-Winthrop Research Inst , Rensselaer, N Y *Research Pharmacologist* (3, 1941)
- Luecke, Richard W**, Ph D Michigan State College, Dept of Agricultural Chemistry, East Lansing *Prof (Research)* (5, 1950)
- Luft, Ulrich C**, M D USAF School of Aviation Medicine, Randolph AFB , Tex *Research Physiologist* (1, 1950)
- Lukens, Francis D W**, M D Univ of Pennsylvania 809 Maloney Clinic, 36th and Spruce Sts , Philadelphia *Assoc Prof of Medicine, Dir of George S Cox Med Research Inst* (1, 1938)
- Lund, E J**, Ph D 1000 Barton Blvd , Austin, Tex *Prof of General Physiology, Univ of Texas* (1, 1930)
- Lundberg, Walter O**, Ph D Hormel Inst, Austin, Minn *Director, Prof of Agricultural Biochemistry, Univ of Minnesota* (2, 1949)
- Lundgren, Harold P**, Ph D U S Dept of Agriculture, Western Regional Research Lab , Albany 6 Calif *Sr Chemist* (2, 1942)
- Lundy, John Silas**, M D Mayo Foundation, Rochester, Minn *Prof of Anesthesiology, Chief of Section on Anesthesia, Mayo Clinic* (3, 1935)
- Lurie, Max B**, M D Henry Phipps Inst , 7th and Lombard Sts , Philadelphia, Pa *Assoc Prof of Exper Pathology* (4, 1934, 6, 1930)
- Lushbaugh, Clarence C**, M D , Ph D Los Alamos Scientific Lab , P O Box 1663, Los Alamos, N Mex *Staff Member, Pathologist, Los Alamos Hospital* (4, 1950)
- Lutz, Brenton R**, Ph D Boston Univ , 675 Commonwealth Ave , Boston, Mass *Prof of Biology* (1, 1925)
- Luyet, Basile J**, Sc D St Louis Univ School of Medicine, St Louis, Mo *Prof of Biology* (1, 1936)
- Lyall, Harold W**, Ph D New York State Dept of Health, Div of Labs and Research, Albany *Asst Dir in charge of Antitoxin, Serum, and Vaccine Labs* (6, 1937)
- Lyman, Carl M**, Ph D A and M College of Texas, Texas Agricultural Exper Station, College Station *Prof and Head of Dept of Biochemistry and Nutrition* (2, 1940)
- Lyman, John F**, Ph D Ohio State Univ , Townsend Hall, Columbus *Prof of Agricultural Chemistry* (2, 1920, 5R, 1933)
- Maaske, Clarence A**, Ph D Univ of Colorado School of Medicine, 4200 E 9th Ave , Denver *Prof of Physiology* (1, 1945)
- Macallum, A Bruce**, M D , Ph D Univ of Western Ontario Med School, London, Ontario, Canada *Research Prof of Biochemistry* (2, 1914)
- MacCardle, Ross C**, Ph D Natl Cancer Inst , Bethesda, Md *Principal Cytologist* (4, 1948)
- MacCorquodale, D W**, Ph D Abbott Labs

- North Chicago, Ill *Head of Biochemical Research* (2, 1934)
- MacFadyen, Douglas A**, M D Presbyterian Hospital, 1753 W Congress St, Chicago 12, Ill *Chairman of Dept of Biochemistry* (2, 1942)
- Macht, David Israel**, M D Sinai Hospital, Baltimore, Md *Consultant Pharmacologist, Research Pharmacologist, Sinai Hospital Labs* (1R, 1916, 3, 1915)
- Macht, Martin B**, Ph D The Jewish Hospital, Cincinnati 29, Ohio (1, 1948)
- MacIntosh, F C**, Ph D McGill Univ, Dept of Physiology, Montreal, Quebec, Canada *Professor and Chairman of Dept* (1, 1950, 3, 1950)
- MacKay, Eaton M**, M D Scripps Metabolic Clinic, La Jolla, Calif (1, 1930)
- MacKay, Ian F S**, Ph D Univ College of the West Indies, Jamaica, British West Indies *Prof of Physiology* (1, 1949)
- Mackenzie, Cosmo G**, Sc.D Univ of Colorado School of Medicine, Dept of Biochemistry, 4200 E 9th Ave, Denver 7 *Professor* (1, 1946, 2, 1946, 5, 1942)
- Mackenzie, George M**, M D Mary Imogene Bassett Hospital, Cooperstown, N Y *Physician-in-Chief, Dir, Otsego County Lab* (6, 1912)
- MacLeod, Colin M**, M D New York Univ College of Medicine, 477 First Ave, New York City *Prof of Bacteriology* (6, 1937)
- MacLeod, Florence L**, Ph D Univ of Tennessee, Knoxville *Prof of Nutrition* (2, 1927, 5, 1933)
- MacLeod, Grace**, Ph D 106 Morningside Drive, New York City 27 *Prof Emeritus of Nutrition, Teachers College, Columbia Univ* (2, 1924, 5R, 1933)
- MacLeod, John**, Ph D Cornell Univ Med College, 1300 York Ave, New York City *Asst Prof of Anatomy* (1, 1942)
- MacNabb, Andrew L**, V S, B V Sc Guelph, Ontario, Canada *Principal, Ontario Veterinary College* (6, 1941)
- MacNider, William deB**, M D, Sc D Univ of North Carolina, Chapel Hill *Kenan Research Prof of Pharmacology* (1, 1912, 2, 1912, 3, 1909, 4, prior to 1920)
- MacPherson, Catherine F C**, Ph D 236 Brock Ave N, Montreal West, Quebec, Canada (2, 1947)
- MacPhillamy, Betty B**, Ph D 145 Greene Ave, Madison, N J *Bacteriologist* (6R, 1944)
- MacVicar, Robert**, Ph D Oklahoma A & M College, Stillwater *Prof and Head of Dept of Agricultural Chemistry Research* (2, 1949)
- Madden, Sidney C**, M D Brookhaven Natl Lab, Upton, L I, N Y *Head, Div of Pathology, Pathologist-in-Chief, Brookhaven Natl Lab Hospital* (4, 1939)
- Maddock, Stephen**, M D Boston City Hospital, Boston, Mass *Dir of Surgical Research Lab, Asst Prof of Surgery, Tufts Med School* (4, 1931)
- Madsen, Louis L**, Ph D Utah State Agricultural College, Dept of Animal Husbandry, Logan *Nutritionist* (5, 1940)
- Magath, Thomas B**, Ph D, M D Mayo Clinic, Rochester, Minn *Head of Div of Clin Labs, Prof of Clin Pathology and Parasitology, Univ of Minnesota, Mayo Foundation* (1, 1928)
- Magill, Thomas P**, M D Long Island College of Medicine, Brooklyn, N Y *Prof of Bacteriology* (6, 1937)
- Magladery, J W**, M D, Ph D, M R C P Johns Hopkins Hospital, Baltimore, Md *Asst Prof of Neurology, Johns Hopkins University* (1, 1950)
- Magnuson, Harold J**, M D, M P H Univ of North Carolina, Chapel Hill *Research Prof of Exper Medicine, Sr Surgeon, USPHS* (4, 1950)
- Magoun, Horace W**, Ph D Univ of California Med School, Los Angeles 24 (1, 1937)
- Mahon, Eleanor Conway**, Ph D Iron River, Mich (4, 1940)
- Main, Roland J**, Ph D Eaton Labs, Inc, Eaton Ave, Norwich, N Y *Med Editor and Consultant in Physiology* (1 1936)
- Maison, George L**, M S, M D Boston Univ Med School, 80 E Concord St, Boston 18, Mass *Prof and Head of Pharmacology Dept* (1, 1939, 3, 1948)
- Major, Randolph T**, Ph D Merck and Co, Inc, Rahway, N J *Vice Pres and Scientific Dir* (2, 1942)
- Malkiel, Saul**, Northwestern Univ Med School, Evanston, Ill *Asst Prof of Medicine, Dir of Allergy Research Lab* (6, 1948)
- Mallory, G Kenneth**, M D Mallory Inst of Pathology, Boston City Hospital, Boston, Mass *Professor* (4, 1940)
- Mallory, Tracy B**, M D Massachusetts General Hospital, Boston *Dir of Pathology and Bacteriology, Prof of Pathology, Harvard Med School* (4, 1937)
- Maloney, Arnold H**, Ph D, M D Howard Univ School of Medicine, Washington, D C *Prof and Head of Dept of Pharmacology* (3, 1932)
- Maltaner, Frank**, Ph D Scarsdale Manor, Scarsdale, N Y *Biochemist, Div of Lab and Research, New York State Dept of Health* (6, 1920)
- Maluf, N S Rustum**, Ph D Columbia Univ, Presbyterian Hospital, Squier Urological Clinic, New York City 32 *Resident in Urology* (1, 1942)
- Man, Evelyn B**, Ph D Yale Univ School of Medicine, 333 Cedar St, New Haven, Conn *1st Prof in Biochemistry Lab, Dept of Medicine* (2, 1936)
- Manerv, Jeanne Forest**, Ph D Univ of Toronto

- Med School Toronto, Ontario, Canada *Demonstration in Biochemistry* (1, 1937)
- Mangun George H, Ph D Henry Ford Hospital, 15713 Heyden St, Detroit 23, Mich *Sr Assoc in Clin Chemistry* (2, 1947)
- Mann Frank C, M A, M D Mayo Clinic, Box 200 Rochester, Minn *Prof of Exper Medicine, Mayo Foundation* (1, 1916, 4R, 1924)
- Mann Frank D, Ph D M D 102-110 2nd Ave, S W Rochester Minn *Consultant in Clinical Pathology Mayo Clinic, Asst Prof of Clinical Pathology Mayo Foundation* (1, 1950, 4, 1950)
- Manning G W M D Ph D Victoria Hospital, London Ontario Canada *Asst Prof of Clin Investigation Dept of Medicine, Lecturer, Dept of Physiology Univ of Western Ontario* (1, 1944)
- Manville, Ira Albert, M D, Ph D 811 N W 19th Ave Portland 9 Ore (1, 1933)
- Manwaring, Wilfred H, M D Stanford Univ, Stanford University, Calif *Prof Emeritus of Bacteriology and Exper Pathology* (4, prior to 1920 6 1917)
- Marbarger John P, Ph D Univ of Illinois Physical Environment Unit, 1853 W Polk St, Chicago 12 *Research Div Aeromed and Physical Environment Lab Assoc Prof of Physiology* (1, 1949)
- Marcus Stanley, Ph D Univ of Utah, Dept of Bacteriology Salt Lake City *Assoc Prof* (6, 1948)
- Marcus Thomas H, A B Johns Hopkins Med School 710 N Washington St, Baltimore, Md *Lecturer in Pharmacology* (3, 1950)
- Marine David M A M D 18 Baltimore Ave, Lenoxville Del 1910, 4, 1913)
- Mark Lester C M D 535 E 14th St, New York City *Assoc Prof Fellow in Anesthesiology* (3, 1949)
- Markes Joseph F Ph D Duke Univ School of Medicine Durham N C *Prof of Anatomy* (1, 1949)
- Markowitz J M D Ph D Univ of Toronto School of Medicine, Toronto, Ontario, Canada *Research Assoc in Physiology* (1, 1929)
- Marmont, George H Ph D Univ of Chicago, Inst of Radiobiology and Biophysics, Chicago 37 Ill 1st *Prof of Physiology* (1, 1941)
- Marmorston, Jessie Inst for Med Research, 4751 Fountain Ave Los Angeles, Calif *Asst Prof of Medicine Univ of Southern California Staff County and Cedars of Lebanon Hospitals* (6, 1932)
- Marrazzi, Amedeo S, M D Med Div, Army Chemical Center, Md *Chief, Toxicology Section* (1, 1949, 3, 1938)
- Marsh, David F, Ph D West Virginia Univ, School of Medicine, Dept of Pharmacology, Morgantown *Head and Prof of Pharmacology* (3, 1946)
- Marsh, Gordon, Ph D State Univ of Iowa, Iowa City *Assoc Prof of Zoology* (1, 1944)
- Marsh, M Elizabeth, Ph D P O Box 17, Soquel, Calif (1, 1929, 5, 1933)
- Marshak, Alfred George, Ph D New York Univ College of Medicine, New York City *Research Assoc* (1, 1940)
- Marshall, Eli Kennerly, Jr, Ph D, M D Johns Hopkins Med School, Baltimore, Md *Prof of Pharmacology and Exper Therapeutics* (1, 1915, 2, 1913, 3, 1915)
- Marshall, Louise Hanson, Ph D Natl Insts of Health, Inst of Exper Biology and Medicine, Bethesda, Md *Physiologist* (1, 1946)
- Marshall, Wade H, Ph D Natl Insts of Health, Bethesda, Md *Research Fellow, Lab of Physical Biology* (1, 1937)
- Martin, Arthur W, Jr, Ph D Univ of Washington, Johnson Hall, Seattle *Executive Officer, Dept of Zoology* (1, 1944)
- Martin, Donald S, M D Univ of Puerto Rico, School of Medicine, San Juan, Puerto Rico (6, 1943)
- Martin, Foster N, Jr, Ph D, M D Tulane Univ Med School, P O Station 20, New Orleans, La *Asst Prof of Pharmacology* (3, 1947)
- Martin, Stephens J, Ph D St Francis Hospital, Hartford, Conn (1, 1933)
- Mars, Walter, Ph D Univ of Southern California School of Medicine, Los Angeles 7, Calif *Assoc Prof of Biochemistry* (2, 1949)
- Mason, Edward C, M D, Ph D Univ of Oklahoma School of Medicine, Oklahoma City *Prof of Physiology* (1, 1935)
- Mason, Eleanor Dewey, Ph D Women's Christian College, Dept of Physiology and Nutrition, Cathedral P O, Madras, India *Prof of Physiology and Nutrition* (1, 1946)
- Mason, Herman C, 131 N Harvey Ave, Oak Park, Ill *Consulting Bacteriologist and Immunologist* (6, 1948)
- Mason, Harold L, Ph D Mayo Clinic, Rochester, Minn *Prof of Physiological Chemistry, Mayo Foundation, Univ of Minnesota* (2, 1941)
- Mason, Howard S, Ph D Natl Insts of Health, Bethesda 14, Md *Cancer Inst Fellow* (2, 1949)
- Mason, Karl Ernest, Ph D Univ of Rochester School of Medicine and Dentistry, Rochester, N Y *Prof of Anatomy* (5, 1941)
- Mason, Morton F, Ph D Parkland Hospital, Oak Lawn at Maple, Dallas 4, Tex *Prof of Pathological Chemistry, Dept of Medicine, Southwestern Med College of the Univ of Texas* (2, 1938)
- Mason, Richard P, M D Army Med Dept, Research and Grad School, Virus and Rickettsial Disease Dept, Washington, D C *Chief, Rickettsial Disease Research Section* (6, 1950)
- Massengale, Oliver N, Ph D Mead Johnson and

- Co, Research Lab, Evansville, Ind *Research Biochemist* (2, 1937)
- Masson, Georges M C**, Ph D Cleveland Clinic Foundation, Research Div, Cleveland, Ohio (1, 1944)
- Mathews, Albert P**, Ph D Marine Biological Lab, Woods Hole, Mass *Carnegie Prof Emeritus of Biochemistry, Univ of Cincinnati* (1R, 1898, 2, 1906)
- Matson, Gustave A**, Ph D Minneapolis Memorial Blood Bank, Inc, 1914 La Salle Ave, Minneapolis, Minn *Director* (6, 1946)
- Matthews, Samuel A**, Ph D Williams College, Williamstown, Mass *Prof of Biology* (1, 1948)
- Mattill, Henry A**, Ph D State Univ of Iowa, Iowa City *Prof and Head of Dept of Biochemistry* (1, 1913, 2, 1909, 5, 1933)
- Mattis, Paul A**, D Sc Western Reserve Univ School of Medicine, Cleveland, Ohio *Asst Prof of Pharmacology* (3, 1946)
- Maurer, Frank W**, Ph D 301 Lake Ave, Newton Highlands 61, Mass (1, 1941)
- Maurer, Fred D**, D V M, Ph D Army Med Dept, Research and Grad School, Army Med Center, Veterinary Div, Washington 12, D C *Chief, Dept Veterinary Virology* (6, 1950)
- Mautz, Frederick R**, M D 10515 Carnegie Ave, Cleveland 6, Ohio (1, 1945)
- Maver, Mary E**, Ph D Natl Cancer Inst, Bethesda 14, Md *Sr Biochemist* (2, 1947)
- Mavor, James Watt**, Ph D 8 Gracewood Park, Cambridge, Mass (1R, 1930)
- Maxfield, Mary E**, Ph D Woman's Med College of Pennsylvania, Dept of Pharmacology, Philadelphia *Assoc Prof* (1, 1947)
- Mayer, Dennis T**, Ph D Univ of Missouri, Dept of Agricultural Chemistry, 211 Schweitzer Hall, Columbia *Assoc Prof* (2, 1950)
- Mayer Manfred M**, Ph D 1331 Howard Rd, Glen Burnie, Md *Assoc Prof of Bacteriology, Johns Hopkins Hospital, School of Hygiene* (6, 1946)
- Maverson, Hymen S**, Ph D Tulane Univ School of Medicine, Station 20, New Orleans, La *Prof and Head of Dept of Physiology* (1, 1928)
- Mavnard, Elliott Allen**, Ph D Univ of Rochester School of Medicine and Dentistry, Rochester 20, N Y *Asst Prof of Pharmacology and Toxicology* (3 1950)
- Maynard, L A**, Ph D Cornell Univ, Ithaca, N Y *Prof of Nutrition and Biochemistry, Dir of School of Nutrition* (2, 1930, 5, 1933)
- Mazur, Abraham**, Ph D City College of New York, 139th St and Convent Ave, New York City 31 *1st Prof in Dept of Chemistry* (2, 1944)
- McCann, William S**, M D, D Sc Univ of Rochester School of Medicine, Rochester N Y *The Charles A Dewey Prof of Medicine* (2, 1923, 5, 1933)
- McCarrell, Jane D** Hood College, Dept of Biology, Frederick, Md (1, 1942)
- McCarty, Maclyn, M D** 66th St and York Ave, New York City 21 *Assoc, Rockefeller Inst for Med Research* (6, 1947)
- McCawley, Elton Leeman**, Ph D Univ of Oregon, Dept of Pharmacology, Portland *Assoc Prof* (3, 1944)
- McCay, Clive M**, Ph D Cornell Univ, Animal Nutrition Lab, Dairy Bldg, Ithaca, N Y *Prof of Nutrition* (2, 1929, 5, 1933)
- McChesney, Evan William**, Ph D Sterling-Winthrop Research Inst, Rensselaer, N Y *Member, Biological Div* (1, 1944)
- McClellan, Walter S**, M D Saratoga Spa, Saratoga Springs, N Y *Med Dir, Assoc Prof of Medicine, Albany Med College* (1, 1931)
- McClendon, J F**, Ph D Route 1, Box 383, Trooper Rd, Norristown, Pa *Research Prof of Physiology, Hahnemann Med College* (1R, 1910, 2, 1914, 5R, 1935)
- McClung, L S** Indiana Univ, Bloomington *Assoc Prof and Chairman of Dept of Bacteriology* (6, 1948)
- McCollum, Elmer Verner**, Ph D Johns Hopkins Univ, Baltimore 18, Md *Prof Emeritus of Biochemistry* (2, 1910, 5, 1933)
- McCollum, Ernestine Becker**, M A Johns Hopkins Univ School of Hygiene, Baltimore 5, Md *Asst Prof of Biochemistry* (5, 1938)
- McCouch, Grayson Prevost**, M D Univ of Pennsylvania School of Medicine, Philadelphia *Assoc Prof of Physiology* (1, 1925)
- McCouch, Margaret Sumwalt**, Ph D Rose Tree Rd, R F D 1, Media, Pa (1, 1934)
- McCoy, Richard H**, Ph D Univ of Pittsburgh, Pittsburgh, Pa *Assoc Research Prof of Chemistry* (2, 1950, 5, 1948)
- McCrea, Forrest D**, Ph D Duke Univ School of Medicine, Durham, N C *Assoc Prof of Physiology and Pharmacology* (1, 1929, 3, 1937)
- McCrudden, Francis H**, M D 19 Stoneleigh Rd, West Newton 65, Mass, *Asst Med Dir, New England Mutual Life Insurance Co* (2, 1906)
- McCulloch, Warren Sturgis**, M A, M D Univ of Illinois College of Medicine, 912 S Wood St, Chicago *Assoc Prof of Psychiatry* (1, 1936)
- McCutcheon, Morton**, M D Univ of Pennsylvania Med School Philadelphia *Prof of Pathology* (4, 1925)
- McDonald, Francis Guy**, Ph D Mead Johnson and Co Research Lab, 2404 W Pennsylvania St, Evansville Ind *Biochemist* (2, 1936, 5, 1947)
- McDonald, Roger K**, M D Baltimore City Hospitals, Cardiovascular Diseases Section, Baltimore 24, Md *Sr 1st Surgeon, USPHS, Natl Heart Inst, Bethesda, Md* (1, 1949)

- McEllroy, William Swindler, M D Univ of Pittsburgh School of Medicine, Pittsburgh, Pa *Dean, Prof of Physiological Chemistry* (2, 1919)
- McElroy, L W Ph D Univ of Alberta, Dept of Animal Science, Edmonton, Canada *Assoc Prof of Animal Husbandry* (5, 1944)
- McElroy, William D, Ph D Johns Hopkins Univ, Baltimore Md *Asst Prof of Biology* (1, 1945)
- McFarland, Ross A, Ph D Harvard Univ School of Public Health, Boston, Mass *Assoc Prof of Industrial Hygiene* (1, 1943)
- McFarlane, William Douglas, Ph D 496 Queen St, E, Toronto, Ontario, Canada *Dir of Research, Canadian Breweries, Ltd and Victory Mills, Ltd* (2, 1933)
- McGinty, Daniel A, Ph D Parke, Davis and Co, Detroit, Mich *Research Physiologist* (1, 1925)
- McGuigan, Hugh Alister, Ph D, M D Univ of Illinois College of Medicine, 1853 W Polk St, Chicago *Prof Emeritus of Pharmacology* (1R, 1907, 2, 1906, 3R, 1913)
- McHargue, J S, Ph D Univ of Kentucky, Dept of Chemistry, Kentucky Agricultural Experiment Station, Lexington *Member Emeritus* (2, 1927)
- McHenry, E W, Ph D Univ of Toronto, School of Hygiene, Toronto, Ontario, Canada *Prof of Public Health Nutrition* (2, 1938, 5, 1935)
- McIntyre A R, Ph D, M D Univ of Nebraska College of Medicine, 42nd and Dewey Ave, Omaha *Prof of Physiology and Pharmacology* (1, 1933 3, 1938)
- McKee, Albert P R F D 7, Iowa City, Iowa *Assoc Prof of Bacteriology, State Univ of Iowa* (6, 1948)
- McKee, Clara M, Squibb Inst for Med Research, New Brunswick, N J *Assoc in Microbiology* (6, 1941)
- McKee, Frank W, M D Univ of Rochester School of Medicine and Dentistry, Rochester, N Y *Instr in Pathology, James E Gleason Fellow* (4, 1947)
- McKee Ralph Wendell, Ph D Harvard Med School 25 Shattuck St, Boston 15, Mass *Asst Prof of Biological Chemistry* (2, 1946)
- McKennis, Herbert, Jr, Ph D Naval C E Research Lab, Port Hueneme, Calif *Assoc Prof of Biochemistry* (2, 1948)
- McKibbin, John M, Ph D State Univ of New York Med Center at Syracuse Univ, Syracuse, N Y *Assoc Prof of Biochemistry* (2, 1948)
- McLain, Paul L, M D Univ of Pittsburgh Med School, Pittsburgh, Pa *Prof of Physiology and Pharmacology* (1, 1948, 3, 1940)
- McLean, Franklin C, Ph D, M D Univ of Chicago, Chicago 37, Ill *Prof of Pathological Physiology* (1, 1914, 2, 1916, 3, 1916)
- McLean, I William, Jr, M D Parke, Davis Lab, Virus Research Div, Detroit, Mich *Research Virologist* (6, 1946)
- McLester, James S, M D Univ of Alabama, 930 S 20th St, Birmingham *Prof of Medicine* (5, 1933)
- McLimans, William F, Ph D Univ of Minnesota, 1834 Chelton Ave, St Paul *Asst Prof of Bacteriology and Immunology* (6, 1949)
- McManus, J F A, M D Univ of Virginia School of Medicine, Charlottesville *Assoc Prof of Pathology* (4, 1948)
- McMaster, Philip D, M D Rockefeller Inst for Med Research, 66th St and York Ave, New York City (4, 1924)
- McMeekin, Thomas L, Ph D U S Dept of Agriculture, Eastern Regional Research Lab, Philadelphia, Pa *Head of Protein Div* (2, 1935)
- McNamara, Bernard P, Ph D Med Div, Army Chemical Center, Md *Asst Chief, Pharmacology Section* (3, 1947)
- McNaught, James Bernard, M D Univ of Colorado School of Medicine, Denver 7 *Prof and Head of Dept of Pathology* (4, 1936)
- McNeil, Crichton, M D Holy Cross Hospital, Salt Lake City, Utah *Pathologist* (6, 1950)
- McPhail, Murchie K, Ph D Natl Defense Board, Suffield Experiment Station, Ralston, Alberta, Canada *Research Scientist* (3, 1941)
- McQuarrie, Irvine, Ph D, M D Univ of Minnesota, Minneapolis *Prof and Head of Dept of Pediatrics* (1, 1949, 4, 1927, 5, 1933)
- McShan, W H, Ph D Univ of Wisconsin, Biology Bldg, Madison 6 *Assoc Prof of Zoology* (2, 1947)
- Medes, Grace, Ph D Lankenau Hospital Research Inst, Philadelphia, Pa *Research Physiological Chemist* (2, 1930)
- Medlar, Edgar M, M D Veterans Admin Hospital, Sunmount, N Y *Chief Lab Pathologist* (4, 1927)
- Meek, Walter J, Ph D Univ of Wisconsin, Madison *Prof of Physiology, Assoc Dean of Med School* (1, 1908)
- Mehl, John W, Ph D Univ of Southern California, 3551 Univ Ave, Los Angeles *Prof of Biochemistry* (2, 1946)
- Meier, Rolf, M D Basle Univ, Pelikanweg 7, Basle, Switzerland *Dir, Biological Research Labs, Ciba A G* (3, 1949)
- Meiklejohn, Gordon, M D 678 Woodmont Ave, Berkeley, Calif *Instr in Medicine, Univ of California Med School* (6, 1948)
- Meister, Alton, M D Natl Cancer Inst, Bethesda 14, Md *Head, Unit on Biochemical Research* (2, 1950)
- Mertes, Joseph Michigan State College, Dept of Physiology and Pharmacology, East Lansing *Asst Prof* (1, 1949)
- Mellon, Ralph R, M S, M D, D P H Western Pennsylvania Hospital, Inst of Pathology, Pittsburgh *Director* (6, 1918)

- Melnick, Daniel**, Ph D The Best Foods, Inc , Research Labs , Bayonne, N J *Chief Technologist* (2, 1940, 5, 1942)
- Melnick, Joseph L**, Ph D Yale Univ School of Medicine, 333 Cedar St , New Haven 11, Conn *Assoc Prof of Microbiology* (2, 1946, 6, 1948)
- Melville, Donald B**, Ph D Cornell Univ Med College, 1300 York Ave , New York City 21 *Assoc Prof of Biochemistry* (2, 1947)
- Melville, Kenneth Ivan**, M D McGill Univ , Montreal, Quebec, Canada *Assoc Prof of Pharmacology* (3, 1931)
- Mendel, Bruno**, M D Univ of Toronto, Banting and Best Dept of Med Research, 100 College St , Toronto 5, Ontario, Canada *Prof of Cellular Physiology* (2, 1947)
- Mendelson, E S**, B A R F D 2, Telford, Pa *Physiologist, Head of Human Engineering Div , Naval Air Exper Station, Philadelphia, Pa* (1, 1949)
- Mendenhall, Walter L**, M S , M D 9 Acacia St , Cambridge, Mass *Retired Prof of Pharmacology, Boston Univ Med School* (1, 1915, 3R, 1917)
- Mendez, Rafael**, M D Natl Inst of Cardiology , Calzada de la Piedad 300, Mexico D F , Mexico *Head of Dept of Pharmacology* (3, 1944)
- Mendlowitz, Milton**, M D 136 E 64th St , New York City 21 *Adjunct Physician in Mt Sinai Hospital of New York* (1, 1949)
- Meneely, George R**, M D Thayer Veterans Admin Hospital, Nashville, Tenn *Asst Prof of Medicine* (4, 1946)
- Meng, H C**, M D , Ph D Vanderbilt Univ Med School, Dept of Physiology, Nashville 4 *Instructor* (1, 1950)
- Menkin, Vally, M A**, M D Temple Univ School of Medicine, Philadelphia, Pa *Assoc Prof of Exper Pathology* (1, 1932, 4, 1932, 6, 1931)
- Menten, Maud L**, M D Ph D 1608 Sixth Ave , New Westminster, B C , Canada *Retired Prof of Pathology, Univ of Pittsburgh* (1, 1915, 4, 1927)
- Mertz, Edwin T**, Ph D Purdue Univ , Dept of Agricultural Chemistry, W Lafayette, Ind *Assoc Prof* (2, 1950)
- Mettier, Stacy R**, M D Univ of California Hospital, San Francisco *Prof of Medicine* (4, 1932)
- Mettler, Fred A**, Ph D M D Columbia Univ College of Physicians and Surgeons, Dept of Neurology, New York City *Assoc Prof of Anatomy* (1, 1937)
- Meyer, Arthur E**, Ph D Fellows Pharmaceutical Lab , New York City (1, 1948)
- Meyer, Curtis E**, Ph D Upjohn Co , Kalamazoo, Mich *Sr Research Chemist* (2, 1942)
- Meyer, Frieda L**, Ph D U S Dept of Agriculture, Bureau of Human Nutrition, Food and Nutrition Div , Washington 25, D C *Nutrition Specialist* (5, 1949)
- Meyer, Karl**, M D , Ph D Columbia Univ College of Physicians and Surgeons, 630 W 168th St , New York City 32 *Assoc Prof of Biochemistry, Dept of Medicine* (2, 1934)
- Meyer, Karl F**, M D , Ph D George W Hooper Foundation, Univ of California Med Center, San Francisco *Director* (4, 1930, 6, 1922)
- Meyer, Roland K**, Ph D Univ of Wisconsin, Biology Bldg , Madison 6 *Prof of Zoology* (1, 1949)
- Meyerhof, Otto**, M D Univ of Pennsylvania School of Medicine, Dept of Physiological Chemistry, Philadelphia *Research Prof of Biochemistry* (2, 1941)
- Mezey, Kaàlman**, M D Calle 84, No 9-68, Bogota, Colombia, South America *Prof of Pharmacology, Javeriana Univ Med School* (3, 1950)
- Michel, Harry O**, Ph D Med Div , Biochemical Section, Army Chemical Center, Md *Biochemist* (2, 1949)
- Mickelsen, Olaf**, Ph D Nutrition Branch USPHS, Washington 25, D C *Chief Chemist* (2, 1944)
- Mider, George Burroughs**, M D Strong Memorial Hospital, Rochester 7, N Y *Prof of Cancer Research* (4, 1940)
- Miles, Walter R**, Ph D Yale Univ , 333 Cedar St , New Haven, Conn *Prof of Psychology, School of Medicine and Inst of Human Relations* (1, 1919)
- Milhorat, Ade T**, M D Cornell Univ Med College, New York City 21 *Asst Prof of Medicine and Instr in Pharmacology, Research Fellow, Russell Sage Inst of Pathology* (1, 1934, 3, 1937, 5, 1935)
- Miller, Augustus Taylor, Jr**, Ph D Univ of North Carolina Med School, Chapel Hill *Assoc Prof of Physiology* (1, 1944)
- Miller, Benjamin F**, M D 71 Mt Vernon St , Boston 8, Mass *Assoc Physician, Massachusetts General Hospital, Clin Prof of Medicine (on leave), George Washington Med School* (2, 1938)
- Miller, Carey D**, M S Univ of Hawaii, Honolulu *Prof of Food and Nutrition, Hawaiian Agricultural Exper Station* (5, 1942)
- Miller, C Phillip**, M S , M D Univ of Chicago, Chicago, Ill *Prof of Medicine, Member, Inst of Radiobiology and Biophysics* (4, 1925, 6, 1928)
- Miller, Edgar G , Jr**, Ph D Columbia Univ , 630 W 168th St , New York City *Prof of Biological Chemistry* (2, 1930)
- Miller, Franklin R**, M D Jefferson Med College and Hospital, Div of Hematology, Philadelphia, Pa *Assoc Prof of Medicine* (4, 1940)
- Miller, Frederick R**, M A , M D Univ of Western Ontario, Faculty of Medicine, London, Ontario, Canada *Research Prof of Neurophysiology* (1, 1905)
- Miller, Gail L**, Ph D Inst for Cancer Research, Dept of General Biochemistry, Hasbrook and

- Hartel Aves, Philadelphia 11, Pa Assoc Member (2, 1949)
- Miller, George H, M D American College of Surgeons, 40 E Erie St, Chicago 11, Ill Dir of Educational Activities (3, 1925)
- Miller, George A, Ph D Harvard Univ, Psycho-Acoustic Lab, Cambridge, Mass Research Fellow (1, 1948)
- Miller, H R, M D Montefiore Hospital, 1020 Park Ave, New York City (1, 1947)
- Miller, James A, Ph D Univ of Wisconsin Med School, McArdle Mem Lab for Cancer Research, Madison 6 Assoc Prof of Oncology (2, 1949)
- Miller, Leon L, Ph D, M D Univ of Rochester School of Medicine and Dentistry, P O Box 287, Crittenden Station, Rochester, N Y Assoc Prof of Radiation Biology and Biochemistry (2, 1947)
- Miller, Lila, Ph D Univ of Michigan, Ann Arbor Asst Prof of Biological Chemistry (2, 1946)
- Miller, Lloyd C, Ph D U S Pharmacopeia, 46 Park Ave, New York City 16 Dir of Pharmacopeial Revision (3, 1938)
- Miller, R C, Ph D Pennsylvania State College, State College Prof of Agricultural and Biological Chemistry (5, 1935)
- Miller, Zelma Baker, Ph D 71 Mt Vernon, Boston, Mass (2, 1940)
- Millman, Nathan, M Sc, Ph D 50 Brookside Ave, Apt 6A, Somerville, N J Research Assoc, Div of Physiology and Pharmacology, Ortho Research Foundation, Raritan, N J (1, 1950)
- Mills, Clarence A, Ph D, M D Cincinnati General Hospital, Cincinnati 29, Ohio Prof of Experimental Medicine, Univ of Cincinnati (1, 1921, 2, 1921)
- Minard, David, Ph D, M D Natl Naval Med Center, Naval Med Research Inst, Bethesda, Md Head, Physiology Div (1, 1950)
- Minot, Annie Stone, Ph D Vanderbilt Univ Med School, Nashville, Tenn Assoc Prof of Biochemistry (1, 1923)
- Mirick, George S, M D Johns Hopkins Univ, School of Medicine, Baltimore, Md Assoc Prof of Medicine (6, 1950)
- Mirsky, Alfred E, Ph D Rockefeller Inst for Med Research, 66th St and York Ave, New York City 21 Member (2, 1941)
- Mirsky, I Arthur, M D The Jewish Hospital, Cincinnati, Ohio Dir, May Inst for Med Research, Asst Prof of Biochemistry, Univ of Cincinnati (1, 1936)
- Mitchell, Harold H, M D 120 Lasky Drive, Beverly Hills, Calif (6, 1943)
- Mitchell, Harold H, Ph D Univ of Illinois, 555 Davenport Hall, Urbana Prof of Animal Nutrition (2, 1919, 5, 1933)
- Mitchell, Helen S, Ph D Univ of Massachusetts, Amherst Dean of School of Home Economics (2, 1925, 5, 1933)
- Mitchell, Philip H, Ph D Brown Univ, Providence 12, R I Robert P Brown Prof of Biology (2, 1909)
- Modell, Walter, M D Cornell Univ Med College, 1300 York Ave, New York City, Asst Prof in Clin Pharmacology (3, 1944)
- Moe, Gordon Kenneth, Ph D, M D New York State Univ School of Medicine, Dept of Physiology, Syracuse 10 Prof and Head of Dept (3, 1944)
- Mohn, James F, M D 24 High St, Buffalo, N Y Asst Prof in Bacteriology and Immunology, Univ of Buffalo School of Medicine (6, 1946)
- Molitor, Hans, M D Merck Inst for Therapeutic Research, Rahway, N J Director (1, 1933, 3, 1942)
- Molland, Jacob, M D, Ph D Univ of Oslo, Oslo, Norway Prof of Pharmacology (3, 1948)
- Molnar, George W, Ph D Med Dept, Field Research Lab, Fort Knox, Ky Physiologist (1, 1949)
- Molomut, Norman, Ph D Biological Labs, 16 Clinton St, Brooklyn 2, N Y Dir of Research and of Labs (6, 1942)
- Montgomery, Hugh, M D Univ of Pennsylvania Hospital, 36th and Spruce Sts, Philadelphia Asst Prof of Clin Medicine (1, 1950)
- Moon, Henry D, M A, M D Univ of California Med School, Div of Pathology, San Francisco 22 Asst Clin Prof of Pathology, Pathologist, V A Hospital, City Pathologist (4, 1950)
- Moon, Virgil H, M Sc, M D Univ of Miami, Med Research Unit, Coral Gables 34, Fla Prof of Pathology (4, 1934)
- Moore, Arthur R, Ph D Univ of Portland, Dept of Biology, Portland, Ore (1, 1912)
- Moore, Carl Vernon, M D Washington Univ School of Medicine, St Louis, Mo Prof of Medicine (4, 1938, 5, 1941)
- Moore, Dan H, Ph D U S Navy No 100, c/o Fleet Post Office, New York City (1, 1948)
- Moore, Lane A, Ph D U S Dept of Agriculture, Div of Nutrition and Physiology, Bureau of Dairy Industry, Beltsville, Md Head, Section of Dairy Cattle Nutrition (5, 1940)
- Moore, Robert A, M D Washington Univ Med School, St Louis, Mo Dean and Prof of Pathology (4, 1929)
- Moore, Robert M, M D Univ of Texas Med School, Galveston (1, 1932)
- Moore, Stanford, Ph D Rockefeller Inst for Med Research, York Ave at 66th St, New York City 21 Assoc Member (2, 1949)
- Moorhouse, Victor Henry K, M B Univ of Manitoba, Winnipeg, Manitoba, Canada Prof of Physiology (1, 1912)
- Morales, Manuel F, Ph D Naval Med Research

- Inst , Bethesda 14, Md *Staff Member, General Physiology* (1, 1950)
- More, Robert H** , M Sc , M D Pathological Inst , 3775 University St , Montreal, Quebec, Canada *Prof of Pathology, McGill Univ* (4, 1949)
- Morehouse, Laurence E** , Ph D Univ of Southern California, Los Angeles 7 *Assoc Prof* (1, 1947)
- Morgan, Agnes Fay** , Ph D Univ of California, Berkeley 4 *Prof of Home Economics, Biochemist, Agricultural Exper Station* (2, 1929, 5, 1933)
- Morgan, Charles F** , Ph D Georgetown Univ School of Medicine, Washington, D C *Prof and Chairman of Dept of Physiology* (1, 1948, 3, 1947)
- Morgan, Clifford T** , Ph D Johns Hopkins Univ , Psychology Dept , Baltimore 18, Md (1, 1943)
- Morgulis, Sergius** , Ph D Univ of Nebraska College of Medicine, Omaha 5 *Prof and Chairman of Dept of Biochemistry* (1, 1914, 2, 1916)
- Morison, Robert S** , M D Rockefeller Foundation, 66th St and York Ave , New York City 21 *Asst Dir of Med Sciences* (1, 1938)
- Moritz, Alan R** , M D Western Reserve Univ , Cleveland, Ohio *Prof of Pathology and Dir of the Institute* (4, 1934)
- Morrell, Clarence Allison** , Ph D Dept of Natl Health and Welfare, 35 John St , Ottawa, Ontario, Canada *Dir , Food and Drug Divisions* (3, 1937)
- Morris, Harold P** , Ph D Natl Cancer Inst , Bethesda 14, Md *Principal Biochemist in Nutrition* (2, 1944, 5, 1943)
- Morris, Marion C** , Ph D Public Health Research Inst of City of New York, Foot of East 15th St , New York City *Assoc in Div of Infectious Diseases* (6, 1936)
- Morrison, Dempsey B** , Ph D Univ of Tennessee College of Medicine, Memphis *Assoc Prof of Chemistry* (2, 1936)
- Morrison, James L** , Ph D Emory Univ School of Medicine, Emory University, Ga *Assoc Prof of Pharmacology* (3, 1944)
- Morrison, Peter R** , Ph D Univ of Wisconsin, Madison 6 *Assoc Prof of Zoology and Physiology* (2, 1950)
- Morse, Minerva** Ph D 5525 Kimbark Ave , Chicago, Ill *Research Assoc, Dept of Pediatrics, Univ of Chicago* (2, 1934)
- Morse, Withrow** , Ph D 32 Manchester Rd , Eastchester, via Tuckahoe, N Y *Consultant* (2, 1914)
- Mortimer, Bernard** , Ph D , M D 25 N Ottawa St Joliet, Ill *Cook County Hospital, Chicago* (1, 1936)
- Morton, John J** , M D Univ of Rochester School of Medicine and Dentistry, Rochester, N Y *Prof of Surgery* (4, 1927)
- Moses, Campbell** , M D , Univ of Pittsburgh School of Medicine, Pittsburgh, Pa *Dir of Ad-*
- dison H Gibson Lab of Applied Physiology* (1, 1948)
- Motley, Hurley L** , Ph D , M D Jefferson Hospital, Philadelphia, Pa *Dir , Cardio-Respiratory Lab , Jefferson Med College and Assoc Prof of Medicine* (1, 1947)
- Moulder, James W** , Ph D Univ of Chicago, Depts of Bacteriology, Parasitology and Biochemistry, 5724 Ellis Ave , Chicago 37, Ill *Asst Prof of Biochemistry* (2, 1950)
- Moulton, C Robert** , Ph D 5602 Dorchester Ave , Chicago, Ill (5, 1933)
- Mountain, Isabel M** Ph D 9 Coolidge Ave , White Plains, N Y (6, 1947)
- Mountcastle, Vernon B** , M D Johns Hopkins Univ Med School, Dept of Physiology, 710 N Washington St , Baltimore 5, Md *Asst Prof of Physiology* (1, 1949)
- Moxon, Alvin L** , Ph D College Station, Brookings, S D *Head, Chemistry Dept , South Dakota Agricultural Exper Station* (2, 1944)
- Moyer, Arden W** , Ph D 407 Mountain View Rd , Englewood, N J *Research Biochemist, Lederle Labs , Pearl River, N Y* (6, 1946)
- Moyer, Carl A** , Ph D 6417 Glenrose Ct , Dallas 1, Tex *Prof of Exper Surgery, Southwestern Med College* (1, 1943)
- Mudd, Stuart, M A** , M D Univ of Pennsylvania, Philadelphia *Prof of Bacteriology* - (1, 1921, 4, 1927, 6, 1927)
- Muehlberger, Clarence W** , Ph D State Health Dept Labs , Lansing, Mich *State Toxicologist* (3, 1928)
- Mueller, J Howard** , Ph D Harvard Med School, 25 Shattuck St , Boston, Mass *Prof of Bacteriology and Immunology* (2, 1922, 4, 1927, 6, 1920)
- Mukherji, B** , D Sc All-India Inst of Hygiene and Public Health, Calcutta *Dir of Biochemical Standardization Lab* (3, 1938)
- Mulder, Arthur G** , Ph D Stritch School of Medicine of Loyola Univ , 706 S Wolcott St , Chicago, Ill *Prof and Chairman of Dept of Physiology* (1, 1937)
- Mulford, Dwight J** , Ph D Kansas Univ Med School, Dept of Biochemistry, Lawrence *Assoc Prof* (2, 1948)
- Mulinos, M G** , M D , Ph D New York Med College, Flower and Fifth Ave Hospitals, Fifth Ave and 105th St , New York City 29 *Assoc Prof of Pharmacology and Physiology* (3, 1931)
- Mull, James W** , Ph D 1870 Grove Ave , Quincy, Ill *Assoc , Quincy Specialties Co* (2, 1937)
- Müller, Otto H** , R N Dr State Univ of New York, Med Center at Syracuse, Dept of Physiology, Syracuse 10 (1, 1947)
- Muller, Robert H** , M D 36 Glen Ridge Pkwy , Montclair, N J *Captain, Med Corps* (6, 1950)
- Mulligan, Richard M** , M D Univ of Colorado

- School of Medicine, 4200 East 9th Ave, Denver
Prof of Pathology (4, 1947)
- Mullin, F J, Ph D Univ of Chicago, Chicago 37,
 Ill *Asst Prof of Physiology* (1, 1937)
- Munro, Muriel Platt, Ph D Jefferson Med Col-
 lege, 1025 Walnut St, Philadelphia 7, Pa *Re-
 search Chemist* (2, 1948)
- Munsell, Hazel E, Ph D Bromatological Lab,
 Natl Inst of Nutrition, Quito, Ecuador *Direc-
 tor* (5, 1933)
- Munson, Paul L, Ph D Harvard School of Dental
 Medicine, 188 Longwood Ave, Boston 15, Mass
Asst Prof of Dental Science (2, 1950)
- Muntwyler, Edward, Ph D State Univ Med
 Center at New York, 350 Henry St, Brooklyn 2,
 N Y *Prof of Biochemistry* (2, 1931)
- Muntz, John A, Ph D 3356 Euclid Heights Blvd,
 Cleveland Heights, Ohio *Asst Prof, Western
 Reserve Univ* (2, 1948)
- Murlin, John R, Ph D Univ of Rochester School
 of Medicine and Dentistry, Rochester, N Y
*Prof Emeritus of Physiology and Dir Emeritus
 of Dept of Vital Economics* (1R, 1906, 2, 1908,
 5, 1933)
- Murphy, Quillian R, M D, Ph D Univ of Wis-
 consin Med School, Dept of Physiology, Mad-
 ison 6 *Asst Prof* (1, 1949)
- Murray, Everitt G D, M A McGill Univ, Mon-
 treal, Quebec, Canada *Prof and Head of Dept
 of Bacteriology and Immunology, Bacteriologist-
 in Chief to the Royal Victoria Children's Memorial
 and Alexandra Hospitals* (6, 1933)
- Murray, F Joseph, Ph D The Wm S Merrell
 Co, Lockland Station, Cincinnati, Ohio *Asst
 Chief Bacteriologist* (6, 1950)
- Mushett, Charles W, Ph D Merck Inst for Ther-
 apeutic Research, Rahway, N J *Head of Dept
 of Pathology* (4, 1948)
- Muus, Jytte, Mag Scient Mount Holyoke Col-
 lege, South Hadley, Mass *Prof of Physiology*
 (2, 1946)
- Myers, Chester N, Ph D 34 Cedar Place, Yonkers
 5, N Y *Chief, Div Chemotherapy, New York
 Skin and Cancer Hospital, Dir of Chemical and
 Clin Research, H A Metz Labs, Inc* (2, 1922)
- Nachmansohn, David, M D Columbia Univ Col-
 lege of Physicians and Surgeons, 630 W 168th
 St, New York City 32 *Research Assoc in Neu-
 rology* (1, 1940)
- Nadler, J Ernest, M D, Med D Sc 80-16 Lefferts
 Blvd, Kew Gardens 15, N Y (3, 1940)
- Nahum, Louis N, M D 1142 Chapel St, New
 Haven, Conn *Asst Prof of Physiology, Yale
 Univ* (1, 1934)
- Najjar, Victor A, M D Johns Hopkins Hospital,
 Baltimore 5, Md *Assoc Prof of Pediatrics*
 (2, 1946)
- Nash, Thomas P, Jr, Ph.D 875 Monroe Ave,
 Memphis, Tenn *Prof of Chemistry, Univ of
 Tennessee College of Medicine, Dean of School
 of Biological Sciences* (2, 1923)
- Nasset, Edmund S, Ph.D Univ of Rochester
 School of Medicine and Dentistry, Rochester,
 N Y *Prof of Physiology* (1, 1932, 5, 1940)
- Nastuk, William L, Ph D Columbia Univ Col-
 lege of Physicians and Surgeons, Dept of Physi-
 ology, 630 W 168th St, New York City 32
Instr in Physiology (1, 1949)
- Natelson, Samuel, Ph D Rockford Memorial Hos-
 pital, 507 Chestnut St, Rockford, Ill *Chairman,
 Dept of Biochemistry* (2, 1950)
- Nathanson, Ira T, M.S, M D Massachusetts
 General Hospital, Boston *Asst in Surgery,
 Instr in Surgery, Harvard Med School* (1, 1943)
- Nathanson, Morris H, M D 6333 Wilshire Blvd,
 Los Angeles, Calif *Assoc Clin Prof of Medi-
 cine, Univ of Southern California School of
 Medicine* (3, 1940)
- Necheles, Heinrich, M D, Ph D Michael Reese
 Hospital, Chicago, Ill *Dir of Dept of Gastro-
 intestinal Physiology, Professorial Lecturer in
 Physiology, Univ of Chicago* (1, 1929)
- Neff, William D, Ph D Univ of Chicago, Dept
 of Psychology, Faculty Exchange, Chicago 37,
 Ill *Assoc Prof* (1, 1950)
- Neil, James M, Ph D Cornell Univ Med Col-
 lege, 1300 York Ave, New York City *Prof
 of Bacteriology and Immunology* (6, 1930)
- Neilson, Charles Hugh, Ph D, M D Humboldt
 Bldg, St Louis, Mo *Assoc Dean and Prof
 of Medicine, St Louis Univ Med School* (2,
 1906)
- Nelson, Arthur A, M D, Ph D Federal Security
 Agency, Food and Drug Admin, Div of Phar-
 macology, Washington 25, D C *Med Officer
 (Pathology)* (4, 1942)
- Nelson, Carl Ferdinand, M D, Ph D Univ of
 Kansas, Dept of Biochemistry, Lawrence *Prof
 of Physiological Chemistry* (2, 1914)
- Nelson, Carl T, M A, M D Harkness Pav
 Presbyterian Hosp, 180 Ft Washington Ave,
 New York City 32 *Dermatologist* (6, 1943)
- Nelson, Erwin E, Ph D, M D Food and Drug
 Admin, Washington 25, D C *Med Dir, Ad-
 junct Clin Prof of Pharmacology, George Wash-
 ington Univ School of Medicine* (3, 1924)
- Nelson, E M, Ph D Food and Drug Admin
 Washington 25, D C *Chief, Div of Nutrition*
 (2, 1927, 5, 1933)
- Nelson, John B, Ph D Rockefeller Inst for Med
 Research, New York City *Assoc Member* (4,
 1934)
- Nelson, John M, Ph D Columbia Univ, New
 York City 27 *Prof Emeritus of Organic Chemis-
 try* (2, 1923)
- Nelson, Marjorie M, Ph D Univ of California,
 Inst of Exper Biology, Berkeley *Research
 Assoc* (5, 1950)

- Nelson, Norton**, Ph D New York Univ College of Medicine, 477 First Ave, New York City 16 *Assoc Prof of Industrial Medicine* (2, 1946)
- Nelson, P Mabel**, Ph D Iowa State College, Ames *Dean, Div of Home Economics* (5, 1934)
- Nelson, Tell, M A**, M D 1415 Kalakauna Ave, Honolulu 19, T.H. *Practicing Physician* (6, 1938)
- Nelson, Victor E**, M S 423 Niagara St, Eau Claire, Wis (2, 1924)
- Nelson, Warren O**, Ph D Univ of Iowa School of Medicine, Iowa City *Prof of Anatomy* (1, 1937)
- Neter, Erwin**, M D Children's Hospital, 219 Bryant St, Buffalo, N Y *Attending Bacteriologist* (6, 1937)
- Nettleship, Anderson**, M D Univ of Arkansas School of Medicine, Little Rock *Prof and Head of Dept of Pathology* (4, 1942)
- Neuberg, Carl**, Ph D, M D Med, Chem D 536 W 113th St, New York City 25 *Research Prof, New York Univ* (2, 1944)
- Neuman, William F**, Ph D, Univ of Rochester School of Medicine and Dentistry, Rochester 20, N Y *Assoc Prof of Pharmacology* (2, 1949)
- Neumann, Charles**, M D 525 E 68th St, New York City 21 *Resident Surgeon, New York Hospital, Instr in Surgery, New York Univ Post-Grad Med School* (1, 1944)
- Neurath, Hans**, Ph D Univ of Washington School of Medicine, Dept of Biochemistry, Seattle 5 *Prof and Exec Officer of Dept* (2, 1940, 6, 1944)
- Neuwelt, Frank**, M D 504 Broadway, Gary, Ind *Research Assoc, Dept of Gastrointestinal Research, Michael Reese Hospital* (1, 1940)
- Neuwirth, Isaac**, Ph D 209 E 23rd St, New York City 10 *Prof of Pharmacology, New York Univ College of Dentistry* (2, 1924, 3, 1931)
- Newman, Elliot V**, M D, Johns Hopkins Hospital, Baltimore, Md *Asst Prof of Medicine* (1, 1948)
- Newman, Henry W**, M D Stanford Med School, Clay and Webster Sts, San Francisco 15, Calif *Assoc Prof of Medicine* (3, 1949)
- Nice, Leonard B**, Ph D Chicago Med School, 710 S Wolcott Ave, Chicago, Ill *Prof of Physiology and Pharmacology* (1, 1921)
- Nicholas, John S**, Ph D Yale Univ, Osborn Zoological Lab, New Haven, Conn *Bronson Prof of Comparative Anatomy* (1, 1927)
- Nicholson, Hayden C**, M S, M D Univ of Arkansas, School of Medicine, Little Rock *Dean* (1, 1932)
- Nickerson, John L**, Ph D Columbia Univ, 630 W 165th St, New York City 32 *Assoc Prof of Physiology* (1, 1945)
- Nickerson, Mark**, Ph D M D Univ of Utah School of Medicine, Salt Lake City *Assoc Prof of Pharmacology* (3, 1947)
- Nicoll, Paul A**, Ph D Indiana Univ, Bloomington *Asst Prof of Physiology* (1, 1945)
- Niemann, Carl G**, Ph D California Inst of Technology, Pasadena 4 *Prof of Organic Chemistry* (2, 1940)
- Nigg, Clara**, Ph D E R Squibb and Sons, New Brunswick, N J *Head of Depts of Bacteriology and Virology, and of Microbiological Development* (6, 1929)
- Nims, Leslie F**, Ph D Brookhaven Natl Lab, Biology Dept, Upton, Long Island, N Y *Acting Head of Dept* (1, 1940)
- Noble, Robert Laing**, M D, Ph D Univ of Western Ontario, Dept of Med Research, London, Ontario, Canada (1, 1941)
- Noell, Werner K**, M D U S A F School of Aviation Medicine, Randolph Field, Tex *Research Physiologist* (1, 1950)
- Nord, F F**, Ph D Fordham Univ, Dept of Organic Chemistry, New York City *Prof of Organic Chemistry and Enzymology* (2, 1940)
- Norris, Earl R**, Ph D Univ of Washington, Seattle *Prof of Biochemistry and Exec Officer of Dept* (2, 1938)
- Norris, L C**, Ph D Cornell Univ, Rice Hall, Ithaca, N Y *Prof of Nutrition* (2, 1939, 5, 1934)
- Northrop, J H**, Ph D, Sc D Univ of California, Berkeley 4 *Visiting Prof of Bacteriology, Member of Rockefeller Inst* (2, 1938)
- Northup, David W**, Ph D West Virginia Univ Med School, Morgantown *Prof of Physiology* (1, 1936)
- Novy, F G**, M D, Sc D 721 Forest Ave, Ann Arbor, Mich *Dean Emeritus of Med School and Prof Emeritus of Bacteriology, Univ of Michigan* (2, 1906, 4R, prior to 1919)
- Nowinski, W W**, Ph D Univ of Texas School of Medicine, Galveston *Research Assoc* (1, 1948)
- Nungester, Walter James**, Ph D, M D Univ of Michigan, Dept of Bacteriology, Ann Arbor *Professor* (6, 1949)
- Oberst, Fred W**, Ph D Med Div, Army Chemical Center, Md *Chief, Gassing Section* (2, 1936, 3, 1949)
- Ochoa, Severo**, M D New York Univ College of Medicine, 477 First Ave, New York City 16 *Prof of Pharmacology* (2, 1942)
- Ogden, Eric** Ohio State Univ College of Medicine, Columbus 10 *Prof of Physiology* (1, 1941)
- O'Hare, James P**, M D 520 Commonwealth Ave, Boston, Mass *Physician, Peter Bent Brigham Hospital, Lecturer in Medicine, Harvard Med School* (4, 1927)
- Ohlson, Margaret A**, Ph D Michigan State College, Dept of Foods and Nutrition, East Lansing *Prof and Head of Dept of Foods and Nutrition* (5, 1945)
- Okey, Ruth**, Ph D Univ of California, 1581 Life

- Sciences Bldg, Berkeley 4 *Prof of Home Economics* (2, 1922, 5, 1933)
- Olcott, Harold S, Ph D U S Dept of Agriculture, Western Regional Research Lab, Albany 6, Calif *Head of Vegetable Processing Div* (2, 1935)
- Oldham, Helen, Ph D Univ of Chicago, Chicago, Ill *Asst Prof, Dept of Home Economics* (5, 1946)
- Oleson, J J, Ph D American Cyanamid Co, Lederle Labs Div, Pearl River, N Y *Head, Dept of Nutrition and Exper Biology* (2, 1950)
- Olitsky, Peter K, M D Rockefeller Inst for Med Research, 66th St and York Ave, New York City *Member* (6, 1917)
- Oliver, Jean Redman, M D Hoagland Lab, 335 Henry St, Brooklyn, N Y *Prof of Pathology, N Y State Med Center at New York City, College of Medicine* (1, 1924, 4, 1924)
- Oliver, Wade W, M D Rockefeller Foundation, New York City *Assoc Dir of Div of Med Sciences* (4, 1925)
- Olmsted, J M D, Ph D Univ of California, Berkeley *Prof of Physiology* (1, 1920)
- Olsen, Norman S, Ph D Thayer Veterans Admin Hospital, Research Lab, Nashville 5, Tenn *Appointment in Dept of Biochemistry, Vanderbilt Univ* (1, 1948, 2, 1949)
- Olson, Byron J, Ph D, M D National Insts of Health, Bethesda, Md *Surgeon, Div of Infectious Diseases* (6, 1948)
- Olson, Carl, Jr, D V M, Ph D Univ of Nebraska, Lincoln *Chairman of Dept of Animal Pathology and Hygiene* (4, 1937)
- Opdyke, David F, Ph D Western Reserve Univ Med School, Cleveland 6, Ohio *Assoc Prof of Physiology* (1, 1945)
- Opie, Eugene L, M D, Sc D Rockefeller Inst for Med Research, 66th St and York Ave, New York City 21 *Member* (1R, 1906, 4, 1913, 6R, 1923)
- Oppenheimer, Enid Tribe 124 E 61st St, New York City *Instr in Physiology, Columbia Univ* (1, 1932)
- Oppenheimer, Ernst, M D Ciba Pharmaceutical Products, Inc, Lafayette Park, Summit, N J *Vice-Pres in charge of Med Research* (3, 1944)
- Oppenheimer, Morton Joseph, Ed M, M D 3400 N Broad St, Philadelphia, Pa *Prof of Physiology, Temple Univ School of Medicine* (1, 1942)
- Orent-Keiles, Elsa, D Sc U S Dept of Agriculture, Bureau of Human Nutrition and Home Economics, Beltsville, Md *Nutrition Chemist, Foods and Nutrition Div* (2, 1935, 5, 1935)
- Ort, John M, Ph D 401 Codwise Ave, New Brunswick, N J *Dir of Research, Carroll Dunham Smith Pharmacal Co* (2, 1932)
- Orten, Aline Underhill, Ph D Wayne Univ College of Medicine, Detroit 26, Mich *Research Assoc, Dept of Physiological Chemistry* (5, 1946)
- Orten, James M, Ph D Wayne Univ College of Medicine, Detroit 26, Mich *Assoc Prof of Physiological Chemistry* (2, 1936, 5, 1937)
- Orth, O Sidney, Ph D, M D Univ of Wisconsin, S M I, Madison *Prof of Pharmacology* (1, 1942, 3, 1944)
- Osborne, Stafford L, Ph D Northwestern Univ Med School, 303 E Chicago Ave, Chicago, Ill *Prof and Chairman of Dept of Physical Medicine* (1, 1941)
- Oser, Bernard L, Ph D Food Research Labs, Inc, 48-14 33rd St, Long Island City 1, N Y *Director* (5, 1945)
- Osler, Abraham G, Ph D Johns Hopkins Hospital, Monument and Wolfe Sts, Baltimore, Md *Dir of Serologic Lab* (6, 1949)
- Oster, Robert H, Ph D Univ of Maryland Med School, Greene and Lombard Sts, Baltimore *Asst Prof of Physiology* (1, 1938)
- Osterberg, Arnold E, Ph D Abbott Labs, Med Dept, N Chicago, Ill *Assoc in Med Dept* (2, 1933)
- Osterhout, Marian I Rockefeller Inst for Med Research, 66th St and York Ave, New York City 21 *Assoc, Div of General Physiology* (1, 1927)
- Osterhout, W J V, Ph D Rockefeller Inst for Med Research, 66th St and York Ave, New York City 21 *Member Emeritus* (1, 1910)
- Otis, Arthur B, Ph D Univ of Rochester, Rochester 7, N Y *Asst Prof of Physiology* (1, 1946)
- Ott, Walther H, Ph D Merck Inst for Therapeutic Research, Rahway, N J *Head of Dept of Biological Control and Poultry Nutrition* (5, 1949)
- Overman, Richard R, Ph D Univ of Tennessee College of Medicine, Division of Physiology, Memphis *Assoc Prof* (1, 1946)
- Owen, Seward E, Ph D 418 S 20th Ave, Maywood, Ill (1, 1938)
- Pace, Donald M, Ph D Univ of Nebraska College of Pharmacy, Dept of Physiology and Pharmacology, Lincoln *Assoc Prof of Physiology* (1, 1944)
- Pace, Nello, Ph D Univ of California, Berkeley 4 *Asst Prof of Physiology* (1, 1947)
- Pack, George T, M D 139 E 36th St, New York City 16 *Fellow in Cancer Research, Memorial Hospital* (1, 1924)
- Packchamian, Ardzoony, Ph D Univ of Texas School of Medicine, Galveston *Assoc Prof of Bacteriology and Tropical Medicine, Dir of Lab of Microbiology* (6, 1943)
- Page, Edouard, Ph D Laval Univ Med School, Dept of Biochemistry, Quebec, Canada (1, 1947)
- Page, Ernest W, M D Univ of California Hos

- pital, Dept of Obstetrics and Gynecology, San Francisco 22 *Asst Prof* (1, 1947)
- Page, Irvine H**, M D Cleveland Clinic Foundation, 2040 E 93rd St, Cleveland 6, Ohio *Dir of Research* (1, 1937, 2, 1932)
- Painter, Elizabeth E**, Ph D Univ of Illinois School of Medicine, 1853 W Polk St, Chicago *Asst Prof of Physiology* (1, 1941)
- Palmer, John W**, Ph D E R Squibb and Sons, Georges Rd, New Brunswick, N J (6, 1949)
- P'An, S Y**, M D, Chas Pfizer and Co, Inc, Dept of Pharmacology, 11 Bartlett St, Brooklyn 6, N Y *In Charge, Dept of Pharmacology* (3, 1941)
- Pangborn, Mary C**, Ph D New York State Dept of Health, Div of Labs and Research, New Scotland Ave, Albany, N Y *Sr Biochemist* (2, 1941)
- Papageorge, Evangeline**, Ph D Emory Univ School of Medicine, Dept of Biochemistry, Emory Univ, Georgia *Assoc Prof* (2, 1950)
- Pappenheimer, A M, Jr**, Ph D New York Univ College of Medicine, 477 First Ave, New York City 16 *Prof of Microbiology* (2, 1941, 6, 1938)
- Pappenheimer, Alwin M**, M D 45 Holden St, Cambridge, Mass *Prof Emeritus of Pathology, Columbia Univ* (4, 1922)
- Pappenheimer, John R**, Ph D Harvard Med School, Boston, Mass *Assoc in Physiology* (1, 1946)
- Papper, E M**, M D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City *Prof of Anesthesiology* (3, 1949)
- Park, Edwards A**, M D Johns Hopkins Hospital, Baltimore, Md *Prof Emeritus of Pediatrics, Johns Hopkins Univ* (4, 1923)
- Parker, George H**, Sc D 16 Berkeley St, Cambridge, Mass *Prof Emeritus of Zoology, Harvard Univ* (1R, 1909)
- Parker, Robert F**, M D Univ Hosps, 2065 Adelbert Rd, Cleveland, Ohio *Assoc Prof of Microbiology and Medicine* (4, 1942, 6, 1935)
- Parkins, William M**, Ph D Route 2, Chariton, Iowa (1, 1939)
- Parpart, Arthur K**, Ph D Princeton Univ, Guyot Hall, Princeton, N J *Prof of Physiology* (1, 1937)
- Parr, Leland W**, Ph D George Washington Univ School of Medicine, 1335 H St NW, Washington, D C *Prof of Bacteriology* (4, 1940)
- Parrack, Horace O**, Ph D Wright-Patterson Air Force Base Aero Med Lab, Dayton, Ohio *Research Physiologist* (1, 1948)
- Parsons, Helen T**, M D, Ph D Univ of Wisconsin, Madison *Prof of Home Economics, in charge of Purnell Research in Nutrition* (2, 1929, 5, 1933)
- Parsons, Robert J**, M D Highland Alameda County Hospital, 2701 14th Ave, Oakland, Calif *Pathologist, Dir of Labs* (4, 1939)
- Paschkis, Karl E**, M D Jefferson Med College, 1025 Walnut St, Philadelphia 7, Pa *Asst Prof of Medicine and Assoc in Physiology, Chief of Endocrine Clinic, Jefferson Hospital* (1, 1942)
- Patt, Harvey M**, Ph D Argonne Natl Lab, Chicago, Ill *Physiologist* (1, 1948)
- Patterson, Thos L**, Ph D, Sc D Wayne Univ College of Medicine, 1512 St Antoine St, Detroit 26, Mich *Research Prof of Physiology, Prof of Oro-Physiology and Dir of Dental Research, Univ of Detroit School of Dentistry* (1, 1920)
- Patterson, Wilbur I**, Ph D Food and Drug Admin, Washington 25, D C *Chief, Organic Analytical Methods Branch, Div of Food* (2, 1948)
- Patton, H D**, Ph D, M D Univ of Washington School of Medicine, Dept of Physiology and Biophysics, Seattle 5 *Assoc Prof* (1, 1947)
- Paul, John R**, M A, M D Yale Univ Med School, 333 Cedar St, New Haven, Conn *Prof of Preventive Medicine* (4, 1927, 6, 1937)
- Pearce, John Musser**, M D 525 E 68th St, New York City 21 *Prof of Pathology, Cornell Univ Med College, Attending Pathologist, New York Hospital* (4, 1942)
- Pearce, Louise**, M D Trevenna Farm, Belle Mead, N J *Assoc Member in Pathology and Bacteriology, Rockefeller Inst for Med Research* (4, 1925)
- Pearcy, Frank**, Ph D, M D 2606 Oak Lawn Ave, Dallas, Tex (1, 1928)
- Pearlman, William H**, Ph D Jefferson Med College, 1025 Walnut St, Philadelphia 7, Pa *Assoc Prof, Biochemistry Dept* (2, 1946)
- Pearse, Herman E**, M D Univ of Rochester School of Medicine and Dentistry, Rochester, N Y *Prof of Surgery* (4, 1932)
- Pearson, Harold E**, M D, M P H Univ of Southern California Med School, Los Angeles *Assoc Prof of Bacteriology, Med Microbiologist, Los Angeles County Hospital* (6, 1948)
- Pearson, P B**, Ph D U S Atomic Energy Commission Div of Biology and Medicine, Washington 25, D C *Chief of Biology Branch* (2, 1944, 5, 1940)
- Pease, Marshall C, Jr**, M D Branchville Rd, R F D 4, Ridgely, Conn *Historian of American Acad of Pediatrics* (6, 1920)
- Peck, Robert L**, Ph D 939 Madison Ave, Plainfield, N J *Sr Chemist, Merck and Co, Inc* (2, 1947)
- Penfield, Wilder G**, M D, D Sc McGill Univ, Montreal, Quebec, Canada *Prof of Neurology and Neurosurgery* (1, 1932)
- Pennington, Mary Engle**, Ph D 233 Broadway, New York City 7 *Consultant in Connection with*

- the Handling, Transportation and Storage of Perishables* (2, 1908)
- Penrod, Kenneth E**, Ph D Duke Univ School of Medicine, Durham, N C *Assoc Prof of Physiology* (1, 1946)
- Peoples, S Anderson**, M D Univ of California, School of Veterinary Medicine, Dept of Pharmacology, Davis *Prof of Comparative Pharmacology* (3, 1937)
- Perkins, John F, Jr**, M D 5621 S Kenwood Ave, Chicago 37, Ill *Asst Prof of Physiology, Univ of Chicago* (1, 1950)
- Perlman, Ely**, M D 77-14 113th St, Forest Hills, L I, N Y *Research Assoc Mt Sinai Hospital* (6, 1944)
- Permar, Howard H**, M D Pathologic Labs, Mercy Hospital, Pittsburgh, Pa *Dir of Labs* (4, 1925)
- Petermann, Mary L**, Ph D Sloan-Kettering Inst for Cancer Research, 444 E 68th St, New York City 21 *Associate* (2, 1947)
- Peters, John P**, M D 123 Marvel Rd, New Haven 15, Conn *John Slade Ely Prof of Medicine, Yale Univ School of Medicine* (2, 1922)
- Peters, Lawrence**, Ph D Western Reserve Univ Med School, Dept of Pharmacology, 2109 Adelbert Rd, Cleveland 6, Ohio *Asst Prof of Pharmacology* (3, 1946)
- Peterson, Lysle H**, M D Univ of Pennsylvania Med School, Dept of Physiology, Philadelphia 4 *Research Fellow in Physiology* (1, 1950)
- Peterson, William H**, Ph D Univ of Wisconsin, Biochemistry Bldg, Madison *Prof of Biochemistry* (2, 1919, 5, 1936)
- Pett, L B**, M D, Ph D Dept of Natl Health and Welfare, Ottawa, Ontario, Canada *Dir of Nutrition* (2, 1937, 5, 1945)
- Pfeiffer, Carl C**, Ph D, M D Univ of Illinois, 1853 W Polk St, Chicago 12 *Prof and Chairman of Dept of Pharmacology* (3, 1938)
- Pfiffner, Joseph J**, Ph D Parke, Davis & Co, Research Labs, Detroit 32, Mich *Research Chemist* (1, 1931, 2, 1931, 5, 1946)
- Phatak, Nilkanth M**, Ph D Univ of Oregon, School of Dentistry, Portland *Assoc Prof and Head of Dept of Pharmacology* (3, 1941)
- Philips, Frederick S**, Ph D Sloan-Kettering Inst for Cancer Research, 444 E 68th Street, New York City 21 *Assoc Member* (3, 1947)
- Phillips, Paul H**, Ph D Univ of Wisconsin, Madison *Prof of Biochemistry* (2, 1940, 5, 1938)
- Phillips, Robert Allan**, M D U S Naval Med Research Unit No 3, American Embassy at Cairo, Egypt, %Navy Pouch Section, Navy Dept Washington 25, D C (1, 1938)
- Pick, Ernst Peter**, M D 19 E 98th St, New York City *Assoc Pharmacologist, Mt Sinai Hospital* (3, 1940)
- Pierce, Harold B**, Ph D Univ of Vermont College of Medicine, Burlington *Prof and Chairman of Dept of Biochemistry* (2, 1929, 5, 1933)
- Pierce, Harold Fisher**, Ph D, M D State Veterans Hospital, Rocky Hill Conn (1, 1928)
- Pierce, Ira H**, Ph D Univ of Iowa, Iowa City *Assoc Prof of Pharmacology* (3, 1933)
- Pigman, William W**, Ph D Univ of Alabama, Med-Dent Schools, Biochemistry Dept, Birmingham *Assoc Prof* (2, 1950)
- Pike, Frank H**, Ph D 437 W 59th St, New York City 19 *Assoc Prof of Physiology, Columbia Univ* (1, 1907)
- Pitcher, J Douglas**, M D City Hospital, Scranton Rd, Cleveland, Ohio *Assoc Prof of Pediatrics, Western Reserve Univ School of Medicine* (3R, 1911)
- Pillemer, Louis**, Ph D Western Reserve Univ, Inst of Pathology, Cleveland, Ohio *Prof of Biochemistry* (2, 1950, 6, 1942)
- Pincus, Gregory**, Sc D Worcester Foundation for Exper Biology, 222 Maple Ave, Shrewsbury, Mass (1, 1935)
- Pincus, I J**, M D Jefferson Med College, Philadelphia, Pa *Assoc in Physiology* (1, 1948)
- Pinkerton, Henry**, M D St Louis Univ School of Medicine, St Louis, Mo *Prof of Pathology* (4, 1931)
- Pinkston, James O**, Ph D American Univ of Beirut, Beirut, Lebanon *Prof of Physiology* (1, 1936, 3, 1939)
- Pinson, Ernest A**, Ph D 2118 C-44th St, Los Alamos, N Mex (1, 1943)
- Pittman, Martha S**, Ph D Manhattan, Kan (5, 1933)
- Pitts, Robert F**, Ph D, M D Cornell Univ Med College, 1300 York Ave, New York City *Prof and Head of Dept of Physiology and Biophysics* (1, 1934)
- Plummer, Albert J**, M D, Ph D Ciba Pharmaceutical Products, Inc, Summit, N J *Sr Pharmacologist* (3, 1950)
- Pollack, Herbert**, Ph D, M D 45 E 66th St, New York City 21 *Assoc Physician and Chief of Metabolism Clinics, Mt Sinai Hospital* (1, 1933, 5, 1935)
- Pomerat, Charles Marc**, Ph D Univ of Texas Med School, Galveston *Prof of Anatomy* (1, 1944)
- Pommerenke, W T**, Ph D M D Univ of Rochester School of Medicine and Dentistry, Rochester 7, N Y *Assoc Prof of Obstetrics and Gynecology* (1, 1947)
- Pond, Samuel E**, Ph D 400 S Main St, East Hartford, Conn *Consulting Engineer, P and W A Div, United Aircraft Corp* (1, 1924)
- Ponder, Eric**, M D, Sc D Nassau Hospital, Mineola, Long Island, N Y (1, 1931)
- Poppen, John R**, M D Aero Med Acceleration

- Lab , Naval Air Development Center, Johnsville, Pa *Director* (1, 1948)
- Popper, Hans**, Ph D , M D Cook County Hospital, 1825 W Harrison St , Chicago 12, Ill *Dir of Dept of Pathology and Scientific Dir of Hektoen Inst for Med Research, Assoc Prof of Pathology, Northwestern Univ Med School* (4, 1942)
- Porter, Eugene L** , Ph D Univ of Texas Med Branch, Galveston *Prof of Physiology* (1, 1913)
- Porter, J R** , Ph D State Univ of Iowa, College of Medicine, Iowa City *Prof of Bacteriology* (6, 1950)
- Porter, Thelma**, Ph D Univ of Chicago, Chicago, Ill *Prof and Chairman of Dept of Home Economics* (5, 1944)
- Post, Robert Lickely**, M D 6310 Vanderbilt Med School, Nashville 4, Tenn *Instr in Physiology* (1, 1950)
- Poth, Edgar J** , M D , Ph D Univ of Texas Med School, Galveston *Prof of Surgery* (1, 1946)
- Potter, Truman S** , M D 117 Northampton Rd , Amherst, Mass *Independent Research Worker* (6, 1939)
- Potter, Van R** , Ph.D Univ of Wisconsin Med School, McArdle Memorial Lab , Madison *Prof of Oncology* (2, 1941)
- Powell, Horace M** , Sc D 5565 Washington Blvd , Indianapolis, Ind *Head Bacteriologist, Eli Lilly & Co* (6, 1934)
- Power, Marschelle H** , Ph D Mayo Clinic, Rochester, Minn *Prof of Physiological Chemistry, Mayo Foundation, Univ of Minnesota* (2, 1932)
- Pratt, Frederick H** , M D Boston Univ School of Medicine, Boston, Mass *Prof Emeritus of Physiology* (1R, 1919)
- Pratt, Joseph H** , M D , Sc D New England Med Center, 25 Bennet St , Boston, Mass *Physician-in-Chief, Boston Dispensary and Joseph H Pratt Diagnostic Clinic, Prof Emeritus of Medicine, Tufts Med School* (1, 1910, 3, 1910, 4, 1927)
- Preisler, Paul W** , Ph D , Washington Univ School of Medicine, 4580 Scott Ave , St Louis 10, Mo *Research Assoc , Biochemistry* (2, 1931)
- Pressman, David**, Ph D Sloan-Kettering Inst for Cancer Research, 444 East 68 St , New York City 21 *Sr Fellow in Cancer Research* (6, 1949)
- Price, Clifford W** , Ph D U S Food and Drug Admin , Washington, D C *Bacteriologist, Antibiotics Analyst, Div of Penicillin Control and Immunology* (6, 1946)
- Prinzmetal, Myron**, M.A., M D 300 S Beverly Drive, Beverly Hills, Calif *Sr Attending Physician, Cedars of Lebanon Hospital, Los Angeles* (3, 1941)
- Prosser, C Ladd**, Ph D Univ of Illinois, Natural History Bldg , Urbana (1, 1935)
- Puestow, Charles B** , M D , Ph D Univ of Illinois College of Medicine, 1853 W Polk St , Chicago *Asst Prof of Surgery* (1, 1934)
- Pugsley, L I** , Ph D Dept of Natl Health and Welfare, Food and Drug Lab , Ottawa, Ontario, Canada *Chief of Lab Service* (2, 1937)
- Putnam, Frank W** , Ph D Univ of Chicago, 947 E 58th St , Chicago 37, Ill *Asst Prof of Biochemistry* (2, 1947)
- Quackenbush, Forrest W** , Ph D R-2, Brookston, Ind *Prof and Head of Dept of Agricultural Chemistry, Purdue Univ* (2, 1946)
- Quaife, Mary L** , Ph D Distillation Products, Inc , Div of Eastman Kodak Co , Rochester 3, N Y *Sr Research Chemist, Dept of Biochemistry* (2, 1948)
- Quastel, J H** , D Sc , Ph D Research Inst , Montreal General Hosp , 3619 University St Montreal, Quebec, Canada *Prof of Biochemistry, McGill Univ* (2, 1948)
- Queen, Frank B** , M D Univ of Oregon School of Medicine, 3181 SW Sam Jackson Park Rd , Portland *Prof of Pathology* (4, 1941)
- Quick, Armand J** , M D , Ph D Marquette Med School, 561 N 15th St , Milwaukee 3, Wis *Prof and Dir of Dept of Biochemistry* (2, 1932, 3, 1937)
- Quigley, J P** , Ph D Univ of Tennessee, Memphis 3 *Prof and Chief of Div of Physiology* (1, 1929, 3, 1945)
- Quilligan, J J** , M D , M B Univ of Michigan, School of Public Health, Observatory St , Ann Arbor *Research Assoc , Dept of Epidemiology, Instr , Dept of Pediatrics* (6, 1950)
- Qumby, Freeman Henry**, Ph D Office of Naval Research, Code 441, Navy Dept , Washington 25, D C *Acting Head, Physiology Branch* (1, 1950)
- Quinby, William C** , M D Harvard Univ Med School, Boston, Mass *Clin Prof of Genito-urinary Surgery* (1R, 1916)
- Quinn, Edmond John**, Ph D 106 N Lee Ave , Rockville Center, Long Island, N Y *Medicinal Sales Div , Merck & Co , Inc , Rahway, N J* (2, 1927, 5, 1933)
- Raab, Wilhelm**, M D Univ of Vermont College of Medicine, Div of Exper Medicine, Burlington *Prof , Head of Division and of Cardiovascular Clin Research Unit* (1, 1949)
- Rabinowitch, I M** , D Sc , M D C M 1020 Med Arts Bldg , Sherbrooke and Guy Sts , Montreal, Quebec, Canada *Assoc Prof of Medicine and Lecturer in Med Jurisprudence and Toxicology, McGill Univ , Dir , Inst for Research, Montreal General Hospital* (2, 1928, 5, 1933)
- Rachele, Julian R** , Ph D Cornell Univ Med College, 1300 York Ave , New York City 21 *Assoc Prof of Biochemistry* (2, 1948)
- Rackemann, Francis M** , M D 263 Beacon St , Boston, Mass *Physician, Massachusetts General*

- Hospital, Lecturer in Medicine, Harvard Med School* (6, 1923)
- Racker, Efraim, M D** New York Univ College of Medicine, New York City 16 *Asst Prof of Microbiology* (2, 1948)
- Raffel, Sidney, Sc D, M D** Stanford Univ, Dept of Bacteriology and Exper Pathology, Stanford Univ, Calif *Asst Prof* (6, 1938)
- Rahn, Hermann, Ph D** Univ of Rochester School of Medicine and Dentistry, Rochester, N Y *Asst Prof of Physiology* (1, 1944)
- Rake, Geoffrey W, M B** Squibb Inst for Med Research, Div of Microbiology, New Brunswick, N J *Head of Div* (6, 1939)
- Rakestraw, Norris W, Ph D** Univ of California, Scripps Inst of Oceanography, LaJolla *Prof of Chemistry* (2, 1925)
- Rakieten, Nathan, Ph D** Bristol Labs, Inc, P O Box 657, Syracuse 2, N Y *Pharmacologist and Toxicologist* (1, 1941)
- Rall, Elaine P, M D** 477 First Ave, New York City *Assoc Prof of Medicine, New York Univ College of Medicine* (1, 1934, 5, 1933)
- Ralston, H J, Ph D** College of Physicians and Surgeons, 344 14th St, San Francisco 3, Calif *Assoc Prof of Physiology* (1, 1947)
- Rammelkamp, Charles H, Jr, M D** Western Reserve Univ, Dept of Preventive Medicine, Cleveland 6, Ohio *Assoc Prof of Medicine* (6, 1943)
- Ramsey, Robert Weberg, Ph D** Med College of Virginia, Richmond *Assoc Prof of Physiology and Pharmacology* (1, 1939)
- Randall, Lowell O, Ph D** Hoffmann-LaRoche, Inc, Nutley 10, N J *Pharmacologist* (2, 1939, 3, 1950)
- Randall, Walter C, Ph D** St Louis Univ School of Medicine, 1402 S Grand Blvd, St Louis, Mo *Instr in Physiology* (1, 1943)
- Randall, William A, Ph D** Food and Drug Admin, Washington 25, D C *Bacteriologist, Div of Penicillin Control and Immunology* (6, 1946)
- Rane, Leo, Ph D** Lederle Labs, Inc, Pearl River, N Y *Dept Head, Normal Blood Plasma* (6, 1942)
- Rantz, Lowell A, M D** Stanford Univ School of Medicine, San Francisco 15, Calif *Assoc Prof of Medicine* (3, 1946)
- Rapoport, Samuel, M D, Ph D** Children's Hospital Research Foundation, Elland and Bethesda, Cincinnati, Ohio *Assoc Prof of Pediatrics and Asst Prof of Biochemistry, Univ of Cincinnati* (2, 1941)
- Rapport, David, M D** 136 Harrison Ave, Boston, Mass *Prof of Physiology, Tufts College Med School* (1, 1922)
- Rapport, Maurice M, Ph D** Columbia Univ College of Physicians and Surgeons, Dept of Biochemistry, 630 W 168th St, New York City 32 *Research Assoc* (2, 1950)
- Raska, Sigwin, Ph D** 545 Graceland Ave, Des Plaines, Ill (1, 1947)
- Rasmussen, A F, Ph D, M D** Univ of Wisconsin Med School, Dept of Med Microbiology, Madison *Assoc Prof* (6, 1949)
- Rasmussen, Andrew Theodore, Ph D** Univ of Minnesota Med School, Minneapolis *Prof of Neurology* (1, 1919)
- Ratcliffe, Herbert L, D Sc** Univ of Pennsylvania, Dept of Pathology, Philadelphia 4 *Assoc Prof of Comparative Pathology, Pathologist, Zoological Society of Philadelphia* (4, 1950)
- Ratner, Bret, M D** 50 E 78th St, New York City *Prof of Pediatrics, New York Univ College of Medicine* (4, 1940, 6, 1928)
- Ratner, Sarah, Ph D** New York Univ College of Medicine, 477 First Ave, New York City 16 *Asst Prof of Pharmacology* (2, 1944)
- Raulston, B O, M D** 200 S Hudson Ave, Los Angeles, Calif *Prof of Medicine and Dir of Clin Teaching and Assoc Dean, Univ of Southern California School of Medicine* (3, 1942)
- Ravdin, I S, M D** Univ of Pennsylvania School of Medicine, Philadelphia *John Rhea Barton Prof of Surgery, Chief Surgeon, Hospital of the Univ of Pennsylvania* (1, 1930, 4, 1930)
- Rawson, Rulon W, M D** Memorial Hospital, New York City *Attending Physician, Chief, Dept of Clin Investigation, Sloan-Kettering Inst* (1, 1947)
- Raymond, Albert L, Ph D** G D Searle and Co, P O Box 5110, Chicago 80, Ill *Vice Pres in charge of Research and Development* (2, 1932)
- Rebuck, John W, M D, Ph D** Henry Ford Hospital, Dept of Labs, Detroit 2, Mich *Assoc in Pathology* (4, 1950)
- Redfield, Alfred C, Ph D** Woods Hole, Mass *Prof of Physiology, Harvard Univ* (1, 1919)
- Reed, Carlos I, Ph D** Univ of Illinois College of Medicine, 1853 W Polk St, Chicago *Prof of Physiology* (1, 1923)
- Reed, Emerson A, Ph D** Hahnemann Med College, Philadelphia, Pa *Asst Prof of Physiology* (1, 1948)
- Reese, John D, M D** North Carolina Sanatorium for the Treatment of Tuberculosis, McCann *Pathologist and Lab Dir* (4, 1949)
- Rehm, Warren S, Jr, Ph D, M D** Univ of Louisville School of Medicine, Louisville, Ky *Prof of Physiology* (1, 1945)
- Reid, Marion Adelaide, Ph D** New Jersey College for Women, New Brunswick *Asst Prof, Dept of Zoology* (1, 1941)
- Reid, Mary E, Ph D** Natl Insts of Health, Bethesda 14, Md *Cytologist* (5, 1947)
- Reumann, Hobart A, M D** Jefferson Hospital, Philadelphia, Pa *Prof of Medicine, Jefferson Med College* (4, 1933)
- Reimann, Stanley P, M D, Sc D** 703 W Phil-

- Ellena St , Mount Airy, Philadelphia, Pa *Dir of Research Inst of Lankenau Hospital, Prof of Oncology, Hahnemann Med College and Hospital, Philadelphia* (1, 1921, 4, 1924)
- Reinecke, Roger M , Ph D , M D Univ of Puerto Rico, School of Medicine, School of Tropical Medicine, San Juan (1, 1947)
- Reineke, E P , Ph D Michigan State College, Dept of Physiology and Pharmacology, East Lansing *Professor* (1, 1950)
- Reiner, John M , Ph D Tufts College Med School, Dept of Physiology, Boston, Mass (1, 1947)
- Reiner, Laszlo, M D , Ph D Wallace and Tiernan Products, Inc , 11 Mill St , Belleville 9, N J *Dir , Pharmaceutical Research* (2, 1942, 6, 1933)
- Reinhardt, William O , M D Univ of California, Berkeley 4 *Assoc Prof of Anatomy* (1, 1950)
- Reinhold, John G , Ph D Univ of Pennsylvania Hospital, 702 Maloney Bldg , Philadelphia 4, Pa *Asst Prof of Physiological Chemistry and Medicine* (2, 1936)
- Rekers, Paul E , M D Inst for Med Research, Cedars of Lebanon Hospital, 4751 Fountain Ave , Los Angeles 27, Calif *Research Assoc* (4, 1950)
- Remington, John W , Ph D Med College of Georgia, Augusta *Asst Prof of Physiology* (1, 1943)
- Remington, Roe E , Ph D , P O Box 1252, Hendersonville, N C *Consultant* (2, 1930, 5R, 1934)
- Renfrew, Alice G , Ph D Mellon Inst of Industrial Research, Univ of Pittsburgh, Pittsburgh, Pa *Sr Fellow* (2, 1939)
- Reynolds, Chapman, M D Louisiana State Univ School of Medicine, New Orleans *Asst Prof of Pharmacology* (3, 1937)
- Reynolds, Orr E , Ph D Office of Naval Research, Med Sciences Div , Washington, D C *Head of Physiology Branch* (1, 1948)
- Reynolds, Samuel R M , Ph D , Sc D 4028 Deepwood Rd , Baltimore 18, Md *Staff Member and Physiologist, Carnegie Inst of Washington, Dept of Embryology* (1, 1932)
- Reznikoff, Paul, M D New York Hospital, 525 E 68th St , New York City *Prof of Clin Medicine, Cornell Univ Med College* (1, 1927)
- Rhines, Ruth, Ph D , M D Univ of Pennsylvania School of Medicine, Dept of Anatomy, Philadelphia 4 *Asst Prof of Anatomy, Instr in Medicine* (1, 1950)
- Rhoads, Cornelius Packard, M D Memorial Hospital, 441 E 68th St , New York City *Dir , Prof of Pathology, Cornell Univ Med College* (4, 1930)
- Rhoads, Jonathan Evans, M D , D Sc Univ of Pennsylvania School of Medicine, Philadelphia 4 *Prof of Surgery, Chief, Surgical Div II, Hospital of the Univ of Pennsylvania* (1, 1946)
- Rice, Christine E , Ph D Animal Diseases Research Inst , Canadian Dept of Agriculture, Hull, Quebec, Canada *Agricultural Scientist* (6, 1938)
- Rice, Harold V , Ph D Univ of Alberta, Edmonton, Alberta, Canada *Prof of Physiology* (1, 1948)
- Rice, James C , Ph D Univ of Mississippi, P O Box 475, University *Prof of Pharmacology* (3, 1941)
- Rich, Arnold Rice, M D Johns Hopkins Hospital, Baltimore, Md *Bazley Prof of Pathology, Johns Hopkins Univ* (4, 1924)
- Richards, Alfred N , Ph D , M D 737 Rugby Rd , Bryn Mawr, Pa *Prof Emeritus of Pharmacology, Univ of Pennsylvania* (1R, 1900, 2, 1906, 3R, 1909)
- Richards, Oscar W , Ph D American Optical Co , Research Lab , P O Box 137, Stamford, Conn *Chief Biologist* (1, 1934)
- Richards, Richard Kohn, M D Abbott Labs , North Chicago, Ill *Assoc Dir of Pharmacologic Research, Prof Lecturer in Pharmacology, Northwestern Univ Med School* (1, 1938, 3, 1947)
- Richardson, Arthur P , M D Emory Univ School of Medicine, Emory University, Ga *Prof and Chairman of Dept of Pharmacology* (3, 1939)
- Richardson, Luther R , Ph D P O Box 102, College Station, Tex *Prof of Biochemistry and Nutrition, Texas A & M College* (5, 1942)
- Richter, Curt P , Ph D Phipps Psychiatric Clinic, Johns Hopkins Hospital, Baltimore, Md *Assoc Prof of Psycho-biology, Johns Hopkins Univ* (1, 1924)
- Richter, Maurice N , M D 303 E 20th St , New York City *Prof of Pathology, Bellevue Med Center, Dir Dept of Pathology, Univ Hospital* (4, 1931)
- Ricketts, Henry T , M D Univ of Chicago, Dept of Medicine, Chicago, Ill *Assoc Prof of Medicine* (1, 1940)
- Riddle, Oscar, Ph D Route 4, Plant City, Fla (1R, 1919)
- Riegel, Byron, Ph D Northwestern Univ , Dept of Chemistry , Evanston, Ill *Professor* (2, 1942)
- Riegel, Cecilia, Ph D Univ of Pennsylvania Hospital, Philadelphia 4, Pa *Asst Prof , Harrison Dept of Surgical Research Univ of Pennsylvania School of Medicine* (2, 1938)
- Rigdon, R H , M D Univ of Texas Med Branch, Galveston *Prof of Exper Pathology* (4, 1941)
- Riggs, Douglas S , M D Harvard Med School, Boston, Mass *Assoc in Pharmacology* (3, 1948)
- Riggs, Lloyd K , Ph D Natl Dairy Research Labs , Oakdale, L I , N Y *Dir of Nutritional Research* (2, 1929)

- Riker, Walter F, Jr, M D Cornell Univ Med College, 1300 York Ave, New York City 21 *Assoc Prof of Pharmacology* (3, 1947)
- Riley, Richard F, Ph D P O box 31, Beverly Hills, Calif *Asst Clin Prof of Radiology* (3, 1950)
- Riley, Richard L, M D 1901 Dixon Rd, Baltimore 9, Md *Dept of Environmental Medicine, Johns Hopkins Univ* (1, 1948)
- Rinehart, James F, M D Univ of California Med School, Parnassus and Third Aves, San Francisco *Prof of Pathology* (4, 1933)
- Ring, Gordon C, Ph D Temple Univ Med School, Broad St, Philadelphia, Pa (1, 1933)
- Rioch, David McKenzie, M D Chestnut Lodge Sanitarium, 500 W Montgomery Ave, Rockville, Md *Dir of Research* (1, 1931)
- Rittenberg, David, Ph D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 *Assoc Prof* (2, 1939)
- Ritzman, E G, A M Univ of New Hampshire, Durham *Research Prof* (5R, 1933)
- Rivers, T M, M D, Sc D Hospital of Rockefeller Inst for Med Research, 66th St and York Ave, New York City *Director* (4, 1925, 6, 1921)
- Robb, Jane Sands, Sc D, M D Syracuse Univ College of Medicine, 761 Irving Ave, Syracuse, N Y *Assoc Prof of Pharmacology* (1, 1924)
- Robbins, Benjamin Howard, M S, M D Vanderbilt Univ School of Medicine, Nashville, Tenn *Assoc Prof of Pharmacology, Prof of Anesthesiology* (3, 1936)
- Robbins, Kenneth C, Ph D Inst of Pathology, 2085 Adelbert Rd, Cleveland, Ohio *Asst Prof of Immunochemistry* (6, 1950)
- Robbins, Mary L, Ph D George Washington Univ School of Medicine, 1335 H St, N W, Washington 5, D C *Asst Prof of Bacteriology* (6, 1946)
- Roberts, Edward F, M D, Ph D Wyeth, Inc, 1600 Arch St, Philadelphia 3, Pa *Dir of Clin Investigation* (6, 1932)
- Roberts, Eugene, Ph D Washington Univ Med School, 4580 Scott Ave, St Louis 10, Mo *Research Assoc in Cancer* (2, 1950)
- Roberts, Joseph T, M D, Ph D Veterans Admin Hospital, Batavia, N Y *Chief of Med Service* (1, 1947)
- Roberts, Lydia J, Ph D Univ of Puerto Rico, Rio Piedras, P R *Prof and Chairman of Dept of Home Economics* (5, 1933)
- Roberts, Sidney, Ph D Univ of California Med School, Los Angeles *Asst Prof of Physiological Chemistry* (1, 1946, 2, 1950)
- Robertson, Elizabeth Chant, M D, Ph D Univ of Toronto, Toronto, Ontario, Canada *Research Fellow in Pediatrics* (5, 1939)
- Robertson, Oswald H, M D Stanford Univ, Dept of Biology, Palo Alto, Calif *Prof of Medicine* (4R, 1932)
- Robinson, Charles Summers, Ph D Vanderbilt Univ Med School, Nashville, Tenn *Prof of Biochemistry* (2, 1925)
- Robinson, Elliott S, M D, Ph D R F D 4, Lacomia, N H (6, 1935)
- Robinson, G Canby, M D, 4712 Keswick Rd, Baltimore, Md *Exec Sec, Maryland Tuberculosis Assoc* (1R, 1912)
- Robinson, Harry J, Ph D, M D Merck Inst for Therapeutic Research, Rahway, N J *Asst Dir* (3, 1946)
- Robinson, Herbert E, Ph D Swift and Co, Research Labs, Union Stock Yards, Chicago 9, Ill *Asst Dir of Research* (5, 1947)
- Robinson, Howard W, Ph D Broad and Ontario Sts, Philadelphia, Pa *Prof of Physiological Chemistry, Temple Univ School of Medicine* (2, 1929)
- Robinson, Sid, Ph D Indiana Univ Med School, Bloomington *Prof of Physiology* (1, 1941)
- Robinson, True W, Ph D Med College of Alabama, Birmingham 5 (1, 1948)
- Roblin, Richard O, Jr, Ph D 1937 W Main St, Stamford, Conn *Dir of Chemotherapy Div, American Cyanamid Co* (2, 1946)
- Robschert-Robbins, F S, Ph D Univ of Rochester School of Medicine and Dentistry, Rochester, N Y *Assoc in Pathology* (1, 1925, 4, 1930)
- Rodbard, Simon, Ph D Michael Reese Hospital, Cardiovascular Dept, 29th and Ellis Aves, Chicago, Ill (1, 1942)
- Roe, Joseph Hiram, Ph D George Washington Univ School of Medicine, Washington, D C *Prof of Biochemistry* (2, 1927, 5, 1933)
- Roeder, Kenneth D, M A Tufts College, Medford, Mass *Assoc Prof of Biology* (1, 1942)
- Roepke, Martin Henry, Ph D Univ Farm, St Paul, Minn *Prof of Veterinary Medicine* (3, 1937)
- Rogers, Charles G, Ph D Oberlin College, Oberlin, Ohio *Prof of Comparative Physiology* (1R, 1911)
- Rogers, Fred T, Ph D, M D Dallas Med and Surgical Clinic, 4105 Live Oak St, Dallas 1, Tex (1, 1917)
- Rogoff, Julius M, M D, Sc D Belle Island, Roanoke, Conn (1, 1916, 3, 1916)
- Roine, Paavo K, Ph D Univ of Helsinki, Helsinki, Finland *Prof of Nutritional Chemistry* (5, 1950)
- Rolfe, Daniel Thomas, M D Meharry Med College, Nashville 8, Tenn *Chairman, Dept of Physiology and Pharmacology, Prof of Physiology* (1, 1950)
- Roll, Paul M, Ph D Sloan-Kettering Inst for Cancer Research, 444 E 68th St, New York City 21 *Assoc Member* (2, 1950)

- Ronzoni, Ethel**, Ph D Washington Univ Med School, 640 S Kingshighway, St Louis 10, Mo *Asst Prof of Biological Chemistry, Neuropsychiatry Dept* (2, 1923)
- Roos, Albert**, M D Washington Univ School of Medicine, Dept of Physiology, Euclid Ave and Kingshighway, St Louis 10, Mo *Instr in Physiology and Surgery* (1, 1949)
- Root, Howard F**, M D 44 Dwight St, Brookline, Mass *Assoc in Medicine, Harvard Med School* (5, 1933)
- Root, Raymond W**, Ph D College of the City of New York, 139th St and Convent Ave, New York City *Assoc Prof of Biology, Head of Div of Physiology* (1, 1949)
- Root, Walter S**, Ph D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 *Prof of Physiology* (1, 1932)
- Rosahn, Paul D**, M D 92 Grand St, New Britain, Conn *Pathologist, New Britain General Hospital, Assoc Clin Prof of Pathology, Yale Univ School of Medicine* (4, 1934)
- Rose, Harry M**, M D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 *Assoc Prof of Bacteriology* (6, 1949)
- Rose, Jerzy E**, M D Johns Hopkins Univ Med School, Dept of Physiology, 710 N Washington St, Baltimore 5, Md *Asst Prof of Physiology and Psychiatry* (1, 1949)
- Rose, William C**, Ph.D Univ of Illinois, Urbana *Prof of Biochemistry* (2, 1912, 5, 1933)
- Rosenblueth, Arturo**, M D Instituto Nacional de Cardiologia, Calzada de la Piedad 300, Mexico D F, Mexico (1, 1932)
- Rosenfeld, Morris**, M D Johns Hopkins School of Medicine, Baltimore, Md *Assoc Prof of Pharmacology and Exper Therapeutics* (3, 1934)
- Rosenow, Edward C**, M D Longview State Hospital, Research Dept, Cincinnati 16, Ohio (4, 1913, 6, 1915)
- Rosenthal, Otto**, M D Univ of Pennsylvania School of Medicine, Philadelphia, Pa *Asst Prof of Cancer Research, Harrison Dept of Surgical Research* (2, 1946)
- Rosenthal, Sanford M**, M D Natl Insts of Health, Bethesda, Md *Chief, Section in Pharmacology and Toxicology, Exper Biology Inst* (3, 1925)
- Rosenthal, S R**, M D, Ph D Univ of Illinois College of Medicine, Chicago *Assoc Prof of Preventive Medicine and Public Health, Dir, Institution for Tuberculosis Research of Univ of Illinois* (1, 1948, 4, 1941)
- Ross, Joseph F**, M D Robert Dawson Evans Memorial, 65 E Newton St, Boston, Mass *Physician, Massachusetts Memorial Hospital, Assoc Prof of Medicine, Boston Univ School of Medicine* (4, 1941)
- Rossiter, R J**, Ph D Univ of Western Ontario, London, Ontario, Canada *Prof of Biochemistry* (2, 1948)
- Rostorfer, Howard Hayes**, Ph D Indiana Univ, Bloomington *Asst Prof of Physiology* (1, 1946)
- Roth, George B**, M D 3814 T St N W, Washington D C *Prof Emeritus of Pharmacology, George Washington Univ* (1R, 1914, 3R, 1911)
- Roth, Grace M**, Ph D Mayo Clinic, Rochester, Minn *Assoc in Clin Physiology* (1, 1939)
- Roth, James Luther d'Aumont**, M D, Ph D Univ of Pennsylvania Grad Hospital, 19th and Lombard Sts, Philadelphia 46 *Research Fellow in Gastroenterology, Grad School of Medicine, Univ of Pennsylvania* (1, 1950)
- Roth, L W**, Ph D Abbott Research Labs, Dept of Pharmacology, North Chicago, Ill *Sr Research Pharmacologist* (1, 1947, 3, 1950)
- Rothchild, Irving**, Ph D Ohio State Univ, Dept of Obstetrics and Gynecology, Univ Hospital, Columbus, Ohio (1, 1949)
- Rothmund, Paul W K**, Dr -Ing Antioch College, Yellow Springs, Ohio *Prof of Chemistry, Research Chemist, C F Kettering Foundation* (2, 1940)
- Rothstein, A**, Ph D Univ of Rochester School of Medicine and Dentistry, Rochester 7, N Y *Asst Prof in Pharmacology, Chief of Physiological Section, Pharmacological Div, Atomic Energy Project* (1, 1949)
- Rous, Peyton**, M D, Sc D Rockefeller Inst for Med Research, York Ave at 66th St, New York City *Member Emeritus* (4, 1913)
- Routh, Joseph I**, Ph D State Univ of Iowa, Iowa City *Assoc Prof of Biochemistry* (2, 1942)
- Rovenstine, Emery Andrew**, M D 477 First Ave, New York City *Prof and Chairman, Dept of Anesthesiology, New York Univ -Bellevue Med Center* (3, 1944)
- Rowntree, Jennie I**, Ph D Univ of Washington, Seattle *Prof of Home Economics* (5, 1933)
- Rowntree, L G**, M D, Sc D 5149 Alton Rd, Miami Beach, Fla (1R, 1911, 2, 1910, 3R, 1908, 4, prior to 1920)
- Rubenstein, Boris B**, Ph D, M D 5121 Ellis Ave, Chicago 15, Ill (1, 1934)
- Rubin, Saul H**, Ph D Hoffmann-LaRoche, Inc, Nutley 10, N J *Dir of Nutrition Labs* (2, 1947, 5, 1947)
- Ruch, Theodore C**, Ph D Univ of Washington, G405 Health Sciences Bldg, Seattle 5 *Prof of Physiology and Biophysics* (1, 1933)
- Ruchman, Isaac**, Ph D Cincinnati General Hospital, Cincinnati, Ohio *Asst Prof of Bacteriology* (6, 1948)
- Rugh, Roberts**, Ph D Columbia Univ, College of Physicians and Surgeons, Dept of Radiology, New York City 32 *Assoc Prof of Radiology* (1, 1950)

- Rusch, Harold Paul, M D Univ of Wisconsin Med School, McArdle Memorial Lab, Madison 6 *Prof of Oncology, Dir of Lab* (4, 1940)
- Rushmer, Robert F, M D Univ of Washington School of Medicine, Dept of Physiology and Biophysics, Seattle 5 *Asst Prof* (1, 1949)
- Rusoff, Louis L, Ph D Louisiana State Univ, Baton Rouge *Assoc Dairy Nutritionist in Exper Station and Assoc Prof of Nutrition* (5, 1948)
- Russell, Jane A, Ph D Emory Univ School of Medicine, Dept of Biochemistry, Emory Univ, Ga (1, 1939)
- Russell, Walter C, Ph D New Jersey Agricultural Exper Station, Rutgers Univ, New Brunswick *Research Specialist and Prof of Agricultural Biochemistry* (2, 1932, 5, 1933)
- Ryan, Andrew Howard, M D Chicago Med School, 710 S Wolcott Ave, Chicago, Ill *Assoc Prof of Physiology and Pharmacology* (1, 1912)
- Rytand, David A, M D Stanford Univ Hospital, San Francisco 15, Calif *Assoc Prof of Medicine, Stanford Univ School of Medicine* (3, 1946)
- Sabin, Albert, M D Children's Hospital Research Foundation, Cincinnati, Ohio *Prof of Research Pediatrics, Univ of Cincinnati* (6, 1946)
- Sabin, Florence R, M D, Sc D 1333 E 10th Ave, Denver 3, Colo *Member Emeritus, Rockefeller Inst* (1R, 1923)
- Sacks, Ernest, M D 28 Malborough Rd, North Haven, Conn *Prof Emeritus of Clin Neurological Surgery, Washington Univ Med School* (1R, 1910)
- Sacks, Jacob, Ph D, M D Brookhaven Natl Lab, Biology Dept, Upton, L I, N Y *Physiologist* (1, 1948, 3, 1933)
- Sah, Peter P T, Ph D Univ of Calif, Div of Pharmacology and Exper Therapeutics, San Francisco *Lecturer in Pharmacology* (3, 1941)
- Sahyun, Melville, Ph D Sahyun Research Lab, 316 Castillo St, Santa Barbara, Calif *Dir and Owner* (2, 1932)
- Sakami, Warwick, Ph D Western Reserve Univ School of Medicine, 2109 Adelbert Rd, Cleveland 6, Ohio *Asst Prof of Biochemistry* (2, 1949)
- Salk, Jonas E, M D Univ of Pittsburgh School of Medicine, Pittsburgh, Pa *Assoc Research Prof of Bacteriology* (6, 1947)
- Salmon, W D, A M Alabama Polytechnic Inst, Auburn *Head, Animal Husbandry and Nutrition* (2, 1929, 5, 1933)
- Salter, William T, M D Yale Univ School of Medicine, 333 Cedar St, New Haven, Conn *Prof of Pharmacology* (1, 1933, 3, 1942, 5, 1934)
- Sammis, Florence E, M D 133 E 58th St, New York City *Physician, Allergy, OPD, New York Hospital* (6, 1943)
- Sampson, John J, M D 2211 Post St, San Francisco, Calif (1, 1932)
- Sampson, Myra M, Ph D Smith College, Northampton, Mass *Prof of Zoology* (5, 1935)
- Samuels, Leo T, Ph D Univ of Utah Med School, Salt Lake City *Prof and Head of Dept of Biological Chemistry* (2, 1941, 3, 1937, 5, 1949)
- Sandels, Margaret R, Ph D Florida State Univ, Tallahassee *Dean of School of Home Economics, Prof of Nutrition* (5, 1933)
- Sandiford, Irene, Ph D Billings Hospital, Univ of Chicago, Chicago, Ill *Asst Prof of Medicine* (2, 1925, 5, 1933)
- Sandow, Alexander, Ph D Washington Square College of Arts and Sciences, New York Univ, New York City *Assoc Prof of Biology* (1, 1945)
- Sandweiss, David J, M D 9739 Dexter Ave, Detroit, Mich *Instr in Clin Medicine, Wayne Univ College of Medicine, Physician, Harper Hospital (OPD)* (1, 1944)
- Sanford, Arthur H, M A, M D Clin Labs, Mayo Clinic, Rochester, Minn *Head of Div of Clin Labs* (6R, 1920)
- Santos, Francisco O, Ph D Univ of the Philippines, Los Banos, Laguna *Prof and Head of Dept of Agricultural Chemistry, College of Agriculture* (5, 1936)
- Saphir, Otto, M D Michael Reese Hospital, 29th St and Ellis Ave, Chicago 16, Ill *Pathologist, Clin Prof of Pathology, Univ of Illinois Med School* (4, 1927)
- Saphra, Ivan, M D Beth Israel Hospital, New York City *Assoc Bacteriologist* (6, 1946)
- Sapirstein, Leo Abraham, Ph D, M D Univ of Southern California, Univ Park, Los Angeles 7 *Asst Prof of Physiology, S A Surgeon (R) USPHS* (1, 1950)
- Sappington, Samuel W, M D, D Sc P O Box 528, Bryn Mawr, Pa *Prof of Pathology, Hahnemann Hospital* (6R, 1913)
- Sarett, Herbert P, Ph D Tulane Med School, 1430 Tulane Ave, New Orleans 13, La *Asst Prof of Biochemistry* (2, 1946, 5, 1947)
- Sarnoff, Stanley J, M D Harvard School of Public Health, 55 Shattuck St, Boston 15, Mass *Asst Prof of Physiology* (1, 1949)
- Sartorius, Otto W, M D Syracuse Univ, College of Medicine, Irving Ave, Syracuse, N Y *Asst Prof of Physiology* (1, 1950)
- Saslaw, Samuel, Ph D, M D Ohio State Univ, Columbus 10 *Asst Prof in Medicine and Bacteriology* (6, 1950)
- Saslow, George, Ph D, M D Washington Univ Med School, Dept of Neuropsychiatry, 640 S Kingshighway, St Louis, Mo *Asst Prof of Psychiatry, Assoc Physician to the Student Health Service* (1, 1936)
- Satterfield, G Howard, A M Univ of North

- Carolina, State College of Agriculture and Engineering, Raleigh *Prof of Biochemistry* (2, 1944, 5, 1941)
- Saul, Leon Joseph, M A , M D Room 1907, 255 S 17th St , Philadelphia 3, Pa (1, 1933)
- Sawyer, Charles H , Ph D Duke Univ School of Medicine, Durham, N C *Prof of Anatomy* (1, 1949)
- Sawyer, Margaret E MacKay, Ph D 142 Lower Albert St , Kingston, Ontario, Canada *Royal Military College* (1, 1935)
- Sawyer, Wilbur A , M D 770 Hilldale Ave , Berkeley 8, Calif (4, 1930)
- Saxton, John A , Jr , M D Snodgrass Lab of Pathology and Bacteriology, 1430 Carroll St , St Louis, Mo *Asst Prof of Pathology, Washington Univ School of Medicine, Lab Dir , Hospital Div , City of St Louis* (4, 1944)
- Sayers, George, Ph D Univ of Utah, Salt Lake City 1 *Assoc Prof of Pharmacology* (1, 1948, 3, 1947)
- Scamman, Richard E , Ph D 172 S E Bedford St , Minneapolis, Minn *Distinguished Service Prof in Grad School* (1R, 1923)
- Scantlebury, Ronald E , Ph D Natl Insts of Health, Div of Research Grants and Fellowships, Bethesda 14, Md (1, 1948)
- Schaefer, Arnold E , Ph D Alabama Polytechnic Inst Lab of Animal Nutrition, Auburn *Assoc Animal Nutritionist* (5, 1950)
- Schales, Otto, D Sc Ochsner Clinic, Prytania and Aline Sts , New Orleans, La *Dir of Chemical Research, Ochsner Foundation, Dir , Biochemical Lab , Ochsner Clinic, Assoc Prof of Biochemistry, Tulane Univ School of Medicine* (2, 1944)
- Scharles, Frederick H , M D 603 El Morado Ct , Ontario, Calif (5, 1935)
- Schattenberg, Herbert John, M S , M D Lab of Clin Pathology, 220-222 Med Arts Bldg , San Antonio, Tex *Director* (4, 1940)
- Schenken, John R , M D Univ of Nebraska College of Medicine, Omaha *Prof of Pathology and Bacteriology* (4, 1942)
- Scherago, Morris, D V M Univ of Kentucky, Lexington *Prof and Head of Dept of Bacteriology* (6, 1948)
- Scherp, Henry W , Ph D Univ of Rochester School of Medicine and Dentistry, Rochester 7, N Y *Assoc Prof of Bacteriology and Immunology* (6, 1940)
- Schick, Bela, M D 17 E 84th St , New York City *Pediatrician, Mt Sinai and Sea View Hospitals* (6, 1924)
- Schiffman, Milton J , Ph D Hoffmann-LaRoche, Inc , Chicago, Ill *Research Consultant* (1, 1943)
- Schiller, Alfred A , M D Univ of Illinois College of Medicine, Chicago *Asst Prof of Physiology* (1, 1948)
- Schiller, Joseph, M D , Ph D 55 West 11th St , New York City 11 *Assoc Prof of Physiology, Long Island Univ* (1, 1949)
- Schlenk, Fritz, Ph D Science Hall, Iowa State College, Ames *Prof of Bacteriology* (2, 1942)
- Schlesinger, M J , Ph D , M D Beth Israel Hospital, 330 Brookline Ave , Boston, Mass *Dir of Pathology, Asst Prof of Pathology, Harvard Med School* (4, 1942, 6, 1921)
- Schlomovitz, Benjamin H , M D 1210 Majestic Bldg , 231 W Wisconsin Ave , Milwaukee, Wis (1, 1919)
- Schlumberger, Hans G , M D Ohio State Univ School of Medicine, Columbus *Assoc Prof in Pathology* (4, 1945)
- Schmeisser, Harry C , M D Univ of Tennessee, Memphis *Prof of Pathology* (4, 1937)
- Schmidt, Carl F , M D Univ of Pennsylvania Med School, Philadelphia *Prof of Pharmacology* (1, 1929, 3, 1924)
- Schmidt, C Robert, Ph D , M D 1027 S Tejon, Colorado Springs, Colo (1, 1940)
- Schmidt, Gerhard, M D Boston Dispensary, 25 Bennett St , Boston, Mass *Sr Research Fellow, Tufts College Med School* (2, 1939)
- Schmidt, L H , Ph D Christ Hospital Inst of Med Research, 2139 Auburn Ave , Cincinnati 19, Ohio *Dir of Research, Assoc Research Prof of Biochemistry, Univ of Cincinnati College of Medicine* (2, 1936, 3, 1946)
- Schmidt-Nielsen, Bodil, D D S Kettering Lab , Univ of Cincinnati, Cincinnati, Ohio *Research Assoc* (1, 1949)
- Schmidt-Nielsen, Knut, Ph D Kettering Lab , Univ of Cincinnati, Cincinnati, Ohio *Asst Prof* (1, 1949)
- Schmitt, Francis Otto, Ph D Massachusetts Inst of Technology, Cambridge *Prof of Biology* (1, 1930)
- Schmitt, Otto H , Ph D Univ of Minnesota, Dept of Physics, Minneapolis 14 *Assoc Prof of Zoology and Physics* (1, 1947)
- Schneider, Charles L , M D , Ph D Wayne Univ College of Medicine, Dept of Physiology, Detroit 26, Mich (1, 1949)
- Schneider, Edward C , Ph D 25 Gordon Place, Middletown, Conn *Prof Emeritus of Biology, Wesleyan Univ* (1R, 1912, 2, 1912)
- Schneider, Howard A , Ph D Rockefeller Inst for Med Research, 66th Street and York Avenue, New York City 21 *Associate* (5, 1947)
- Schneider, John J , Ph D , M D Jefferson Med College, 1025 Walnut St , Philadelphia 7, Pa *Research Assoc , Expr Medicine* (2, 1950)
- Schneerson, S Stanley, M D Mount Sinai Hospital, 2 East 100th St , New York City 29 *Assoc Bacteriologist* (6, 1946)
- Schoenbach, Emanuel B , M D Johns Hopkins School of Hygiene, 615 N Wolfe St , Baltimore,

- Md Assoc Prof of Preventive Medicine (6, 1941)
- Schoepfle, Gordon M, Ph D Washington Univ School of Medicine, St Louis, Mo Asst Prof of Physiology (1, 1943)
- Scholander, P F, M D, Ph D Swarthmore College, Dept of Zoology, Swarthmore, Pa Research Biologist (1, 1947)
- Schradeck, Constant E, M D P O Box 98, Newton Highlands, Mass Retired (6, 1921)
- Schreiner, Oswald, Ph D 21 Primrose St, Chevy Chase 15, Md Collaborator, U S D A (2, 1908)
- Schroeder, E F, Ph D G D Searle & Co, P O Box 5110, Chicago 80, Ill Research Biochemist (2, 1938)
- Schroeder, Henry A, M D Dept of Internal Medicine, Hypertension Div, Washington Univ, 640 S Kingshighway, St Louis 10, Mo (1, 1947)
- Schubert, Maxwell, Ph.D New York Univ College of Medicine, 477 First Ave, New York City 10 Adjunct Assoc Prof of Chemistry (3, 1947)
- Schuck, Cecelia, Ph D Purdue Univ, Dept of Home Economics, Lafayette, Ind Prof of Nutrition (5, 1941)
- Schueler, Fred W, Ph D State Univ of Iowa College of Medicine, Dept of Pharmacology, Iowa City Asst Prof (3, 1949)
- Schultz, Edwin William, M D Stanford Univ, 743 Cooksey Lane, Stanford, Calif Prof of Bacteriology and Exper Pathology (4, 1927, 6, 1928)
- Schultz, Fred H, Jr, Ph D Commercial Solvents Corp, Terre Haute, Ind Dir, Pharmaceutical Research (3, 1948)
- Schultz, W H, Ph D 241 W Broadway, Long Beach, N Y Prof Emeritus of Pharmacology, Univ of Maryland (1R, 1907, 3R, 1909)
- Schultze, Max O, Ph D Univ of Minnesota, Div of Agricultural Biochemistry, St Paul 8 Professor (2, 1938)
- Schweigert, B S, Ph D Univ of Chicago, American Meat Inst Foundation, 939 E 57th St, Chicago 37, Ill Asst Prof of Biochemistry, Head, Div of Biochemistry and Nutrition, American Meat Inst Foundation (2, 1949, 5, 1950)
- Schweizer, Malvina, Ph D New York Univ, Washington Square College of Arts and Sciences, New York City Asst Prof of Biology (1, 1944)
- Schwerma, Henry, Ph D 107 Upper Terr, San Francisco 17, Calif (1, 1948)
- Schwert, George W, Ph D Duke Univ School of Medicine, Dept of Biochemistry, Durham, N C Asst Prof (2, 1949)
- Schwimmer, Sigmund, Ph D Enzyme Research Lab, 800 Buchanan St, Albany 6, Calif Chemist, U S Bureau of Agricultural and Industrial Chemistry (2, 1947)
- Scott, Charles Covert, Ph D, M D Inlow Clinic, Shelbyville, Ind Internist (3, 1945)
- Scott, David Alymer, Ph D Connaught Labs, Univ of Toronto, Toronto 5, Ontario, Canada Research Member (2, 1935)
- Scott, Donald, Jr, Ph.D Univ of Pennsylvania Med School, 243 Anatomy Bldg, Philadelphia 4 Asst Prof, Dept of Anatomy, Assoc, Dept of Neurology (1, 1950)
- Scott, Ernest L, Ph D 64 South St, Bogota, N J Assoc Prof Emeritus of Physiology, Columbia Univ (1R, 1914, 2, 1915)
- Scott, Frederick Hughes, Ph D, M B Univ of Minnesota, Minneapolis Prof Emeritus of Physiology (1R, 1908, 2, 1909)
- Scott, James K, M D Univ of Rochester School of Medicine and Dentistry, Atomic Energy Project, Rochester 7, N Y Chief, Pathology Section, AEC, Assoc in Pathology (4, 1949)
- Scott, John C, Ph D Hahnemann Med College, Philadelphia, Pa Prof and Head of Dept of Physiology (1, 1936)
- Scott, R W, M A, M D City Hospital, Cleveland, Ohio Physician in Chief, Prof of Clin Medicine, Western Reserve Univ (1, 1917, 3, 1917)
- Scott, V Brown, Ph D, M D Inlow Clinic, Shelbyville, Ind Internist, Div of Medicine (1, 1941)
- Scott, W W, Ph D, M D Brady Urological Inst, Johns Hopkins Hospital, Baltimore 5, Md (1, 1943)
- Scrimshaw, Nevin S, Ph D, M D Instituto de Nutricion de Centro America y Panama, Guatemala City, Guatemala, Central America Dir of Div of Nutrition, Pan American Sanitary Bureau (5, 1949)
- Scudi, John V, Ph D Nepera Chemical Co, Inc, Nepera Park, Yonkers 2, N Y Dir of Research (2, 1942, 3, 1950, 5, 1945)
- Seager, Lloyd D, M S, M D Univ of Arkansas Med. School, Little Rock Prof and Head, Dept of Physiology and Pharmacology (3, 1939)
- Sealock, Robert R, Ph D Iowa State College, Ames Assoc Prof of Chemistry (2, 1940, 5, 1941)
- Seastone, C V, Jr, M D Univ of Wisconsin Med School, Madison Prof and Chairman of Med Bacteriology (6, 1939)
- Sebrell, W H, Jr, M D Natl Insts of Health, Bethesda 14, Md Dir, Natl Insts of Health, Professorial Lecturer on Nutrition, George Washington Univ (2, 1938, 5, 1937)
- Seecof, David P, M D 1970 Daly Ave, Bronx, New York City (4, 1927)
- Seegal, Beatrice Carrie, M D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 Assoc Prof of Bacteriology (6, 1949)

- Seegal, David**, M D Maimonides Hospital, Brooklyn, N Y *Dir of Med Services* (6, 1930)
- Seegers, Walter H**, Ph D Wayne Univ College of Medicine, 1512 St Antoine St, Detroit 26, Mich *Prof of Physiology* (1, 1947, 2, 1941)
- Seever, Maurice Harrison**, Ph D, M.D Univ of Michigan, Ann Arbor *Prof of Pharmacology* (1, 1933, 3, 1930)
- Segaloff, Albert**, M D Alton Ochsner Med Foundation, 3503 Prytania St New Orleans, La *Dir of Endocrine Research* (4, 1946)
- Seibert, Florence B**, Ph D Univ of Pennsylvania, Henry Phipps Inst, 7th and Lombard Sts, Philadelphia *Assoc Prof of Biochemistry* (2, 1925)
- Seidell, Atherton**, Ph D 2301 Connecticut Ave, Washington, D C *Special Expert, Natl Insts of Health* (2, 1924)
- Seifter, Joseph**, M D Wyeth Inst of Applied Biochemistry, Philadelphia, Pa *Director* (3, 1940)
- Seifter, Sam**, Ph D New York State Univ Med Center at New York City, 350 Henry St, Brooklyn 2, N Y *Assoc Prof of Biochemistry* (2, 1946)
- Selkurt, Ewald E**, Ph D Western Reserve Univ School of Medicine, Cleveland 6, Ohio *Assoc Prof of Physiology* (1, 1945)
- Selle, Wilber Arthur**, Ph D Univ of California at Los Angeles Med School, Los Angeles *Prof and Head, Dept of Biophysics* (1, 1938)
- Sellers, E A**, M D, Ph D Univ of Toronto, Dept of Physiology, Toronto, Ontario, Canada *Assoc Prof of Physiology, Assoc Prof in Banting and Best Dept of Med Research* (1, 1947)
- Selye, Hans**, M D, Ph D Inst of Exper Medicine and Surgery, Univ of Montreal, Montreal, Quebec, Canada *Prof and Dir* (1, 1934)
- Sendroy, Julius, Jr**, Ph D Naval Med Research Inst, Natl Naval Med Center, Bethesda 14, Md *Chief Chemist* (2, 1928)
- Sevag, M G**, Ph D Univ of Pennsylvania School of Medicine, Dept of Bacteriology, Philadelphia 4 *Assoc Prof of Bacteriology* (2, 1949, 6, 1941)
- Sevringhaus, Elmer L**, M A, M D Hoffmann-La Roche Inc, Nutley 10, N J *Dir of Clin Research, Clin Prof of Medicine, New York Med College* (2, 1923, 5, 1939)
- Shaffer, C Boyd**, Ph D Mellon Inst, Pittsburgh 13, Pa *Sr Fellow* (3, 1950)
- Shaffer, Morris F**, Ph D Tulane Univ School of Medicine, Dept of Bacteriology, New Orleans, La *Professor* (4, 1939, 6, 1937)
- Shaffer, Philip A**, Ph D Washington Univ Med School, St Louis 10, Mo *Distinguished Service Prof of Biological Chemistry* (2, 1906)
- Shanes, Abraham M**, Ph D Natl Insts of Health, Exper Biology and Medicine Inst, Bethesda 14, Md (1, 1946)
- Shank, Robert E**, M D Washington Univ School of Medicine, St Louis 10, Mo *Prof of Preventive Medicine* (2, 1947)
- Shannon, James A**, Ph D, M D Squibb Inst for Med Research, New Brunswick, N J *Director* (1, 1933, 3, 1945)
- Shapiro, Herbert**, Ph D Henry Phipps Inst, 7th and Lombard Sts, Philadelphia 47, Pa *Research Assoc* (1, 1937)
- Sharpless, George R**, Sc D Lederle Labs, Pearl River N Y *Research Biochemist* (5, 1942)
- Shaw, J C**, Ph D Univ of Maryland, Dept of Dairy Husbandry, College Park, Md *Professor* (1, 1947)
- Shaw, James H**, Ph D Harvard School of Dental Medicine, Boston, Mass *Assoc in Nutrition* (5, 1948)
- Shaw, Myrtle**, Ph D 11 S Lake Ave Albany, N Y *Sr Bacteriologist, Div of Labs and Research, N Y State Dept of Health* (6, 1937)
- Shay, Harry**, M D Temple Univ School of Medicine, Philadelphia Pa *Dir of Fels Research Inst and Clin Prof of Medicine* (1, 1944)
- Shear, M J**, Ph D Natl Cancer Inst, Bethesda, 14, Md *Chief of Chemotherapy Section* (2, 1930)
- Sheard, Charles**, Ph D Mayo Foundation, Rochester, Minn *Chief of Div of Physics and Biophysical Research, Prof of Physiological Optics and Biophysics, Univ of Minnesota* (1, 1925)
- Sheehan Donal**, M D, D Sc New York Univ, Bellevue Med Center, 477 First Ave, New York City (1, 1938)
- Shelesnyak, M C**, Ph D Weizmann Inst of Science, Dept of Exper Biology, Rehovoth, Israel *Sr Scientist* (1, 1948)
- Shelley, Walter B**, Ph D, M D 353 Inwood Rd, Ardmore, Pa (1, 1946)
- Shemin, David**, Ph D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 *Assoc Prof of Biochemistry* (2, 1944)
- Sheppard, C W**, Ph D Oak Ridge Natl Lab, Biology Div, Oak Ridge, Tenn *Physicist* (1, 1950)
- Sheppard, Fay**, M S Univ of Oklahoma Med School Oklahoma City *Instr in Biochemistry* (2, 1936)
- Sherman, F G**, Ph D Brown Univ, Dept of Biology, Providence 12, R I *Asst Prof* (1, 1950)
- Sherman, Henry C**, Ph D Columbia Univ, New York City *Mitchell Prof Emeritus of Chemistry* (1R, 1923, 2, 1906, 5R, 1933)
- Sherman, William C**, Ph D Ralston Purina Co, St Louis, Mo *Head of Biological Labs* (5, 1949)
- Sherwin, Carl Paxson**, Sc D, M D, Dr P H 6 Carstensen Rd Scarsdale, N Y *Dir of Meta-*

- bolic Service, St Vincent's Hospital, Assoc Physician, French Hospital* (2, 1917)
- Sherwood, Noble P**, Ph D, M D Univ of Kansas, 517 Snow Hall, Lawrence *Prof of Bacteriology* (6, 1928)
- Sherwood, Thomas Cecil**, Ph D, M D 1824 Robert St, New Orleans, La *Southern Baptist Hospital Staff Member, Internal Medicine* (1, 1938)
- Shettles, Landrum B**, Ph D, M D Columbia Univ, College of Physicians and Surgeons, Box 330, 622 W 168th St, New York City (1, 1946)
- Shudeman, Frederick E**, Ph D, M D Univ of Michigan, Dept of Pharmacology, Ann Arbor *Assoc Prof* (3, 1944)
- Shuls, Maurice E**, Sc D 600 W 168th St, New York City 32 *Asst Prof of Nutrition, School of Public Health, Columbia Univ* (5, 1950)
- Shimkin, Michael Boris**, M D Univ of California Med School, San Francisco *Dir, Lab of Exper Oncology, Laguna Honda Home* (4, 1940)
- Shinowara, George Y**, Ph D Ohio State Univ College of Medicine, Columbus *Assoc Prof, Dept of Pathology, Chem Pathologist, Univ Hospital* (2, 1949)
- Shipley, Reginald A**, M D Western Reserve Univ School of Medicine, Cleveland 6, Ohio *Asst Prof of Medicine* (1, 1945)
- Shipley, Robert E**, M D Lilly Lab for Clin Research, Indianapolis City Hospital, Indianapolis, Ind (1, 1945)
- Shive, William**, Ph D Univ of Texas, Austin *Assoc Prof of Chemistry* (2, 1948)
- Shlaer, Simon**, Ph D Box 1663, Los Alamos, N M (1, 1938)
- Shock, Nathan W**, Ph D Baltimore City Hospitals, Baltimore 24, Md *Chief, Section on Gerontology, USPHS, Natl Insts of Health, Bethesda, Md* (1, 1942)
- Shoemaker, Harold A**, Ph D Univ of Oklahoma School of Medicine, 801 N E 13th St, Oklahoma City *Prof of Pharmacology* (3, 1941)
- Shope, Richard E**, M D Ridge Road, Kingston, N J *Merck Inst for Therapeutic Research* (4, 1934)
- Shorr, Ephraim**, M D New York Hospital, 525 E 68th St, New York City *Asst Attending Physician, Asst Prof of Medicine, Cornell Univ Med College* (1, 1931, 3, 1942)
- Shrigley, E W**, Ph D, M D Indiana Univ Med Center, Dept of Microbiology, 1040-1232 W Michigan St, Indianapolis 7 (6, 1946)
- Shwartzman, Gregory**, M D 230 E 50th St, New York City *Head of Dept of Bacteriology, Mount Sinai Hospital, Prof of Bacteriology, Columbia Univ* (4, 1929, 6, 1930)
- Sichel, F J M**, Ph D Univ of Vermont College of Medicine, Burlington *Prof and Chairman of Dept of Physiology and Biophysics* (1, 1939)
- Sickles, Grace M**, B A 2201 Twelfth St, Troy, N Y *Assoc Bacteriologist, Div of Labs and Research, New York State Dept of Health* (6, 1932)
- Sickles, Gretchen R**, A B New York State Dept of Health, Div of Labs and Research, Albany, N Y *Asst Bacteriologist* (6, 1937)
- Siebenmann, Charles O**, D Eng Connaught Med Research Labs, Univ of Toronto, Toronto 5, Ontario, Canada *Research Assoc, Assoc in Hygiene and Preventive Medicine* (3, 1946)
- Siebert, Walter J**, M D Lutheran Hospital, St Louis, Mo *Dir of Labs, Pathologist, Asst Prof, Washington Univ* (4, 1932)
- Siegmund, Otto Hanns**, D V M 409 B St, Davis, Calif *Jr Veterinarian, Asst Prof, Univ of California School of Veterinary Medicine* (3, 1950)
- Sigel, M Michael**, Ph D Children's Hospital, 1740 Bainbridge St, Philadelphia 46, Pa *Assoc in Virology, Univ of Pennsylvania* (6, 1950)
- Silber, Robert H**, Ph D Merck Inst, Rahway, N J *Head, Dept of Biochemistry* (2, 1948)
- Silberberg, Martin**, M D Snodgrass Lab of Pathology, 1430 Carroll St, St Louis 4, Mo *Asst Prof of Pathology, Washington Univ School of Medicine, Pathologist, Hospital Div, City of St Louis* (4, 1944)
- Silberberg, Ruth**, M D Snodgrass Lab of Pathology, 1430 Carroll St, St Louis 4, Mo *Asst Prof of Pathology, Washington Univ School of Medicine, Pathologist, Hospital Div, City of St Louis* (4, 1944)
- Silverman, Milton**, Ph D Natl Insts of Health, Bethesda 14, Md *Scientist, Exper Biology and Medicine Inst* (2, 1950)
- Silvette, Herbert**, Ph D Meharry Med College, Nashville, Tenn *Visiting Prof of Pharmacology* (1, 1933, 3, 1940)
- Simmonds, Sofia**, Ph D Yale Univ, Osborn Botanical Lab, New Haven 11, Conn *Asst Prof of Microbiology and Biochemistry* (2, 1948)
- Simonds, James P**, Ph D, M D 2033 W Morse Ave, Chicago 45, Ill *Prof Emeritus of Pathology, Northwestern Univ Med School* (4, prior to 1920)
- Smonsens, Daisy G**, Ph D Los Angeles County Hospital, 1200 N State St, Los Angeles 33, Calif *Chemist and Toxicologist, Instr in Medicine, Univ of Southern California Med School* (2, 1949)
- Simonson, Ernst**, M D Univ of Minnesota, Lab of Physiological Hygiene, Stadium South Tower, Minneapolis 14 *Assoc Prof of Physiology* (1, 1941)
- Simpson, Miriam E**, Ph D, M D Univ of California, Berkeley *Prof of Anatomy* (1, 1946)
- Singal, Sam A**, Ph D Med College of Georgia, Augusta *Assoc Prof of Biochemistry* (2, 1948)
- Singer, Thomas P**, Ph D Western Reserve Univ,

- Cleveland, Ohio *Asst Prof of Biochemistry* (2, 1948)
- Sizer, Irwin W, Ph D Massachusetts Inst of Technology, Cambridge *Assoc Prof of Physiology* (1, 1944)
- Skinner, John Taylor, Ph D The Grapette Co, Camden, Ark *Chief Chemist* (2, 1946)
- Slaughter, Donald, M D Univ of South Dakota School of Med Sciences, Vermillion *Dean* (3, 1938)
- Slonaker, James R, Ph D 334 Kingsley Ave, Palo Alto, Calif *Prof of Physiology, Leland Stanford Jr Univ* (1R, 1917)
- Smadel, Joseph Edwin, M D Army Med Dept, Research and Grad School, Washington 12, D C *Chief, Dept of Virus and Rickettsial Diseases* (4, 1940, 6, 1937)
- Small, James C, M D 101 S 39th St, Philadelphia, Pa *Assoc in Medicine, Univ of Pennsylvania Grad School of Medicine* (4, 1927)
- Smetana, Hans F, M D Armed Forces Inst of Pathology, 7th St and Independence Ave, Washington 25, D C *Chief, Dept of Pathology* (4, 1934)
- Smith, Arthur H, Ph D Wayne Univ College of Medicine, Detroit 26, Mich *Prof of Physiological Chemistry* (2, 1921, 5, 1933)
- Smith, Austin Edward, M D American Med Assoc, 535 N Dearborn St, Chicago, Ill *Editor, J A M A* (3, 1942)
- Smith, Clarence A, Ph D Standard Brands, Inc, 595 Madison Ave, New York City *Technical Dir, Agricultural Dept* (1, 1921)
- Smith, David T, M D Duke Hospital, Durham, N C *Prof of Bacteriology and Assoc Prof of Medicine* (5, 1943, 6, 1949)
- Smith, Dietrich Conrad, Ph D Univ of Maryland School of Medicine, Lombard and Greene Sts, Baltimore *Assoc Prof of Physiology* (1, 1937)
- Smith, Douglas E, Ph D Argonne Natl Lab, P O Box 5207, Chicago 80, Ill (1, 1947)
- Smith, Edwin L, Ph D Univ of Texas Dental School, Houston *Prof of Physiology* (1, 1948)
- Smith, Elinor Van Dorn, Ph D Smith College, Northampton, Mass *Assoc Prof of Bacteriology* (6, 1940)
- Smith, Elizabeth R B, Ph D % Dr Paul K Smith, 1335 H St, N W, Washington 5, D C (2, 1938)
- Smith, Emil L, Ph D Univ of Utah School of Medicine, Salt Lake City 1 *Prof of Biochemistry, Research Prof of Medicine* (2, 1946)
- Smith, Erma A, Ph D, M D S H Camp Co, Jackson, Mich (1, 1928)
- Smith, Falconer, Ph D 6920 Tilden Lane, Rockville, Md *Scientist (officer), USPHS, Research in Radiobiology* (1, 1950)
- Smith, George H, Ph D Yale Univ School of Medicine, New Haven, Conn *Prof of Immunology and Asst Dean, Chairman of Dept of Bacteriology, Yale Univ* (6, 1918)
- Smith, H P, M S, M D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 *Delafield Prof of Pathology* (1, 1937, 4, 1925)
- Smith, Homer W, Sc D New York Univ, Bellevue Med Center, 477 First Ave, New York City *Prof of Physiology* (1, 1923, 2, 1930)
- Smith, Janice M, Ph D Univ of Illinois, Dept of Home Economics, Urbana *Prof and Chief of Nutrition* (5, 1947)
- Smith, John R, A M, M D Washington Univ School of Medicine, St Louis 10, Mo *Asst Prof of Medicine* (1, 1947)
- Smith, Lawrence Weld, M D 119 E 26th St, New York City 10 (4, 1927)
- Smith, Lee Irvin, Ph D Univ of Minnesota School of Chemistry, Minneapolis 14 *Prof and Chief of Div of Organic Chemistry* (2, 1942)
- Smith, Margaret Cammack, Ph D El Encanto Estates, Tucson, Ariz (5, 1933)
- Smith, Margaret H D, M D, B es lettres Tulane Univ, 1430 Tulane Ave, New Orleans, La *Asst Prof of Pediatrics* (6, 1950)
- Smith, Maurice I, M D 8415 Lynbrook Dr, Bethesda, Md *Principal Pharmacologist, Retired, USPHS* (1R 1920, 3R, 1916)
- Smith, Paul K, Ph D George Washington Univ School of Medicine, 1335 H St, N W, Washington 5, D C *Prof of Pharmacology and Exec Officer of Dept* (2, 1937, 3, 1937)
- Smith, Paul W, Ph D Univ of Oklahoma School of Medicine, 801 E 13th St, Oklahoma City *Assoc Prof of Pharmacology* (1, 1933)
- Smith, Philip Edward, Ph D Columbia Univ, 630 W 168th St, New York City 32 *Prof of Anatomy* (1, 1923)
- Smith, Ralph G, M D, Ph D Federal Security Agency, Food and Drug Admin, Washington 25, D C *Med Officer, Chief of New Drug Section* (3, 1929)
- Smith, R Blackwell, Jr, Ph D Med College of Virginia, Richmond 19 *Assoc Prof of Pharmacology* (3, 1944)
- Smith, Sedgwick E, Ph D Cornell Univ, Dept of Animal Husbandry, Ithaca, N Y *Animal Physiologist* (5, 1945)
- Smith, Susan Gower, M A Duke Univ, Durham, N C *Assoc in Dept of Medicine and Nutrition, School of Medicine* (5, 1939)
- Smith, Sybil L, A M 1421 44th St, N W, Washington, D C (5, 1940)
- Smith, Wilbur Kenneth, M D Univ of Rochester School of Medicine and Dentistry, Rochester, N Y *Assoc Prof of Anatomy* (1, 1939)
- Smith, Willie W, Ph D Natl Insts of Health, Bethesda, Md *Physiologist* (1, 1941)
- Smithburn, Kenneth C, M D Rockefeller Foun-

- dation, 49 W 49th St , New York City *Staff Member, International Health Div* (6, 1937)
- Smolens, Joseph, B S Children's Hospital, 1740 Bainbridge St , Philadelphia, Pa (6, 1943)
- Smythe, C V , Ph D 5000 Richmond St , Philadelphia, Pa *Head of Biochemistry, Rohm & Haas Co* (2, 1934)
- Snape, William J , M D Jefferson Med College, Philadelphia, Pa *Assoc in Physiology* (1, 1948)
- Snell, Albert M , M D Palo Alto Clinic, 300 Homer St , Palo Alto, Calif (4, 1930)
- Snell, Esmond E , Ph D Univ of Wisconsin, Madison 6 *Prof of Biochemistry* (2, 1942, 5, 1946)
- Snider, Ray S , Ph D Northwestern Univ School of Medicine, 303 E Chicago Ave , Chicago 11, Ill *Assoc Prof of Anatomy* (1, 1949)
- Snyder, Charles D , Ph D 4709 Keswick Rd , Baltimore, Md *Prof Emeritus of Exper Physiology, Johns Hopkins Univ* (1R, 1907)
- Snyder, Franklin Faust, M D Boston Lying-In Hospital, Boston, Mass (1, 1936)
- Snyder, John Crayton, M D Harvard School of Public Health, 695 Huntington Ave , Boston, Mass *Prof of Microbiology* (6, 1950)
- Sobel, Albert E , Ph D Jewish Hospital of Brooklyn, 555 Prospect Pl , Brooklyn 16, N Y *Head of Dept of Chemistry, Special Lecturer, Long Island College of Medicine* (2, 1939)
- Sobin, Sidney S , M D , Ph D 405 N Bedford Drive, Beverly Hills, Calif *Research Assoc in Physiology, Univ of Southern California* (1, 1949)
- Sobotka, Harry H , Ph D Mount Sinai Hospital, Fifth Ave and 100th St , New York City 29 *Head of Dept of Chemistry* (2, 1932, 5, 1933)
- Solandt, Donald Young, M D , Ph D Univ of Toronto, Toronto, Ontario, Canada *Prof of Physiology in charge of Biophysics, Faculty of Medicine, Prof and Head of Dept of Hygiene, School of Hygiene* (1, 1937)
- Sollmann, Torald, M D 14327 Superior Rd , Cleveland, Ohio *Dean and Prof Emeritus of Pharmacology, Western Reserve Univ* (1R, 1902, 2, 1906, 3, 1908)
- Solotorovsky, Morris, Ph D 203 W 5th St , Plainfield, N J *Merck Inst for Therapeutic Research, Rahway, N J , Research Assoc in Chemotherapy* (6, 1946)
- Somogyi, Michael, Ph D 216 S Kingshighway, St Louis, Mo *Biochemist, Jewish Hospital of St Louis* (2, 1927)
- Sonnenschein, Ralph R , M D , Ph D Univ of Illinois, College of Medicine, Chicago 12 *Research Asst in Psychiatry* (1, 1950)
- Soskin, Samuel, M D , Ph D Michael Reese Hospital, 29th St and Ellis Ave , Chicago 16, Ill *Med Dir and Dir of Research Inst , Dean, Postgrad School* (1, 1930, 5, 1933)
- Soule, Malcolm H , Sc D Univ of Michigan, Ann Arbor *Prof of Bacteriology, and Chairman of Dept of Bacteriology* (4, 1927, 6, 1925)
- Spain, Will C , M D 116 E 53rd St , New York City *Chn Prof of Medicine, Post-Grad Med School, Columbia Univ* (6, 1923)
- Spaulding, Earle H , Ph D Temple Univ , School of Medicine, Broad St at Ontario, Philadelphia 40, Pa *Prof of Bacteriology, Head of Dept* (6, 1950)
- Speelman, C R , Ph D Dept of Commerce, Civil Aeronautics Admin , Washington 25, D C (1, 1940)
- Specht, Heinz, Ph D Natl Insts of Health, Bethesda, Md *Scientist, USPHS* (1, 1941)
- Spector, Harry, Ph D Quartermaster Food and Container Inst for the Armed Forces, 1849 W Pershing Rd , Chicago 9, Ill *Chief, Nutrition Div* (5, 1950)
- Speirs, Mary, Ph D Georgia Agricultural Exper Station, Experiment *Head of Dept of Home Economics* (5, 1949)
- Sperry, Roger W , Ph D Univ of Chicago, Dept of Anatomy, Chicago 37, Ill (1, 1945)
- Sperry, Warren M , Ph D New York State Psychiatric Inst , 722 W 168th St , New York City 32 *Principal Research Biochemist, Assoc Prof of Biological Chemistry, Columbia Univ* (2, 1929, 5, 1933)
- Spicer, Samuel S , M D Natl Insts of Health, Bethesda 14, Md *Commissioned Officer, USPHS, Surgeon* (1, 1950)
- Spiegel, Ernest A , M D Temple Univ School of Medicine, Broad and Ontario Sts , Philadelphia, Pa *Prof of Exper Neurology* (1, 1936)
- Spiegel-Adolf Mona, M D Temple Univ School of Medicine, Broad and Ontario Sts Philadelphia, Pa *Prof and Head of Dept of Colloid Chemistry* (2, 1933)
- Spiegelman, Sol, Ph D Univ of Illinois, Dept of Bacteriology, Urbana (1, 1946)
- Spies, Tom D , M D Hillman Hospital, Birmingham, Ala *Dir of Nutrition Clinic* (3, 1941, 4, 1940, 5, 1938)
- Spink, Wesley W , M D Univ of Minnesota Hospital, Minneapolis *Assoc Prof of Medicine, Univ of Minnesota Med School* (6, 1940)
- Spohn, Adelaide, Ph D Elizabeth McCormick Memorial Fund, 848 N Dearborn St , Chicago, Ill *Nutritionist* (5, 1933)
- Spoor, Herbert J , Ph D , M D 6705A 186th Lane, Flushing, L I , N Y *Dir , Dermatological Research, New York Med College* (1, 1945)
- Sprent, J F A , Ph D Univ of Toronto, 43 Queens Park, Toronto 5, Ontario, Canada *Asst Prof , Sr Research Fellow, Ontario Research Foundation* (6, 1949)

- Sprinson, David B**, Ph D Columbia Univ College of Physicians and Surgeons, Dept of Biochemistry, 630 W 168th St, New York City 32 *Research Assoc* (2, 1949)
- Sproul, Edith E**, M D Columbia Univ College of Physicians and Surgeons, Dept of Pathology, 630 W 168th St, New York City 32 *Assoc Prof* (4, 1941)
- Sprunt, Douglas H**, M D, M S Univ of Tennessee, Memphis *Prof of Pathology, Head of Dept. of Pathology and Bacteriology* (4, 1934, 6, 1936)
- Stacy, Ralph W**, Ph D Ohio State Univ, Dept of Physiology, Hamilton Hall, Columbus 10 *Asst Prof* (1, 1949)
- Stadie, William C**, M D 821 Maloney Clinic, 36th and Spruce Sts, Philadelphia 4, Pa *John Herr Musser Prof of Research Medicine, Univ of Pennsylvania* (2, 1922)
- Stanley, Wendell M**, Ph D Univ of California, Rm 223, Forestry Bldg, Berkeley 4 *Prof and Chairman of Dept of Biochemistry, Dir of Virus Lab* (2, 1936)
- Stannard, James Newell**, Ph D Univ of Rochester Med School, Atomic Energy Project, Rochester 7, N Y *Assoc Prof of Radiation Biology* (1, 1938)
- Stansly, Philip G**, Ph.D American Cyanamid Co, Stamford Research Labs, Stamford, Conn *Microbiologist, Chemotherapy Dept* (2, 1950)
- Stare, Fredrick J**, Ph D, M D 695 Huntington Ave, Boston 15, Mass *Prof of Nutrition, Harvard Univ* (2, 1937, 5, 1942)
- Starr, Isaac**, M D Hospital of the Univ of Pennsylvania, Philadelphia *Hartzell Prof of Research Therapeutics* (1, 1929, 3, 1942)
- Stavitsky, Abram B**, Ph D, V.M D Western Reserve Univ School of Medicine, 2109 Adelbert Rd, Cleveland 6, Ohio *Asst Prof of Microbiology* (6, 1950)
- Stavraky, George W**, M D Univ of Western Ontario, Med School, London, Ontario, Canada *Prof of Physiology* (1, 1937, 3, 1944)
- Stead, Eugene A, Jr**, M D Duke Univ, Dept of Medicine, Durham N C (1, 1945)
- Stearns, Genevieve**, Ph D State Univ of Iowa, College of Medicine, Iowa City *Research Prof of Pediatrics* (2, 1932, 5, 1937)
- Steel, Matthew**, Ph D New York State Univ Med Center at New York City, 350 Henry St, Brooklyn, N Y *Prof of Biological Chemistry* (2, 1909)
- Steele, J Murray**, M D Third (N Y U) Research Div, Goldwater Memorial Hospital, Welfare Island, New York City *Assoc Prof of Medicine, New York Univ, Dir of 3rd Med Div, Welfare Hospital* (1, 1936)
- Steenbock, Harry**, Ph D Univ of Wisconsin, Madison *Prof of Biochemistry* (2, 1912, 5, 1933)
- Steffee, C Harold**, M D, Ph D U S Marine Hospital, Dept of Pathology, New Orleans 15, La *Resident* (4, 1950)
- Steggerda, F R**, Ph D Univ of Illinois, 416 Natural History Bldg, Urbana *Assoc Prof of Physiology* (1, 1934)
- Stehle, Raymond Louis**, Ph D McGill Univ, Faculty of Medicine, Montreal, Quebec, Canada *Prof of Pharmacology* (2, 1920, 3, 1922)
- Steigman, Alex J**, M S, M D Louisville General Hospital, E Chestnut St, Louisville 21, Ky *Consultant Clin Epidemiologist, Nat'l Foundation for Infantile Paralysis* (6, 1949)
- Steigmann, Frederick**, M S, M D 348 S Hamlin Ave, Chicago, Ill *Assoc in Medicine, Univ of Illinois, College of Medicine, Assoc Attending Physician, Cook County Hospital* (3, 1942)
- Steiman, S E**, Ph D, M D 23 Broad St, Lynn, Mass *Asst Physician, Metropolitan State Hospital, Waltham, Mass* (1, 1939)
- Stein, George J**, Ph D 406th Med General Lab APO 500 %P M, San Francisco, Calif *Chief, Section of Bacteriology and Serology* (6, 1947)
- Stein, William Howard**, Ph D Rockefeller Inst for Med Research, 66th and York Ave, New York City 21 *Assoc in Chemistry* (2, 1946)
- Steinbach, H Burr**, Ph D Univ of Minnesota, Dept of Zoology, Minneapolis (1, 1934)
- Steinberg, Bernhard**, M.D Toledo Hospital Inst of Med Research, Toledo, Ohio *Dir of Toledo Hospital Inst, Chief Pathologist and Dir of Labs, Toledo Hospital* (4, 1928, 6, 1946)
- Steiner, Paul E**, M D Univ of Chicago, Chicago, Ill *Prof of Pathology* (4, 1939)
- Steinhardt, Jacinto**, Ph.D 4004 Laird Pl, Chevy Chase 15, Md *Dir, Operations Evaluation Group, Mass Inst of Technology* (2, 1939)
- Steinhaus, Arthur H**, Ph D 5315 Drexel Ave, Chicago, Ill *Prof of Physiology, George Williams College, Hyde Park* (1, 1928)
- Stekol, Jakob A**, D Sc Lankenau Hospital Research Inst, Philadelphia 30, Pa *Assoc Member* (2, 1936)
- Stern, Kurt G**, Ph D Polytechnic Inst of Brooklyn, 99 Livingston St, Brooklyn 2, N Y *Adjunct Prof of Biochemistry* (2, 1938)
- Stetten, DeWitt, Jr**, M D, Ph D Public Health Research Inst of The City of New York, Inc, Foot of E 15th St, New York City 9 *Chief, Div of Nutrition and Physiology* (2, 1944)
- Stetten, Marjorie R**, Ph D Public Health Research Inst of The City of New York, Inc, Foot of E 15th St, New York City 9 *Assoc, Div of Nutrition and Physiology* (2, 1947)
- Stevens, S Smith**, Ph D Harvard Univ, Memorial Hall, Cambridge, Mass *Prof of Psychology, Dir of Psychological Labs* (1, 1937)
- Stevenson, James A F**, M A, M D C.M Univ of Western Ontario, Dept of Med Research,

- London, Ontario, Canada *Assoc Prof, Lecturer in Physiology* (1, 1950)
- Stewart, Dorothy R**, Ph D Rockford College, Rockford, Ill (1, 1947)
- Stewart, Fred W**, M D Memorial Hospital, 444 E 68th St, New York City *Pathologist, Prof of Pathology, Cornell Med School* (4, 1928)
- Stewart, Harold L**, M D Natl Cancer Inst, Bethesda, Md *Chief of Pathology Section* (4, 1936)
- Stewart, Wellington B**, M D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City *Assoc in Pathology* (4, 1950)
- Stewart, Winifred Bayard**, M D, M A 1930 Spruce St, Philadelphia, Pa *Prof of Neurology, Woman's Med College of Pennsylvania* (1, 1941)
- Stuckney, J Clifford**, Ph D West Virginia Univ School of Medicine Morgantown *Assoc Prof of Physiology* (1, 1944)
- Stiebeling, Hazel K**, Ph D U S Dept of Agriculture, Washington, D C *Chief, Bureau of Human Nutrition and Home Economics* (5, 1933)
- Stier, Theodore J B**, Ph D Indiana Univ Med School, Bloomington *Assoc Prof of Physiology* (1, 1938)
- Still, Eugene U**, Ph D Box 991, Sarasota, Fla (1, 1929)
- Stummel, Benjamin F**, Ph D Rees Stealy Med Research Fund, Ltd, 2001 Fourth Ave, San Diego, Calif *Research Biochemist* (2, 1947)
- Stock, Aaron H**, M D Children's Hospital of Pittsburgh 125 De Soto St, Pittsburgh, Pa (6, 1947)
- Stockton, Andrew Benton**, M D 655 Sutter St, San Francisco, Calif *Asst Clin Prof of Medicine* (3, 1931)
- Stoerk, Herbert C**, M D Merck Inst for Therapeutic Research Rahway, N J *Head of Dept of Cancer Research* (4, 1948)
- Stoesser, Albert V**, M D, Ph D Univ of Minnesota Med School, 1409 Willow St, Loring Park, Minneapolis *Clin Assoc Prof of Pediatrics* (6, 1949)
- Stohlman, Edward F**, LL B Natl Insts of Health, Bethesda, Md *Assoc Pharmacologist* (3, 1948)
- Stokinger, Herbert E**, Ph D A E Project, Univ of Rochester, P O Box 287, Station 3, Rochester N Y *Assoc Prof of Pharmacology, Chief of Industrial Hygiene and Toxicology* (3, 1950, 6, 1947)
- Stokstad, E L Robert**, Ph D Lederle Labs, Pearl River, N Y *Asst Dir, Nutrition and Physiology Dept* (2, 1947, 5, 1942)
- Stoland, O O**, Ph D 1845 Learned Ave, Lawrence, Kan *Prof of Physiology and Pharmacology, Univ of Kansas* (1, 1913)
- Stone, Gilbert C H** Ph D City College of New York, Dept of Chemistry, Convent Ave and 140th St, New York City *Asst Prof* (2, 1950)
- Stone, William E**, Ph D Univ of Wisconsin, Dept of Physiology, Madison *Asst Prof of Physiology* (1, 1945)
- Stormont, Robert T**, Ph D, M D American Med Assoc, 535 N Dearborn St, Chicago, Ill *Sec of Council on Pharmacy and Chemistry* (3, 1941)
- Storvick, Clara A**, Ph D Oregon State College, School of Home Economics, Corvallis, Ore *Assoc Prof of Foods and Nutrition* (5, 1947)
- Stotz, Elmer, H**, Ph D Univ of Rochester School of Medicine and Dentistry, Rochester 20, N Y *Prof of Biochemistry* (2, 1939)
- Stoughton, Roger W**, Ph D Mallinckrodt Chemical Works, 3600 N Second St, St Louis, Mo *Research Chemist* (3, 1939)
- Strong, Frank M**, Ph D Univ of Wisconsin, Dept of Biochemistry, Madison 6 *Prof of Biochemistry* (2, 1941)
- Struck, Harold Carl**, Ph D Creighton Univ School of Medicine, 302 N 14th St, Omaha 2, Nebr (1, 1940)
- Stuart, Charles A**, Ph D 372 Lloyd Ave, Providence, R I *Assoc Prof of Biology, Brown Univ* (6, 1935)
- Stumpf, Paul K**, Ph D Univ of California, Div of Plant Biochemistry, 3048 Life Science Bldg, Berkeley 4 *Asst Prof* (2, 1950)
- Sturgis, Cyrus Cressey**, M D Simpson Memorial Inst, Ann Arbor, Mich *Dir of Thomas Henry Simpson Memorial Inst for Med Research, Chairman, Dept of Medicine, Univ of Michigan Hospital* (4, 1927)
- Sturkie, Paul D**, Ph D Rutgers Univ, New Brunswick, N J *Assoc Research Specialist in Poultry Husbandry, Assoc Prof of Poultry Husbandry* (1, 1948)
- Stutzman, Jacob W**, Ph D, M D Boston Univ School of Medicine, Boston, Mass *Assoc Prof of Pharmacology* (1, 1946, 3, 1948)
- Sugg, John Y**, Ph D Cornell Univ Med College, 1300 York Ave, New York City *Assoc Prof of Bacteriology and Immunology* (6, 1938)
- Sulkin, S Edward**, Ph D Southwestern Med College, Dallas, Tex *Prof and Chairman of Dept of Bacteriology and Immunology* (6, 1944)
- Sullivan, Michael Xavier**, Ph D Chemo-Medical Research Inst, Georgetown Univ, 37th & O Sts, N W, Washington, D C *Dir and Research Prof of Chemistry* (2, 1909)
- Sulzberger, Marion B**, M D 999 5th Ave, New York City *Assoc Clin Prof of Dermatology and Syphilology, N Y Post-Grad Med School of Columbia Univ, Assoc Dir of Skin and Cancer Unit of N Y Post-Grad Hospital* (6, 1936)
- Summerson, William H**, Ph D Med Div, Army

- Chemical Center, Md *Chief, Biochemistry Section* (2, 1942)
- Sumner, J B, Ph D Cornell Univ, Ithaca, N Y *Dir, Enzyme Chemistry Lab* (2, 1919)
- Sunderman, F William, M D, Ph D Grady Memorial Hospital, 36 Butler St, S E Atlanta 3, Ga *Dir, Clin Labs, Prof, Clin Medicine, Emory Univ* (2, 1931)
- Sundstroem, Edward S, M D Univ of California, Berkeley 4 *Prof Emeritus of Biochemistry* (2, 1919)
- Sure, Barnett, Ph D Univ of Arkansas, Fayetteville *Head of Dept and Prof of Agricultural Chemistry* (2, 1923, 5, 1933)
- Sutherland, Earl W, Jr, M D Washington Univ School of Medicine, Dept of Biological Chemistry, St Louis 10, Mo *Asst Prof* (2, 1950)
- Sutherland, George F, M D Duke Univ School of Medicine, Durham, N C (1, 1939)
- Sutton, T Scott, Ph D Ohio State Univ, Columbus *Prof, Dir of Inst of Nutrition and Food Technology* (5, 1936)
- Svirbely, Joseph L, Ph D 1002 Fawcett Ave, McKeesport, Pa (3, 1945)
- Swain, Robert E, Ph D 634 Mirada Ave, Stanford Univ, Calif *Prof Emeritus of Chemistry* (2, 1909)
- Swann, Howard G, Ph D Univ of Texas Med School, Dept of Physiology, Galveston *Asst Prof of Physiology* (1, 1940)
- Swanson, Marjorie A, Ph D Bowman Gray School of Medicine, Dept of Biochemistry, Winston-Salem 7, N C *Asst Prof* (2, 1950)
- Swanson, Pearl P, Ph D Iowa State College, Ames *Prof of Foods and Nutrition, Dept of Foods and Nutrition* (5, 1933)
- Swanson, William W, M S, M D 2376 E 71st St, Chicago, Ill *Asst Prof of Pediatrics, Northwestern Univ* (2, 1938)
- Sweeney, H Morrow, Ph D Aero-Med Lab, Wright-Patterson Air Force Base, Dayton, Ohio (1, 1939)
- Sweet, J E, M D, Sc D Unadilla, N Y *Prof Emeritus of Surgical Research, Cornell Univ Med College* (1R, 1913)
- Swendseid, Marian E, Ph D Univ of Michigan, Simpson Memorial Inst, Ann Arbor *Research Biochemist* (2, 1950)
- Swift, Homer, M D, D Sc 888 Park Ave, New York City *Member, Rockefeller Inst for Med Research, Physician to Hospital of Rockefeller Inst for Med Research* (6, 1920)
- Swift, Raymond W, Ph D Pennsylvania State College, State College *Prof and Head of Dept of Animal Nutrition* (5, 1934)
- Swingle, Wilbur Willis, Ph D Princeton Univ, Princeton, N J *Prof of Biology* (1, 1924)
- Swinyard, Ewart A, Ph D Univ of Utah, Salt Lake City *Prof of Pharmacy, School of Pharmacy* (3, 1948)
- Sydenstricker, V P, M D Med College of Georgia, Augusta *Prof of Medicine* (5, 1944)
- Sykes, Joseph F, Ph D U S Dept of Agriculture, Bureau of Dairy Industry, Beltsville, Md *Physiologist* (1, 1942)
- Syvertson, Jerome T, M D Univ of Minnesota, Minneapolis, Minn *Prof and Head, Dept of Bacteriology and Immunology* (4, 1940, 6, 1947)
- Szego, Clara M, Ph D Univ of California, Med School, Los Angeles *Asst Prof of Zoology* (1, 1946)
- Szent-Gyorgyi, Albert, Ph D, M D Natl Insts of Health, Bethesda 14, Md (2, 1949)
- Szepsenwol, Josel, M D Emory Univ School of Medicine, Emory University, Ga *Asst Prof of Anatomy* (1, 1948)
- Tabor, Herbert, M D Natl Insts of Health, Pharmacology Section, Exper Biology and Medicine Inst, Bethesda 14, Md *Surgeon, USPHS* (3, 1947)
- Tager, Morris Western Reserve Univ, Cleveland, Ohio *Dept of Microbiology* (6, 1948)
- Tainter, M L, M A, M D Sterling-Winthrop Research Inst, 33 Riverside Ave, Rensselaer, N Y *Director* (1, 1929, 3, 1927)
- Talbot, Samuel Armstrong, Ph D Johns Hopkins Hospital, Baltimore, Md *Instr in Physiological Optics, Johns Hopkins Univ* (1, 1940)
- Taliaferro, William H, Ph D Univ of Chicago, Dept of Bacteriology, Chicago, Ill *Ehakim H Moore Distinguished Service Prof of Parasitology and Dean of Div of Biological Sciences* (6, 1930)
- Tannenbaum, Albert, M D Michael Reese Hospital, 29th St & Ellis Ave, Chicago, Ill *Dir of Dept of Cancer Research* (4, 1942)
- Tarr, H L A, Ph D Fisheries Exper Station, 898 Richards St, Vancouver, B C, Canada *Sr Bacteriologist, Dept of Bacteriology* (2, 1950)
- Tarver, Harold, Ph D Univ of California, Div of Biochemistry, Berkeley 4 *Asst Prof of Biochemistry* (2, 1947)
- Tashiro, Shiro, Ph D, M D Univ of Cincinnati College of Medicine, Cincinnati, Ohio *Prof of Biochemistry* (1, 1913, 2, 1913)
- Tatum, Arthur L, Ph D, M D Service Memorial Inst, Univ of Wisconsin, Madison *Prof of Pharmacology* (1, 1913, 3, 1919)
- Tatum, Edward L, Ph D Stanford Univ, School of Biological Sciences, Stanford University, Calif *Prof of Biology* (2, 1947)
- Tauber, Henry, Ph D V D Venereal Disease Exper Lab, USPHS, Chapel Hill, N C *Sr Biochemist Assoc Prof of Exper Medicine, School of Public Health Univ of North Carolina* (2, 1933)
- Taylor, A N, Ph D Univ of Oklahoma School

- of Medicine, Oklahoma City *Asst Prof and Chairman of Dept of Physiology* (1, 1948)
- Taylor, Alton R**, Ph D Parke, Davis & Co, Detroit 32, Mich *Sr Researcher, Research Div* (2, 1947)
- Taylor, Craig L**, Ph D Univ of California, Dept of Engineering, Los Angeles *Assoc Prof of Engineering* (1, 1945)
- Taylor, Dermot B**, M B, B Ch, B A O Univ of California Med Center, San Francisco 22 *Assoc Prof of Pharmacology* (3, 1950)
- Taylor, Fred A**, Ph D 320 E North Ave, N S, Pittsburgh, Pa *Biochemist, Singer Memorial Lab* (2, 1933)
- Taylor & Haywood M**, Ph D Duke Univ School of Medicine, Durham, N C *Prof of Toxicology and Assoc Prof of Biochemistry, Biochemist and Toxicologist to Duke Hospital* (4, 1942)
- Taylor, Henry Longstreet**, Ph D Univ of Minnesota, School of Public Health, Minneapolis *Prof of Physiological Hygiene* (1, 1944)
- Taylor, John Fuller**, Ph D Washington Univ School of Medicine, Euclid and Kingshighway, St Louis, Mo *Asst Prof of Biological Chemistry* (2, 1944)
- Taylor, M Wight**, Ph D New Jersey Agricultural Exper Station, New Brunswick *Assoc Biochemist in Nutrition and Assoc Prof of Agricultural Biochemistry, Rutgers Univ* (5, 1944)
- Taylor, Norman Burke**, M D Univ of Toronto, Toronto 5, Ontario, Canada *Prof of Physiology* (1, 1922)
- Taylor, Richard M**, Rockefeller Foundation, 66th St and York Ave, New York City *Member Staff, Internatl Health Div* (6, 1949)
- Taylor, Robert D**, M D Clinical Research Div, Cleveland Foundation, Cleveland 6, Ohio *Member* (1, 1945)
- Teague, Robert S**, Ph D, M D Medical College of Alabama, Dept of Pharmacology, Birmingham 5 *Prof and Chairman of Dept of Pharmacology* (3, 1942)
- Templeton, Roy D**, B S 133 Handley St, San Antonio, Tex (1, 1935)
- Ten Broeck, Carl**, M D 94 Battle Rd, Princeton, N J (4R, 1932)
- Tepley, Lester J**, Ph D Univ of Wisconsin, Enzyme Inst, 1702 University Ave, Madison *Project Assoc* (2, 1950)
- Tepperman, Jay**, M D State Univ of New York School of Medicine, Dept of Pharmacology, Syracuse, N Y *Assoc Prof of Pharmacology* (1, 1944)
- Terplan, Kornel L**, M D Univ of Buffalo School of Medicine, Buffalo, N Y *Prof of Pathology* (4, 1935)
- Terry, Roger**, M D Univ of Rochester School of Medicine and Dentistry, Dept of Pathology, Rochester 7, N Y *Instr in Pathology* (4, 1949)
- Thannhauser, S J**, M D, Ph D Pratt Diagnostic Hospital, 30 Bennet St, Boston, Mass *Assoc Chief, Prof of Clin Medicine, Tufts Med School* (2, 1937)
- Thatcher, Jonathan S**, M D, Ph D State College of Washington, Finch Memorial Hospital, Pullman *Staff Physician* (1, 1949)
- Thauer, Rudolf**, M D 5628 Uber St, Philadelphia 41, Pa *Physiologist, Research Work in N A M C U S Navy* (1, 1949)
- Thayer, Sidney Allen**, Ph D 1402 S Grand Blvd, St Louis 4, Mo *Assoc Prof of Biochemistry, St Louis Univ School of Medicine* (2, 1933)
- Therman, Per-Olof**, M D 111 N 49th St, Philadelphia 39, Pa *Neurophysiologist, Pennsylvania Hospital, Asst Prof, Univ of Pennsylvania* (1, 1950)
- Thienes, Clinton H**, M D, Ph D Univ of Southern California School of Medicine, Los Angeles *Prof and Head of Dept of Pharmacology and Toxicology* (3, 1928)
- Thomas, Arthur W**, Ph D Columbia Univ, New York City 27 *Prof of Chemistry* (2, 1924)
- Thomas, Byron H**, Ph D Iowa State College, Ames *Prof and Head of Animal Chemistry and Nutrition, Iowa Agric Exper Station* (5, 1933)
- Thomas, Caroline Bedell**, M D Johns Hopkins Hospital, Baltimore, Md *Assoc Prof of Medicine, Johns Hopkins Univ School of Medicine* (1, 1939)
- Thomas, J Earl**, M D Jefferson Med College, Philadelphia, Pa *Prof of Physiology* (1, 1922, 3, 1924)
- Thomas, Lewis**, M D Tulane Univ Med School, New Orleans, La *Assoc Prof of Medicine* (6, 1950)
- Thompson, Marvin R**, Ph D 67 Greenwich Ave, Stamford, Conn (3, 1944)
- Thompson, Randall L**, Sc D, M D Indiana Univ, Med Center, Indianapolis 7 *Prof of Bacteriology* (6, 1937)
- Thompson, William R**, Ph D 1 Darroch Rd, Delmar, N Y *Sr Biochemist, Div of Labs and Research, New York State Dept of Health* (2, 1934)
- Thomson, David Landsborough**, Ph D McGill Univ, Montreal, Quebec, Canada *Prof of Biochemistry and Dean of Faculty of Grad Studies and Research* (2, 1929)
- Thorn, George Widmer**, M D Peter Bent Brigham Hospital, Boston, Mass *Prof of Medicine, Harvard Univ* (1, 1939)
- Thorp, W T S**, D V M, M S Natl Insts of Health, Bethesda, Md *Chief, Section on Comparative Pathology and Hematology* (4, 1948)
- Tidwell, Herbert C**, Ph D Southwestern Med

- School, 2211 Oak Lawn Ave, Dallas, Tex *Prof and Chairman of Dept of Biochemistry* (2, 1948)
- Tillett, William S, M D New York Univ College of Medicine, Dept of Bacteriology, 477 First Ave, New York City *Prof of Medicine* (6, 1927)
- Tilt, Jennie, Ph D 1011 Lincoln Blvd, Santa Monica, Calif (5, 1939)
- Tipson, R Stuart, Ph D Mellon Inst of Industrial Research, Univ of Pittsburgh, Pittsburgh, Pa *Sr Fellow, Dept of Research in Pure Chemistry* (2, 1937)
- Tipton, Samuel R, Ph D Univ of Tennessee, Dept of Zoology, Knoxville *Prof of Zoology* (1, 1940)
- Tislow, Richard, M D Schering Corp, Bloomfield, N J *Dir of Biological Labs* (1, 1944, 3, 1950)
- Titus, Harry W, Ph D Lime Crest Research Lab, RFD 1, Newton, N J *Technical Counsellor and Dir of Nutritional Research* (2, 1929, 5, 1933)
- Tobias, Julian M, M D Univ of Chicago, Chicago, Ill *Instr in Physiology* (1, 1944)
- Tocantins, Leandro Maués, M D Jefferson Med College, Philadelphia, Pa *Assoc Prof of Medicine* (1, 1939)
- Todd, Wilbert R, Ph D Univ of Oregon Med School, Portland, Ore *Assoc Prof of Biochemistry* (2, 1948, 5, 1948)
- Todhunter, Elizabeth Neige, Ph D Univ of Alabama, University *Prof of Nutrition* (5, 1939)
- Toennies, Gerrit, Ph D Inst for Cancer Research and Lankenau Hospital Research Inst, Philadelphia 11, Pa *Head, Dept of General Biochemistry* (2, 1934)
- Tolle, Chester D, Ph D Federal Security Agency, Food and Drug Admin, Washington, D C *Sr Biochemist* (5, 1942)
- Toman, James E P, Ph D Abbott Laboratories, Pharmacological Labs, North Chicago, Ill (1, 1945, 3, 1950)
- Tomarelli, Rudolph M, Ph D Wyeth, Inc, Nutritional Div, Mason, Ingham County, Mich *Sr Nutritional Investigator* (2, 1950, 5, 1950)
- Tomlinson, Wray Joseph, M D 1017 E 4th Ave, Mitchell, S Dak *Chief of Labs, St Joseph and Methodist State Hospitals, Consulting Pathologist, St Johns Hospital, Huron* (4, 1945)
- Tompkins, Edna H, M D Cancer Research Inst, New England Deaconess Hospital, 195 Pilgrim Rd, Boston 15, Mass *Research Assoc* (4, 1941)
- Torda, Clara, Ph D, M D Cornell Univ Med College, New York City *Research Assoc in Pharmacology* (1, 1943, 3, 1944)
- Toth, Louis A, Ph D Louisiana State Univ School of Medicine, Dept of Physiology, New Orleans 13 *Assoc Prof of Physiology* (1, 1940)
- Totter, John R, Ph D Univ of Arkansas School of Medicine, Little Rock *Assoc Prof, Dept of Biochemistry* (2, 1946)
- Tourtellotte, Dee, D Sc Charles B Knox Gelatin Co, 4th and Erie Sts, Camden, N J *Head of Nutrition Lab* (5, 1935)
- Tower, Sarah Sheldon, M D, Ph D Johns Hopkins Univ, Baltimore, Md *Asst Prof of Psychiatry* (1, 1932)
- Trager, William, M D, Ph D Rockefeller Inst for Med Research, 66th and York Ave, New York City 21 *Associate* (4, 1947)
- Traub, Frederick B, M D 405 E 72nd St, New York City 28 *Assoc Bacteriologist, Jewish Hospital of Brooklyn* (6, 1946)
- Travell, Janet, M D Cornell Univ Med College, New York City *Asst Prof of Clin Pharmacology* (3, 1933)
- Travis, Lee Edward, Ph D Univ of Southern California, Los Angeles *Prof of Psychology and Dir of Psychological Center, Major, YAAF (Yuma, Ariz)* (1, 1929)
- Treadwell, Carleton R, Ph D George Washington Univ School of Medicine, Dept of Biochemistry, 1335 H St, N W, Washington, D C *Assoc Prof of Biochemistry* (2, 1944, 5, 1949)
- Treffers, Henry P, Ph D Yale Med School, Dept of Immunology, New Haven, Conn *Assoc Prof of Immuno-chemistry* (6, 1942)
- Trimble, Harry C, Ph D Harvard Med School, 25 Shattuck St, Boston, Mass *Asst Prof of Biological Chemistry* (2, 1929, 5, 1936)
- Turnit, Hans Joachim, M D Med Div, Army Chemical Center, Md *Research Biophysicist* (1, 1950)
- Tuba, Jules, Ph D Univ of Alberta, Dept of Biochemistry, Edmonton, Alberta, Canada *Assoc Prof* (2, 1950)
- Tuft, Louis H, M D 1530 Locust St, Philadelphia, Pa *Asst Prof of Medicine, Temple Univ Med School, Chief of Clinic of Allergy and Applied Immunology, Temple Univ Hospital* (6, 1928)
- tum Suden, Caroline, Ph D Med Div, Toxicology, Army Chemical Center, Md (1, 1936)
- Tunturi, Archie Robert, Ph D Univ of Oregon Med School, Portland *Asst Prof of Anatomy* (1, 1946)
- Tuohy, Edward B, M S, M D Georgetown Univ Hospital, 3800 Reservoir Rd, Washington, D C *Prof of Anesthesiology* (3, 1941)
- Turner, Abby H, Ph D Mount Holyoke College, South Hadley, Mass *Prof of Physiology* (1R, 1928)
- Tuttle, Waid Wright, Ph D State Univ of Iowa, Iowa City *Prof of Physiology* (1, 1925)
- Tweedy, Wilbur R, Ph D Veterans Admin Hospital, Radioisotope Unit, Hines, Ill *Assoc Director* (2, 1931)
- Tyler, Albert, Ph D California Inst of Tech-

- nology, Pasadena, Calif *Assoc Prof of Embryology* (6, 1946)
- Tytell, Alfred A, Ph D Univ of Cincinnati College of Medicine, Eden and Bethesda Aves Cincinnati 19, Ohio *Asst Prof of Biological Chemistry* (6, 1950)
- Tyler, David B, Ph D Carnegie Inst of Washington, Dept of Embryology, Wolfe and Madison Sts, Baltimore 5, Md *Member of Staff* (1, 1943)
- Udenfriend, Sidney, Ph D Natl Insts of Health, Natl Heart Inst, Dept of Chemical Pharmacology, Bethesda 14, Md *Biochemist* (2, 1950)
- Umbreit, Wayne W, Ph D Merck Inst for Therapeutic Research, Rahway, N J *Asst Dir* (2, 1947)
- Ungar, Georges, M D, D Sc Rheumatic Fever Research Inst, 3026 S California Ave, Chicago 8, Ill *Research Assoc* (1, 1950)
- Unna, Klaus R W, M D Univ of Illinois College of Medicine, 1853 W Polk St, Chicago 12, Ill *Assoc Prof, Dept of Pharmacology* (1, 1941, 3, 1944, 5, 1942)
- Upton, Morgan, Ph D Rutgers Univ, Dept of Psychology, New Brunswick, N J (1, 1934)
- Urban, Frank, Ph D, M D 302 Northern Bldg, Green Bay, Wis *Physician and Surgeon* (2, 1932)
- Utter, Merton F, Ph D Western Reserve Univ, Dept of Biochemistry, Cleveland, Ohio *Assoc Prof of Physiological Chemistry* (2, 1946)
- Vahlteich, Ella McCollum, Ph D 310 Walnut St, Englewood, N J (5, 1933)
- Valle, J R, M D Escola Paulista de Medicina, Caixa Postal 144-A, Sao Paulo, Brazil *Prof of Pharmacology* (3, 1947)
- Vander Brook, Milton J, Ph D The Upjohn Co, Kalamazoo 99, Mich *Pharmacologist* (3, 1950)
- Vanderscheer, James, Ch E 136 Linwood Ave, Ridgewood, N J *Research Chemist, Lederle Labs* (6, 1946)
- Van Dyke, H B, Ph D, M D Columbia Univ College of Physicians and Surgeons, 630 W 168th St New York City 32 *Hosack Prof of Pharmacology* (1, 1925, 3, 1927)
- van Harrevelt, Anthonie, M A, M D California Inst of Technology, Pasadena *Prof of Physiology* (1, 1941)
- Van Liere, Edward J, M D, Ph D West Virginia Univ School of Medicine, Morgantown *Prof of Physiology and Dean* (1, 1927)
- Van Middlesworth, Lester, Ph D Univ of Tennessee, Memphis *Instr in Physiology* (1, 1948)
- Van Slyke, Donald D, Ph D, M D Brookhaven Natl Lab, Upton, L I, N Y *Asst Dir, Depts of Biology and Med Sciences, Member Emeritus, Rockefeller Inst* (2, 1908)
- van Wagenen, Gertrude, Ph D Yale Univ School of Medicine, New Haven, Conn *Assoc Prof* (1, 1932)
- van Wagtendonk, Willem J, Ph D Indiana Univ Dept of Zoology, Bloomington *Assoc Prof* (2, 1946)
- Van Winkle, Walton, Jr, M D American Med Assoc, 535 N Dearborn St, Chicago 10, Ill *Sec'y, Therapeutic Trials Committee* (3, 1939)
- Varney, Philip L Washington Univ School of Medicine, St Louis, Mo *Asst Prof of Bacteriology* (6, 1948)
- Vars, Harry M, Ph D Univ of Pennsylvania Med School, Harrison Dept of Surgical Research, Philadelphia *Assoc Prof of Physiological Chemistry* (2, 1935, 5, 1935)
- Velick, Sidney Frederick Washington Univ School of Medicine, Dept of Biochemistry, Euclid Ave and Kingshighway, St Louis 10, Mo *Asst Prof of Biochemistry* (2, 1946)
- Vennesland, Birgit, Ph D Univ of Chicago, Dept of Biochemistry, Chicago, Ill *Assoc Prof* (2, 1944)
- Venning, Eleanor H., Ph D Univ Clinic, Royal Victoria Hospital, Pine Ave, Montreal, Quebec, Canada *Asst Prof of Medicine, McGill Univ* (2, 1938)
- Vestling, Carl Swensson, Ph D Univ of Illinois, Noyes Lab, Urbana, Ill *Asst Prof of Biochemistry* (2, 1946)
- Vickery, Hubert B, Ph D Connecticut Agric Exper Station, New Haven *Biochemist in Charge, Dept of Biochemistry, Lecturer in Physiological Chemistry, Yale Univ* (2, 1923)
- Victor, Joseph, M D Camp Detrick, Frederick, Md *Chief, Pathology Branch* (4, 1935)
- Villee, Claude A, Jr, Ph D Harvard Med School, 25 Shattuck St, Boston 15, Mass *Asst Prof of Biological Chemistry* (2, 1948)
- Virtue Robert W, Ph D, M D Denver General Hospital, Denver 4, Colo *Anesthesiologist* (2, 1939)
- Visscher, Frank E, Ph D Upjohn Co, Kalamazoo 99, Mich *Research Scientist, Dept of Pharmacology and Endocrinology* (1, 1947)
- Visscher, Maurice B, Ph D, M D Univ of Minnesota, Minneapolis *Prof and Head of Dept of Physiology* (1, 1927)
- Voegtlin, Carl, Ph D Univ of Rochester Med School, Rochester, N Y *Lecturer in Pharmacology* (1R, 1908, 2, 1908, 3, 1908)
- von Haam, Emmerich, M D Ohio State Univ, Columbus *Prof of Pathology* (4, 1938)
- Von Oettingen, W F, M D, Ph D Natl Insts of Health, Lab of Physical Biology, Exper Biology and Medicine Inst, Bethesda, Md *Med Officer, Toxicology* (3, 1925)
- Voris, LeRoy, Ph D Natl Research Council, 2101 Constitution Ave, Washington 25, D C *Exec Sec, Food and Nutrition Board* (5, 1949)
- Vorwald, Arthur J, Ph D, M D Saranac Lake,

- N Y Dir, Edward L Trudeau Foundation and Saranac Lab (4, 1937)
- Vos, Bert J, Ph D, M D Food and Drug Admin, Div of Pharmacology, Washington, D C Pharmacologist (3, 1941)
- Wachstein, Max, M D St Catherine's Hospital, Brooklyn, N Y Dir of Lab, Research Asst, Mt Sinai Hospital (4, 1947)
- Waddell, James, Ph D E I duPont de Nemours & Co, New Brunswick, N J Dir of Biological Lab (2, 1930, 5, 1935)
- Wadsworth, Augustus B, M D Manchester, Vt (4, 1935, 6, 1920)
- Waelsch, Heinrich, M D, Ph D 722 West 168th St, New York City 32 Assoc Research Biochemist, N Y State Psychiatric Inst and Hospital, Assoc Prof of Biochemistry, Columbia Univ (2, 1941)
- Wagman, Irving H, Ph D Jefferson Med College, Dept of Physiology, Philadelphia 7, Pa Asst Prof of Physiology (1, 1946)
- Wainio, Walter W, Ph D Rutgers Univ, Bureau of Biological Research, Box 515, New Brunswick, N J Assoc Research Specialist (2, 1950)
- Waisman, Harry A, M D, Ph D Univ of Illinois Med School, 1819 W Polk St, Chicago 12 Asst Prof of Pediatrics (2, 1944)
- Wakeman, Alfred J, Ph D Hatfield Hill Rd, Bethany, Conn Retired (2, 1906)
- Wakerlin, George E, Ph D, M D Univ of Illinois Med School, 1853 W Polk St, Chicago Prof and Head of Dept of Physiology (1, 1933, 3, 1934)
- Wakim, Khalil G, M D, Ph D Mayo Clinic, Rochester, Minn Consultant, Prof of Physiology, Mayo Foundation (1, 1942)
- Waksman, Byron H, M D 52 Phillips St, Boston 14, Mass Research Fellow, Massachusetts General Hospital, Teaching appointment, Harvard Med School (6, 1950)
- Walcott, William W, Ph D Columbia Univ College of Physicians and Surgeons, Dept of Physiology, New York City 32 Instructor (1, 1947)
- Wald, George, Ph D Harvard Univ, Biological Labs, Cambridge, Mass (1, 1934, 2, 1950)
- Walker, A Earl, M D 601 N Broadway, Baltimore 5, Md Prof of Neural Surgery, The Johns Hopkins Univ (1, 1950)
- Walker, Arthur M, M D Veterans Admin, Washington 25, D C (1, 1932, 3, 1939)
- Walker, Burnham S, Ph D, M D Boston Univ School of Medicine, 80 E Concord St, Boston, Mass Prof of Biochemistry (2, 1940)
- Walker, Ernest Linwood, Sc D 50 Winchester Dr, Menlo Pk, Calif (3R, 1931)
- Walker, Harry A, Ph D Emory Univ School of Medicine, Emory University, Ga Asst Prof of Pharmacology (3, 1948)
- Walker, Sheppard M, Ph D Univ of Louisville School of Medicine, 101 W Chestnut St, Louisville, Ky Asst Prof of Physiology (1, 1946)
- Wallen-Lawrence, Zonja, Ph D 4534 W Pine Blvd, St Louis 8, Mo (2, 1937)
- Walter, Annabel W 29 Perry St, New York City 14 Bacteriologist, New York City Dept of Health, Bureau of Labs (6, 1946)
- Walter, Carl W, M D Harvard Med School, 25 Shattuck Street, Boston, Mass Asst Clin Prof of Surgery, Sr Assoc in Surgery, Peter Bent Brigham Hosp (4, 1942)
- Walters, Orville S, Ph D, M D McPherson, Kan Physician (1, 1936)
- Walton, Robert P, Ph D, M D Med College of the State of South Carolina, Charleston Prof of Pharmacology (3, 1933)
- Walton, Seth T, V M D, Ph D Lab, Veterans Hospital, Oteen, N C Dir of Labs and Research (6, 1936)
- Walzer, Matthew, M D 20 Plaza St, Brooklyn, N Y Attending in Allergy, Jewish Hospital of Brooklyn (6, 1924)
- Wang, Chi Che, Ph D U S Veterans Admin Hospital, Hines, Ill Research Biochemist in charge of Metabolic Lab, Assoc Prof of Dept of Exper Medicine, Northwestern Univ Med School, Chicago, Ill (2, 1922, 5, 1933)
- Wang, Shih-Chun, M D, Ph D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 Assoc Prof of Physiology (1, 1943)
- Wangeman, Clayton P, M D Broadway Med Center, Associated Anesthesiologists, Seattle, Washington (3, 1946)
- Wangensteen, Owen Harding, M D Univ Hospital, Minneapolis 14, Minn Prof of Surgery, Univ of Minnesota (1, 1947, 4, 1931)
- Ward, Arthur A, Jr, M D Univ of Washington, School of Medicine, Div of Neurosurgery, Seattle Asst Prof of Surgery, Head of Div (1, 1949)
- Ward, Walter E, Ph D, M D Cutter Labs Fourth and Parker Sts, Berkeley 1, Calif Assoc Med Dir (6, 1947)
- Ware, Arnold G, Ph D Los Angeles County Hospital, Main Lab, 1200 N State St, Los Angeles 33, Calif Head Chemist, Asst Prof of Biochemistry, Univ of Southern California (2, 1949)
- Warner, Emory D, M D Med Labs Bldg, Iowa City, Ia Prof of Pathology (4, 1937)
- Warner, Robert C, Ph D New York Univ College of Medicine, 477 First Ave, New York City 16 Asst Prof of Chemistry (2, 1946)
- Warren, James V, M D Emory Univ School of Medicine, Atlanta, Ga Prof of Physiology, Assoc Prof of Medicine (1, 1947)
- Warren, Joel 7415 Lannhurst St, Chevy Chase, Md Chief, Virus Research Section, Army Med Dept Research and Grad School (6, 1949)

- Warren, Marshall R, Ph D Univ of Tennessee College of Medicine, Memphis *Assoc Prof of Pharmacology* (3, 1948)
- Warren, Shields, M D 195 Pilgrim Rd, Boston, Mass *Prof of Pathology, Harvard Med School, Dir, Div of Biology and Medicine, USAEC* (4, 1929)
- Wartman, William Bechmann, M D Northwestern Univ, 303 East Chicago Ave, Chicago 11, Ill *Morrison Prof and Chairman of Dept of Pathology* (4, 1940)
- Wasteneys, Hardolph, Ph D Univ of Toronto, Toronto, Ontario, Canada *Prof and Head of Dept of Biochemistry* (2, 1915)
- Waterman, Robert E, B S Schering Corp, 86 Orange St, Bloomfield, N J *Vice-Pres* (2, 1940)
- Waters, Ralph Milton, M D R R #3, Box 456, Orlando, Fla (3, 1937)
- Watson, Cecil J, M D, Ph D University Hospital, Dept of Medicine, Minneapolis, Minn *Prof and Head of Dept of Medicine* (4, 1941)
- Watson, Dennis W Univ of Minnesota Med School, Dept of Bacteriology and Immunology, Minneapolis *Assoc Prof* (6, 1949)
- Watson, John B, Ph D Box 526, Westport, Conn (1, 1907)
- Watts, Daniel T, Ph D Univ of Virginia Med School, Dept of Pharmacology, Charlottesville *Assoc Prof of Pharmacology* (3, 1950)
- Waud, Russell A, M D, Ph D Univ of Western Ontario Med School, London, Ontario, Canada *Prof and Head of Dept of Pharmacology* (3, 1931)
- Waugh, David F, Ph D Massachusetts Inst of Technology, Dept of Biology and Biological Engineering, Cambridge *Asst Prof of Physical Biology* (1, 1943)
- Way, E Leong, Ph D Univ of California Med Center, San Francisco, Calif *Asst Prof of Pharmacology* (3, 1947)
- Wearn, Joseph T, M D Lakeside Hospital, Cleveland Ohio *Dir of Medicine, Prof of Medicine, Western Reserve Univ* (1, 1921)
- Weatherby, J H, Ph D Med College of Virginia, Dept of Physiology and Pharmacology, Richmond, Va *Assoc Prof of Pharmacology* (3, 1941)
- Weber, Clarence J, M D, Ph D 5641 Tahoe Lane, Kansas City 3, Kans *Chief, Lab Service, V A Hospital, Wadsworth* (2, 1931)
- Webster, Bruce, M D, C M Cornell Univ Med College, 1300 York Ave, New York City *Asst Prof of Medicine, Assoc Attending Physician, New York Hospital* (5, 1935)
- Weed, Lewis H, M D, Ph D Natl Research Council, 2101 Constitution Ave, Washington, D C (1R, 1919)
- Wégria, René, M D Presbyterian Hospital, Dept of Medicine, 622 W 168th St, New York City *Asst Prof of Medicine* (1, 1941, 3, 1950)
- Weichert, Charles K, Ph D Univ of Cincinnati, Cincinnati, Ohio *Prof of Zoology* (1, 1935)
- Weichselbaum, Theodore E, Ph D Washington Univ School of Medicine, Dept of Surgery, 4580 Scott Ave, St Louis 10, Mo *Research Assoc* (2, 1950)
- Weil, Alfred J, M D The Bronx Hospital, New York City *Dir of Dept of Bacteriology* (6, 1940)
- Weil, Arthur, M D 952 5th Ave, New York City (4, 1940)
- Weil, Leopold, Ph D U S Dept of Agriculture, Eastern Regional Research Lab, Chestnut Hill Station, Philadelphia 18, Pa *Chemist* (2, 1942)
- Weil, Marvin Lee, M D Army Med Dept Research and Grad School, Army Med Center, Washington, D C *Captain, Med Corps* (6, 1950)
- Weinhouse, Sidney, Ph D Lankenau Hospital Research Inst, Fox Chase, Philadelphia 11, Pa *Head, Metabolic Chemistry* (2, 1948)
- Weir, Everett G, Ph D 231 S 13th Ave, Maywood, Ill (1, 1941)
- Weiser, Russell S, Ph D Univ of Washington School of Medicine, Seattle 5, Washington *Assoc Prof of Microbiology* (6, 1948)
- Weiss, Charles, Ph D, M D Jewish Hospital, York and Tabor Rds, Philadelphia, Pa *Dir of Labs* (4, 1934, 6, 1920)
- Weiss, Emil, M D, Ph D 5036 Bernard St, Chicago, Ill *Pathologist, People's Hospital* (6, 1927)
- Weiss, Paul, Ph D Univ of Chicago, Chicago, Ill *Prof of Zoology* (1, 1936)
- Welch, Arnold D, Ph D, M D Western Reserve Univ School of Medicine, Cleveland, Ohio *Prof and Dir of Dept of Pharmacology* (2, 1950, 3, 1942, 5, 1944)
- Welch, Henry, Ph D U S Food and Drug Admin, Rm 6171 S Agriculture Bldg, Washington, D C, *Chief of Div of Penicillin Control and Immunology* (6, 1932)
- Weld, Charles Beecher, M A, M D Dalhousie Univ, Halifax, N S, Canada *Prof of Physiology* (1, 1936)
- Weld, Mrs Julia T Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City *Research Assoc in Pathology* (6, 1920)
- Welker, William H, Ph D 534 N Elmwood Ave, Oak Park, Ill *Prof Emeritus of Biological Chemistry, Univ of Illinois College of Medicine* (2, 1906)
- Weller, Carl Vernon, M D 1130 Fair Oaks Parkway, Ann Arbor, Mich *Prof and Chairman of Dept of Pathology, Univ of Michigan* (4, 1923)

- Wells, Herbert S , M D Univ of Minnesota, Minneapolis 14 *Prof of Clin Physiology* (1, 1932)
- Wells, Joseph Albert, M D , Ph D Northwestern Univ Med School, Chicago, Ill *Assoc Prof of Pharmacology* (3, 1944)
- Welsh, John H , Ph D Harvard Univ , Biological Labs , 16 Divinity Ave , Cambridge 38, Mass *Assoc Prof of Zoology* (1, 1945)
- Wendel, William B , Ph D Tulane Univ School of Medicine, 6501 St Charles Ave , New Orleans 15, La *Prof and Head of Dept of Biochemistry* (2, 1932)
- Werber, Erna A , Ph D 44 W 83rd St , New York City *Dir of Research Lab , Jewish Hospital of Brooklyn* (6, 1948)
- Werkman, C H , Ph D Iowa State College, Science Hall, Ames *Prof and Head of Dept of Bacteriology* (2, 1942)
- Werle, Jacob M , M D 2000 W 25th St , Cleveland 13, Ohio *Surgeon* (1, 1943)
- Werner, Harold W , Ph D The Wm S Merrell Co , Lockland Station, Cincinnati, Ohio *Dir Pharmacology Research* (3, 1942)
- Wertenberger, Grace E , Ph D Univ of Indiana, Dept of Physiology, Bloomington (1, 1943)
- Werthessen, Nicholas T , Ph D Shrewsbury, Mass Worcester Foundation for Exper Biology *Sr Fellow* (1, 1946)
- Wertz, Anne W , Ph D Univ of Massachusetts, Nutrition Research Labs , Home Economics Dept , Amherst *Prof , Research, Home Economics Nutrition* (5, 1950)
- Wescoe, W Clarke, M D Cornell Univ Med College, 1300 York Ave , New York City 21 *Asst Prof of Pharmacology* (3, 1949)
- Wesson, Laurence G , Ph D Forsyth Dental Infirmary, Boston, Mass *Research Biochemist* (2, 1929, 3, 1932)
- Wesson, Laurence G , Jr , Ph D New York Univ College of Medicine, Dept of Physiology, New York City 16 *Asst Prof* (1, 1949)
- West, Edward S , Ph D Univ of Oregon Med School, Portland *Prof of Biochemistry* (2, 1925)
- West, Harold D , Ph D Meharry Med College, Nashville 8, Tenn *Prof and Head of Dept of Biochemistry* (2, 1946)
- Westerfeld, Wilfred Wiedey, Ph D State Univ of New York, Med Center at Syracuse Univ , Syracuse 10, N Y *Prof of Biochemistry* (2, 1944)
- Westfall, B A , Ph D Univ of Missouri School of Medicine, Dept of Physiology and Pharmacology, Columbia *Prof of Pharmacology* (1, 1949, 3, 1950)
- Weston, Raymond E , M D , Ph D Montefiore Hospital, Med Div , New York City 67 *Asst in Medicine* (1, 1947)
- Weymouth, Frank W , Ph D Stanford Univ , Calif *Prof and Exec of Dept of Physiology* (1R, 1917)
- Wheeler, Albert H , M S , M S P H , Dr P H Univ of Michigan Hospital, Serology Lab , Ann Arbor *Research Assoc* (6, 1950)
- Wheeler, George W , M D New York Hospital, 525 E 68th St , New York City *Asst Dir* (6, 1920)
- Wheeler, Mary W , M A New York State Dept of Health, Div of Labs and Research, Albany *Assoc Bacteriologist* (6, 1933)
- Wheelon, Homer, M S , M D Univ of Washington, Seattle, Wash *Prof of Medicine* (1, 1919)
- Whipple, George H , M D , Sc D Univ of Rochester, Rochester, N Y *Prof of Pathology and Dean of School of Medicine and Dentistry* (1, 1911, 4, 1913)
- White, Abraham, Ph D Univ of California School of Medicine, Los Angeles 24 *Prof and Chairman of Dept of Physiological Chemistry* (2, 1934, 5, 1937)
- White, Alan G C , Ph D Tulane Univ , Dept of Biochemistry, New Orleans, La *Asst Prof of Biochemistry* (2, 1949)
- White, Colin, B S Med School, Hospital Centre, Dept of Physiology, Birmingham 15, England (1, 1949)
- White, Florence R , Ph D % Dr Julius White, Natl Insts of Health, Natl Cancer Inst , Bethesda 14, Md *Biochemist* (2, 1946)
- White, Frank D , Ph D Univ of Manitoba, Faculty of Medicine, Bannatyne Ave , Winnipeg, Manitoba, Canada *Prof of Biochemistry, Biochemist, Winnipeg General Hospital* (2, 1931)
- White, Harvey Lester, M D Washington Univ Med School, St Louis 10, Mo *Prof of Physiology* (1, 1923)
- White, Julius, Ph D Natl Insts of Health, Natl Cancer Inst , Bethesda 14, Md *Head Chemist* (2, 1937)
- White, Paul Dudley, M D Massachusetts General Hospital, Boston, Mass *Physician (in charge of Cardiac Clinics and Lab), Lecturer in Medicine, Harvard Med School* (3, 1921)
- Whitehead, Richard W , M A , M D Univ of Colorado School of Medicine, 4200 E Ninth Ave , Denver *Prof of Physiology and Pharmacology* (1, 1933, 3, 1928)
- Whitehorn, William V , M D Univ of Illinois College of Medicine, Chicago *Asst Prof of Applied Physiology* (1, 1947)
- Whittenberger, James L , M D Harvard School of Public Health, Dept of Physiology, 55 Shattuck St , Boston 15, Mass *Asst Prof and Head of Dept of Physiology* (1, 1949)
- Wiener, Alexander S , M D 64 Rutland Rd Brooklyn, N Y *Bacteriologist and Serologist to Office of Chief Med Examiner of New York*

- City, Head of Transfusion Div, Jewish Hospital of Brooklyn* (6, 1932)
- Wiercinski, Floyd J**, Ph D Hahnemann Med College, 235 N 15th St, Philadelphia, Pa *Asst Prof in Physiology* (1, 1950)
- Wiersma, Cornelis A G**, Ph D California Inst of Technology, Pasadena *Assoc Prof of Physiology* (1, 1941)
- Wiese, Alvin C**, Ph D Univ of Idaho, Dept of Agricultural Chemistry, Moscow *Prof and Head of Dept* (5, 1950)
- Wiggers, Carl J**, M D, Sc D Western Reserve Univ Med School, Cleveland, Ohio *Prof and Dir of Physiology* (1, 1907, 3R, 1909)
- Wiggers, Harold C**, Ph D Union Univ, Albany Med College, Dept of Physiology and Pharmacology Albany 3, N Y *Prof and Chairman of Dept* (1, 1938)
- Wigodsky, Herman S**, Ph D, M D 420 E Houston St, San Antonio 5, Tex (1, 1943)
- Wikler, Abraham**, M D USPHS Hospital, Lexington, Ky *Sr Surgeon (R)* (3, 1944)
- Wilber, Charles G**, Ph D St Louis Univ, 1402 S Grand Blvd, St Louis 4, Mo *Dir of Biological Labs* (1, 1947)
- Wilde, Walter S**, Ph D Tulane Univ School of Medicine, Station 20, New Orleans, La *Assoc Prof of Physiology* (1, 1944)
- Wilder, Russell M**, Ph D, M D Mayo Clinic, Rochester, Minn *Prof of Medicine, Mayo Foundation, Univ of Minnesota* (1, 1921, 4R, 1924, 5, 1933)
- Wiley, Frank H**, Ph D Federal Security Agency, Food and Drug Admin, Washington 25, D C *Chief Div of Pharmaceutical Chemistry* (2, 1933)
- Wilhelmi, Alfred E**, Ph D Emory Univ School of Medicine, Emory Univ, Ga *Prof of Biochemistry* (2, 1942)
- Wilhelmj, Charles Martel**, M D Creighton Univ School of Medicine, Omaha, Neb *Prof of Physiology* (1, 1931)
- Wilkerson, Vernon A**, M D, Ph D Med Arts Bldg, 61 K St, N W, Washington, D C *Biochemical Consultant* (2, 1936)
- Williams, Carroll M**, Ph D, M D Harvard Univ, Biological Labs, Cambridge, Mass *Asst Prof of Zoology* (1, 1947)
- Williams, Harold H**, Ph D Cornell Univ, Fernow Hall, Ithaca, N Y *Prof of Biochemistry* (2, 1938, 5, 1936)
- Williams, Horatio B**, M D, Sc D Box 893, Greenwich, Conn *Dalton Prof Emeritus of Physiology, Columbia Univ* (1, 1912)
- Williams, J W**, Ph D Univ of Wisconsin, Chemistry Bldg, Madison *Prof of Chemistry* (2, 1944)
- Williams, Ray D**, M S, M D 6834 Waterman St, St Louis, Mo *Asst Prof of Clin Medicine, Washington Univ* (5, 1941)
- Williams, Robert Hardin**, M D Univ of Washington, Dept of Medicine, Seattle *Prof and Exec Officer* (4, 1940)
- Williams, Robert R**, D Sc 297 Summit Ave, Summit, N J *Dir of Grants, Research Corp* (2, 1919, 5, 1941)
- Williams, Roger J**, Ph D Univ of Texas, Dept of Chemistry, Austin *Prof of Chemistry, Dir of Biochemical Inst* (2, 1931)
- Williams, W Lane**, Ph D Univ of Minnesota Med School, Minneapolis 14 *Assoc Prof of Anatomy* (1, 4, 1947)
- Wills, J H**, Ph D Med Div, Pharmacology Section, Army Chemical Center, Md (1, 1943)
- Wilson, Armine T Alfred I DuPont Inst**, Wilmington 99, Del *Chief Bacteriologist, Pediatrician-in-Chief* (6, 1949)
- Wilson, David Wright**, Ph D Univ of Pennsylvania Med School, Philadelphia *Benjamin Rush Prof of Physiological Chemistry* (1, 1915, 2, 1915)
- Wilson, Eva D**, Ph D 256 E Irvin Ave, State College, Pa (5, 1947)
- Wilson, Frank N**, M D Univ Hospital, Ann Arbor, Mich *Prof of Medicine, Univ of Michigan* (4, 1925)
- Wilson, J Walter**, Ph D Brown Univ, Dept of Biology, Providence 12, R I *Chairman of Dept of Biology, F L Day Prof of Biology* (1, 1949, 4, 1950)
- Wilson, John W**, Ph D Wright-Patterson Air Force, Aero-Med Lab, Dayton, Ohio *Research Physiologist* (1, 1948)
- Wilson, Karl M**, M D Univ of Rochester School of Medicine, Rochester, N Y *Prof of Obstetrics and Gynecology* (4, 1927)
- Wilson, P W**, Ph D Univ of Wisconsin, Dept of Agricultural Bacteriology, Madison *Prof of Agricultural Bacteriology* (2, 1939)
- Wilson, Robert H**, Ph D U S Dept of Agriculture, Western Regional Research Lab, 800 Buchanan St, Albany, Calif *Sr Pharmacologist* (3, 1937)
- Winder, Claude V**, Sc D Parke Davis and Co, Detroit, Mich *Research Pharmacologist* (1, 1938, 3, 1948)
- Windle, William Frederick**, Ph D Univ of Pennsylvania Med School, Philadelphia *Prof of Anatomy* (1, 1937)
- Winkenwerder, Walter LaF**, M D 1014 St Paul St, Baltimore, Md *Assoc in Medicine, Johns Hopkins Med School* (6, 1938)
- Winnick, Theodore**, Ph D Univ of Iowa Med School, Iowa City *Assoc Prof of Biochemistry, Radiation Research Lab* (2, 1946)
- Winter, Charles A**, Ph D Merck Inst for Therapeutic Research, Rahway, N J *Research Assoc* (1, 1940)
- Winter, Irwin Clinton**, Ph D, M D G D Searle

- and Co , P O Box 5110, Chicago 80, Ill *Dir of Clin Research* (3, 1941)
- Winternitz, M C , M D Natl Research Council, 2101 Constitution Ave , Washington 25, D C *Chairman, Div of Med Sciences* (4, 1913)
- Wintersteiner, Oskar, Ph D Squibb Inst for Med Research, New Brunswick, N J *Member, Head, Div of Organic Chemistry* (2, 1930)
- Wintrobe, Maxwell Myer, M D , Ph D 175 E 21st St , S , Salt Lake City 15, Utah *Prof and Head, Dept of Internal Medicine, Univ of Utah College of Medicine, Physician-in-Chief, Salt Lake County General Hospital* (4, 1940)
- Winzler, Richard J , Ph D Univ of Southern California Med School, Dept of Biochemistry, Los Angeles 7 *Professor* (2, 1946)
- Wiseman, Bruce Kenneth, M D Ohio State Univ , Kinsman Hall, Columbus *Prof and Chairman of Dept of Medicine, Asst Dir of Med Research* (4, 1932)
- Wislocki, George B , M D Harvard Univ Med School, 25 Shattuck St , Boston, Mass *Parkman Prof of Anatomy* (1, 1924)
- Wissler, Robert W , M D , Ph D Albert Merritt Billings Hospital, 950 E 59th St , Chicago 37, Ill *Asst Prof of Pathology, Univ of Chicago* (4, 1949)
- Witebsky, Ernest, M D Buffalo General Hospital, 100 High St , Buffalo, N Y *Prof and Head of Dept of Bacteriology and Immunology* (6, 1935)
- Witlich, Fred W , M D 401 LaSalle Med Bldg , Minneapolis 2, Minn *Sec-Treas of American College of Allergists, Chairman of Exec Committee, International Assoc of Allergists* (6, 1944)
- Wolbach, S Burt, M D Children's Hospital, Boston, Mass *Shattuck Prof Emeritus of Pathological Anatomy, Harvard Med School, Dir of Nutritional Research, Children's Hospital* (4, prior to 1920)
- Wolf, Abner, M D Columbia Univ College of Physicians and Surgeons, New York City 32 *Assoc Prof of Neuropathology* (4, 1948)
- Wolf, Arnold Veryl, Ph D Union Univ , Albany Med College, Albany, N Y *Assoc Prof of Physiology* (1, 1946)
- Wolf, Stewart, M D Cornell Univ Med College, New York City *Asst Prof of Medicine* (1, 1948)
- Wolff, Harold G , M D , M A New York Hospital, 525 E 68th St , New York City *Assoc Prof of Medicine, Cornell Univ Med College, Assoc Attending Physician, New York Hospital* (1, 1930, 3, 1942)
- Wolff, William A , Ph D Wake Forest College, Bowman Gray School of Medicine, Winston-Salem 7, N C *Asst Prof of Biochemistry* (2, 1947)
- Womack, Madelyn, Ph D U S Dept of Agriculture, Agricultural Research Admin , Foods and Nutrition Div , Washington 25, D C *Biochemist* (5, 1947)
- Wood, Earl H , Ph D , M D Mayo Foundation, Rochester, Minn *Assoc Prof of Physiology, Grad School, Univ of Minnesota, Consultant in Physiology, Mayo Clinic* (1, 1943, 3, 1949)
- Wood, Harland G , Ph D Western Reserve Univ , Dept of Biochemistry, Cleveland 6, Ohio *Prof of Biochemistry* (2, 1944)
- Wood, Horatio C , Jr , M D , Ph M 319 S 41st St , Philadelphia 4, Pa *Prof of Pharmacology, Philadelphia College of Pharmacy and Science* (3R, 1908)
- Wood, John L , Ph D Univ of Tennessee School of Biological Sciences, 875 Monroe Ave , Memphis 3 *Assoc Prof of Chemistry* (2, 1947)
- Woodbury, Robert A , Ph D , M D Univ of Tennessee, Dept of Pharmacology, Memphis *Prof and Chief of Div of Pharmacology* (1, 1936, 3, 1941)
- Woods, Alan C , M D Johns Hopkins Hospital, Wilmer Ophthalmological Inst , Baltimore, Md *Dir of Inst and Ophthalmologist-in-Chief, Acting Prof of Ophthalmology, Johns Hopkins Univ* (6, 1918)
- Woods, Ella, Ph D Univ of Idaho, Moscow *Home Economist, Exper Station* (2, 1925, 5, 1933)
- Woods, Lauren A , M D , Ph D Univ of Michigan, Dept of Pharmacology, Ann Arbor *Asst Prof* (3, 1949)
- Woodward, Alvalyn E , Ph D Univ of Michigan, Ann Arbor *Asst Prof of Zoology* (1, 1932)
- Woodyatt, Rollin T , M D 237 E Delaware Place, Chicago, Ill *Prof of Medicine, Rush Med College, Univ of Chicago* (2, 1912)
- Wooley, Jerald G Natl Insts of Health, Exper Biology and Medicine Inst , Bethesda, Md *Sr Biologist* (6, 1949)
- Woolley, D Wayne, Ph D Rockefeller Inst for Med Research, 66th St and York Ave , New York City *Member* (2, 1946, 5, 1941)
- Woolpert, Oram C , M D , Ph D Camp Detrick, Frederick, Md *Tech Dir of Biological Div , Chemical Corps* (6, 1947)
- Woolsey, Clinton N , M D Univ of Wisconsin, Service Memorial Insts , Madison *Charles Sumner Slichter Research Prof of Neurophysiology* (1, 1938)
- Wortis, S Bernard, M D New York Univ College of Medicine, Dept of Psychiatry, New York City 16 *Prof and Chairman of Dept of Psychiatry, Dir of Psychiatric Div of Bellevue Hospital* (1, 1947)
- Wright, Angus, M D Univ of Southern California Med School, 657 S Westlake Ave , Los Angeles *Pathologist, California Hospital* (4, 1935)
- Wright, Arthur W , M D Albany Med College, New Scotland Ave , Albany, N Y *Prof of Pathology and Bacteriology* (4, 1941)

- Wright, Charles Ingham, Ph D Natl Insts of Health, Bethesda, Md *Sr Pharmacologist, USPHS* (1, 1935, 3, 1936)
- Wright, George G, Ph D Camp Detrick, Frederick, Md *Chief, Special Procedures Branch, S-Div* (6, 1943)
- Wright, Harold N, Ph D Univ of Minnesota, Minneapolis *Prof of Pharmacology* (3, 1931)
- Wright, Lemuel D, Ph D Sharp and Dohme, Inc, Med Research Div, Glenolden, Pa, *Dir of Nutritional Research* (2, 1946, 5, 1946)
- Wright, Paul A, Ph D Univ of Michigan, Dept of Zoology, Ann Arbor *Asst Prof* (1, 1950)
- Wright, Sydney L, Ph D Endsmeet Farm, Wyncote, Pa (2, 1933)
- Wu, Hsein, Ph D Med College of Alabama, Birmingham 5 *Visiting Prof of Biochemistry* (2, 1950)
- Wulff, V J, Ph D Univ of Illinois, Dept of Zoology and Physiology, Urbana *Asst Prof of Physiology, Special Research Asst* (1, 1949)
- Wulzen, Rosalind, Ph D Oregon State College, Corvallis *Asst Prof of Zoology* (1R, 1916)
- Wyckoff, Ralph W G, Ph D USPHS, Natl Insts of Health, Bethesda, Md *Sr Scientist* (6, 1940)
- Wyman, Jeffries, Jr, Ph D Harvard Univ, Biological Labs, Cambridge, Mass *Assoc Prof of Zoology* (1, 1928)
- Wyman, Leland C, Ph D Boston Univ, College of Liberal Arts, 675 Commonwealth Ave, Boston, Mass *Prof of Biology* (1, 1927)
- Wynne, Arthur M, Ph D Univ of Toronto, Dept of Biochemistry, Toronto, Ontario, Canada *Prof of Biochemistry* (2, 1940)
- Yager, Robert H, V.M.C. Army Med Dept Research and Grad School, Army Med Center, Washington 12, D C *Lt Col, Dir, Veterinary Div* (6, 1950)
- Yerkes, Robert M, Ph D Yale Univ School of Medicine, New Haven, Conn *Prof Emeritus of Psychobiology, Yale Univ* (1R, 1904)
- Yonkman, Frederick F, Ph D, M D Ciba Pharmaceutical Products, Inc, Summit, N J *Dir of Research at Ciba, Lecturer in Pharmacology, Columbia Univ College of Physicians and Surgeons* (3, 1931)
- Youmans, John B, M D Vanderbilt Univ School of Medicine, Nashville, Tenn *Dean* (5, 1948)
- Youmans, William Barton, Ph D, M D Univ of Oregon Med School, Portland *Prof of Physiology* (1, 1939, 3, 1949)
- Young, A G, Ph D, M D 520 Commonwealth Ave, Boston, Mass *Asst Prof of Therapeutics, Boston Univ School of Medicine, Med Dir of Corey Hill Hospital, Brookline* (3, 1925)
- Young, E G, Ph D Natl Research Council of Canada, Maritime Regional Lab, Halifax, N S *Director* (2, 1925)
- Youngburg, Guy E, Ph D Route 2, Box 268 Mesa, Ariz *Emeritus* (2, 1927)
- Youngner, Julius Stuart, M S, Sc.D Univ of Pittsburgh, School of Medicine, Virus Research Lab, Pittsburgh, Pa *Asst Research Prof of Bacteriology* (6, 1950)
- Yuile, Charles L, M D, C M Univ of Rochester School of Medicine and Dentistry, Rochester, N Y *Assoc Prof of Pathology* (4, 1941)
- Zamecnik, Paul, M D Massachusetts General Hospital, Fruit St, Boston 14, Mass *Asst Prof of Medicine and Tutor in Biochemical Sciences, Harvard Univ* (2, 1950)
- Zapp, John A, Jr, Ph D 916 Harper Ave, Drexel Hill, Pa *Asst Dir, Haskell Lab of Industrial Toxicology, E I duPont de Nemours & Co* (1, 1950)
- Zarrow, M X, Ph D Purdue Univ, Dept of Biological Sciences, Lafayette, Ind (1, 1949)
- Zechmeister, L, Dr Ing California Inst of Technology, Pasadena *Prof of Organic Chemistry* (2, 1941)
- Zeckwer, Iselde T, M D Univ of Pennsylvania School of Medicine, Philadelphia *Assoc Prof of Pathology* (1, 1934, 4, 1927)
- Zeidman, Irving, M D Univ of Pennsylvania School of Medicine, Dept of Pathology, Philadelphia *Associate* (4, 1949)
- Zeldis, Louis J, M D Brookhaven Natl Lab, Med Dept, Upton, Long Island, N Y *Pathologist, Div of Pathology and Brookhaven Natl Lab Hospital* (4, 1945)
- Zilversmit, D B, Ph D Univ of Tennessee, Dept of Physiology, Memphis 3 *Instructor* (1, 1949)
- Zimmerman, Harry M, M D Montefiore Hospital, Gun Hill Rd, New York City 67 *Chief, Lab Div, Prof of Pathology, College of Physicians and Surgeons, Columbia Univ* (4, 1933)
- Zirkle, Raymond E, Ph D Univ of Chicago, Chicago, Ill *Prof of Botany and Dir of Inst of Radiobiology and Biophysics* (1, 1948)
- Zittle, Charles A, Ph D U S Dept of Agriculture, Eastern Regional Research Lab, Philadelphia 18, Pa *Chemist, Protein Div* (2, 1946)
- Zucker, Marjorie B, Ph D New York Univ College of Dentistry, Dept of Physiology, 209 E 23rd St, New York City 10 (1, 1947)
- Zweifach, Benjamin W, Ph D New York Hospital and Cornell Univ Med College, Dept of Medicine, 525 E 68th St, New York City 21 *Asst Prof of Physiology* (1, 1945)
- Zwemer, Raymund L, Ph D 5003 Battery Lane, Bethesda 14, Md *Chief, Science Div, Library of Congress, Washington, D C* (1, 1930)

SUMMARY OF MEMBERSHIP

The American Physiological Society	1238
American Society of Biological Chemists	882
American Society for Pharmacology and Experimental Therapeutics	458
The American Society for Experimental Pathology	352
American Institute of Nutrition	357
The American Association of Immunologists	384
Total membership of Federation	3671

DECEASED MEMBERS

Babkin, B P (1, 1924) May 3, 1950	Kurotchkin, Timothy J (6, 1946) April 29, 1949
Baudisch, Oskar (2, 1931) March 28, 1950	Lehmann, Gerhard (3, 1939) November 1948
Bazett, Henry C (1, 1921) July 11, 1950	Munro, F L (2, 1948) April 8, 1950
Boivin, Andre (6, 1949) March 1, 1950	Murphy, James B (4, prior to 1920) August 24, 1950
Cope, Otis M (1, 1929) January 28, 1950	Pemberton, Ralph (5, 1933) June 1949
Emmett, Arthur D (2, 1908, 5, 1933) June 11, 1947	Perlzweig, William A (2, 1924, 5, 1944) December 10, 1949
Friedemann, Ulrich (6, 1938) November 15, 1949	Petersen, William F (4, 1923) August 21, 1950
Gray, Samuel H (4, 1939) August 18, 1949	Pohlman, Augustus G (1, 1934) March 31, 1950
Groat, William A (6, 1917) 1945	Reed, Howard S (2, 1909) May 12, 1950
Gutierrez-Noriega, Carlos (3, 1948) November 1950	Robbie, W A (1, 1947) September 7, 1949
Haythorn, Samuel R (4, 1925) December 6, 1949	Stillman, Ernest G (6, 1930) December 16, 1949
Hertz, Saul (4, 1935) August 2, 1950	Toomey, John A (6, 1943) January 1, 1950
Howe, Percy R (5, 1935) February 28, 1950	

LIST OF MEMBERS BY LOCATION

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ALABAMA POLYTECHNIC INST

R W Engel, 5 W D Salmon, 2, 5
E L Hove, 5 A E Schaefer, 5

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A E Casey, 4

HILLMAN HOSP

T D Spies, 3, 4, 5

MED COLLEGE OF ALABAMA

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J M Bruhn, 1 T W Robinson, 1
E B Carmichael, 1, 2 R S Teague, 3
R L Driver 1 3 H Wu 2

UNIV OF ALABAMA

J S McLester, 5 E N Todhunter, 5
W W Pigman, 2

Alabama—Unclassified

R A Lambert 4

ALASKA

L Irving, 1

ARIZONA

Tucson

UNIV OF ARIZONA

A R Kemmerer, 5

Arizona—Unclassified

B O Barnes, 1 G E Youngburg, 2
A L Lieberman, 1

ARKANSAS

Camden

THE GRAPETTE CO

J T Skinner, 2

Fayetteville and Little Rock

UNIV OF ARKANSAS

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P L Day, 2, 5 B Sure 2 5
F E Emery, 1 H C Nicholson, 1
M C Kik, 5 J R Totter, 2
A. Nettleship, 4

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A K Balls 2 H Lineweaver, 2
F DeEds 2 3 H P Lundgren, 2
O H Emerson 2 H. S Olcott, 2
H L Fraenkel Conrat, 2 S Schwimmer 2
J D Greaves, 2 R H. Wilson 3

Berkeley

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W E Ward, 6

STATE DEPT PUBLIC HEALTH

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M Calvin, 2 A F Morgan, 2, 5
F H Carpenter, 2 M M Nelson, 5
I L Chaikoff, 1 J H Northrop, 2
M Doudoroff, 2 R Okey, 2, 5
H M Evans, 1 J M D Olmsted, 1
H O L Fischer, 2 N Pace 1
C R Grau, 5 W O Reinhardt, 1
D M Greenberg 2, 5 M E Simpson, 1
W Z Hassid 2 W M Stanley, 2
H B Jones, 1 P K. Stumpf, 2
P L Kirk, 2 E S Sundstroem, 2
C A Knight, 2 H Tarver, 2

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H C Bradley, 1, 2

Beverly Hills

H. B Friedgood, 1 R F Riley, 3
H H. Mitchell, 6 S S Sobin, 1
M Prinzmetal, 3

Davis

UNIV OF CALIFORNIA

H H Cole, 1 F H Kratzer, 5
J B Enright 6 S A Peoples, 3
H Goss 2, 5 O H Siegmund, 3
M Kleiber, 1 5

La Jolla

SCRIPPS INST OF OCEANOGRAPHY

N W Rakestraw, 2

SCRIPPS METABOLIC CLINIC

E M Mackay, 1

Los Angeles

BIO SERVICE LABS

O J Golub, 6

CEDARS OF LEBANON HOSP

N B Friedman, 4 E Haas 2
H Goldblatt 1 4 P E Rekers, 4

CHILDREN'S HOSP

R E Knutti 4

COLLEGE OF MED EVANGELISTS

H. A Davis, 4

LOS ANGELES COUNTY HOSP

H E Pearson, 6 A G Ware, 2
D G Simonsen 2

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J M Adams, 4 S Roberts, 1 2
T H Bullock, 1 W A Selle, 1
F Crescentelli, 1 C M Szego, 1
M S Dunn, 2 C L Taylor 1
T L Jahn, 1 A White, 2 5
H W Magoun, 1

UNIV OF SOUTHERN CALIFORNIA

W H Bachrach 1 J Marmorston, 6
F M Baldwin, 1 W Marx 2
J W Buchanan, 1 J W Mehl, 2
H J Deuel, Jr, 1, 2, 5 L E Morehouse, 1
D R Drury, 1 L A Sapirstein, 1
B H Ershoff, 5 C H Thienes, 3
E Geiger, 5 L E Travis 1
P O Greeley, 1 R J Winzler, 2
C Hyman, 1 A Wright, 4

VETERANS ADMINISTRATION

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LOS ANGELES—UNCLASSIFIED

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W G Clark 1 3 B O Raulston 3
T J Haley, 3

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R J Parsons, 4

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Palo Alto

PALO ALTO CLINIC

A M. Snell, 4

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H. Borsook, 2 A. Tyler, 6
D H Campbell, 6 A Van Harreveld, 1
J W Dubnoff, 2 C A. G Wiersma, 1
N H. Horowitz, 2 L Zechmeister, 2
C G Niemann, 2

Port Hueneeme

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B F Shimmel, 2

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G R. Bisland, 4 M Friedman, 1
S O Byars, 1

STANFORD UNIV

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A. J. Cox, Jr, 4 H. W. Newman, 3
W C Cutting, 3 L A Rantz, 3
R H Dreisbach, 3 D A. Ryland, 3

UNIV OF CALIFORNIA

H R. Bierman, 3 B Labet, 1
W L Bostick, 4 S Lindsay 4
J L Carr, 4 S R Mettier, 4
J J Eiler, 2 K. F Meyer, 4 6
H. W Elliott, 3 H D Moon, 4
N E Freeman, 1 E W Page, 1
L D Greenberg, 2 J F Rinehart, 4
N Halliday, 5 P P T Sah, 3
C H Hine, 3 M B Shumkin, 4
E Jawetz, 6 D B Taylor 3
W J Kerr 3 E L Way, 3
H I Kohn, 1

UNCLASSIFIED

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R P Ellrod 6 G J Stein, 6
H E Foster 6 A B Stockton, 3
J J Sampson, 1

Santa Barbara

COTTAGE HOSP

F E Bischoff, 2, 5 A E Koehler, 2

SAHYUN LABS

M Sahyun, 2

Santa Monica and Vallejo

KABAT KAISER INST

O L Huddleston, 1 M Levine, 6

UNCLASSIFIED

J Tilt, 5

Stanford

STANFORD UNIV

J P Baumberger, 1
C M Child, 1R
J M Crismon, 1
G A Feigen, 1
C S French, 1, 2
F A Fuhrman, 1
R Grant, 1
A C Griffin, 2
V E Hall, 1
H S Loring, 2

J M Luck, 2
W H Manwaring, 4, 6
S Raffel, 6
O H Robertson, 4R
E W Schultz, 4, 6
J R Slonaker, 1R
R E Swain, 2
E L Tatum, 2
T W Weymouth, 1R

California—Unclassified

B W Beadle, 2
G W Clark, 2
P M Dawson, 1R
R A Kocher, 2

W S Lawrence, 3
M E Marsh, 1, 5
F H Scharles, 5
E L Walker, 3R

COLORADO

Denver

DENVER GENERAL HOSP

R Virtue, 2

UNIV OF COLORADO

F A Cajon, 2, 5
F D'Amour, 1
W B Draper, 1, 3
H H Gordon, 5
R M Hill, 2
J H Holmes, 1
R C Lewis, 2, 5

B B Longwell, 2
C A Maske, 1
C G Mackenzie, 1, 2, 5
J B McNaught, 4
R M Mulligan, 4
R W Whitehead, 1, 3

DENVER—UNCLASSIFIED

H J Corper, 2
G K Lewis, 5

F R Sabin, 1R

Fort Collins

COLORADO A & M COLLEGE

F X Gassner, 1

Colorado—Unclassified

J M Beazell, 1
H Bourquin, 1

E S Fetcher, 1
C R Schmidt, 1

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East Hartford

UNITED AIRCRAFT CORP

S E Pond, 1

Greenwich

ST LUKE'S CONVALESCENT HOSP

A A Albanese, 2

GREENWICH—UNCLASSIFIED

H L Amoss, 4, 6

H B Williams, 1

Hartford

CONN STATE DEPT OF HEALTH

O R Benham, 6

INST OF LIVING

W T Liberson, 1

ST FRANCIS HOSP

S J Martin, 1

Middletown

WESLEYAN UNIV

R A Cortner, Jr, 5

UNCLASSIFIED

E G Schneider, 1R, 2

New Haven

CONN AGRIC EXPR STATION

C I Bliss, 3
R B Hubbell, 2, 5

H B Vickery, 2

NEW HAVEN HOSP

D C Darrow, 2

PIERCE FOUNDATION

L P Herrington, 1

YALE UNIV

R J Anderson, 2
G A Bartsell, 1
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F G Blake, 4, 6
W D Blake, 1
E J Boell, 1
D N Bonner, 2
D D Bonnycastle, 3
J R Brobeck, 1
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H G Cassidy, 2
G R Cowgill, 1, 2, 5
E C Curnen, 6
J English, Jr, 2
J S Fruton, 2
J F Fulton, 1
S Gelfan, 1
R H Green, 6
H S N Greene, 4
H W Haggard, 1, 2
R G Harrison, 1
D I Hitchcock, 2

H D Hoberman, 2
M E Howard, 4, 6
W A Krehl, 2, 5
L S Kubie, 4
D M Kydd, 5
H Lamport, 1
R B Livingston, 1
C N H Long, 1, 2
E B Man, 2
J L Melnick, 2, 6
W R. Miles, 1
L N Nahum, 1
J S Nicholas, 1
J R Paul, 4, 6
J P Peters, 2
P D Rosahn, 4
W T Salter, 1, 3, 5
S Simmonds, 2
G H. Smith, 6
H P Treffers, 6
G Van Wagenen, 1
R M Yerkes, 1R

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E G Boettiger, 1

Connecticut—Unclassified

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K. Blunt 2	J M Rogoff, 1 3
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W J Elser 6	A J Wakeman, 2
C S Leonard 3	J B Watson, 1

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ALFRED E DUPONT INST

P B Hamilton 2	J A Zapp, Jr, 1
A T Wilson 6	

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L W Parr 4	C R Treadwell, 2, 5

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T Koppanyi, 1, 3	E B Tuohy 3
C F Morgan, 1, 3	

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M Harris, 2

HOWARD UNIV

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J L Johnson 1	

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L Voris, 5	

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E M Hewston 5	H K. Stiebeling 5
M J Horn 2	M Womack, 5

AIR FORCE, SGO

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ARMY, CWS

J H Defandorf, 3	H A Kuhn, 3
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ARMED FORCES INST OF PATHOLOGY

H G Grady, 4	H F Smetana, 4
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ARMY MED CENTER

H C Batson, 6	H L Ley, Jr 6,
W S Gochenour Jr, 6	R P Mason 6
D E Gregg, 1	F D Maurer 6
M Hilleman, 6	J E Smadel, 4, 6
E B Jackson, 6	M L Weil, 6
J F Kent, 6	R H Yager, 6
L R Kuhn, 6	

ARMY, SGO

C J Koehn, 5

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P B Pearson, 2, 5

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R L Grant, 2	W A. Randall, 6
O L Kline, 5	R G Smith, 3
E P Laug 2, 3	C D Tolle, 5
A J Lehman, 3	B J Vos, 3
A. A. Nelson, 4	H Welch, 6
E E Nelson, 3	F H Wiley, 2

NAVY, MED DEPT

A R Behnke 1	D H Moore, 1
H T Karsner, 4	R. A Phillips, 1

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L T Fairball, 2	

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A H Lawton, 1, 3	A M Walker, 1, 3
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District of Columbia—Unclassified

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D D Donahue 3	S L Smith, 5
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UNIV OF MIAMI

C Brooks, 1, 3	E Larson, 1, 3
D H Cook, 2	V H Moon, 4

Gainesville

UNIV OF FLORIDA

T J Cunha, 5	G K Davis, 5
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W H Eddy, 2, 5R

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U S NAVAL AIR STATION

A. Graybiel, 1

Tallahassee

FLORIDA STATE UNIV

K. Dittmer, 2

M R Sandels, 5

Florida—Unclassified

G R Crisler, 1

N Fell, 6

N Glickman, 1

J W Jobling, 4R

O Riddle, 1R

L G Rowntree, 1R, 2, 3R 4

E U Still, 1

R M Waters, 3

GEORGIA

Atlanta

EMORY UNIV

W F Friedewald, 4

J V Waiten, 1

GRADY MEMORIAL HOSP

F W Sunderman, 2

Augusta

MED COLLEGE OF GEORGIA

R P Ahlquist, 3

L Allen, 1

A. P. Briggs, 2

P Dow, 1

W K. Hall, 2

W F Hamilton, 1

J W Remington, 1

S A Singal 2

V P Sydenstrcker, 5

Emory University

EMORY UNIV

H W Ades, 1

S W Gray, 1

J Haldi 1

M Hines 1

G C Knowlton, 1

G T Lewis 2

J L Morrison, 3

E Papageorge 2

A P Richardson 3

J A Russell 1

J Szepeenvol 1

H A Walker 3

A E Wilhelm, 2

Experiment

GEORGIA AGRIC EXPR STATION

M Speirs, 5

Georgia—Unclassified

G Bachmann, 1R

HAWAII

Honolulu

G O Burr 2 5

W A Gortner, 2

A R Lamb 2 5

C D Miller 5

T Nelson 6

IDAHO

Moscow

UNIV OF IDAHO

T B Keith, 5

A. C Wiese, 5

E Woods, 2, 5

ILLINOIS

Chicago

AMERICAN COLLEGE OF SURGEONS

G H Miller, 3

AMERICAN DENTAL ASSN

J R Doty, 2

AMERICAN MED ASSN

F T Jung, 1

R Hussey, 4

A E Smith, 3

R T Stormont, 3

W Van Winkle, Jr, 3

ARMOUR LABS

E E Hays, 2

ARMY, QMC, FOOD & CONTAINER INST

G H Berryman, 1

H L Fevold 2

H D Lightbody, 2

H Spector, 5

AEC, ARGONNE LAB

A M Brues, 1

H Lusco, 4

H M Patt, 1

D E Smith 1

ALBERT MERRITT BILLINGS HOSP

R W Wissler, 4

BJORKSTEN RESEARCH LABS

E L Gustus 2

CHICAGO LYING-IN HOSP

M E Davis, 3

W J Dieckmann, 3

CHICAGO MED SCHOOL

G Clark, 1

P P Foa 1

L B Nice 1

A H Ryan, 1

CHILDREN'S MEMORIAL HOSP

T B Friedman 6

COOK COUNTY HOSP

B Mortimer 1

H Popper 4

ELIZABETH McCORMICK MEM FUND

A Spohn, 5

G D SPARLE & Co

R T Dillon 2

D M Green 1 3

W E Hambourger 3

A L Raymond 2

E F Schroeder, 2

I C Winter 3

GEORGE WILLIAMS COLLEGE

A H. Steinhaus 1

HEKTOEN RESEARCH INST

O Felsenfeld, 6

L Hektoen 6H

W S Hoffmann 2

HOFFMANN LA ROCHE, INC.

M J Schiffrin, 1

LAB FOR CLIN PHYSIOLOGY

E Jacobson 1

LOYOLA MED SCHOOL

A G Mulder, 1

MICHAEL REESE HOSP

C Cohn, 1	S Rodbard, 1
H S Guterman, 1	B B Rubenstein 1
R Isaacs 4	O Saphir, 4
L N Katz, 1	S Soskin, 1, 5
R Levine, 1	A Tannenbaum, 4
H Necheles, 1	

MT SINAI HOSP

I Davidsohn 4 6

NORTHWESTERN UNIV

J H Annegers, 1	O Hepler, 4
J M Beazell 1	K K. Jones, 1
W C Buchbinder, 1	E W Knox, 2
H Bull, 2	A J Kosman, 1
C A Dragstedt 1, 3	M M Kunde, 1
C J Farmer, 2	J H Last, 3
L S Fosdick, 2	A Lein 1
S Freeman, 1	S L Osborne, 1
T E Friedemann, 2	J P Simonds, 4
J S Gray, 1	R S Snider, 1
R R Greene 1	W W Swanson, 2
H Greengard, 1	W B Wartmann, 4
F S Grodins, 1	J A Wells 3
S C Harris, 1	

PEOPLE'S HOSP

E Weiss, 6

PRESBYTERIAN HOSP

R G Gould 2	A Hiller 2
G Hass 4	D A MacFayden 2

RHEUMATIC FEVER RESEARCH INST

A F Coburn, 6 G Ungar 1

SWIFT & Co

E Eagle 1 H E Robinson, 5

UNIV OF CHICAGO

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J C Allen 1	L R Dragstedt, 1
A S Alving 1	K P Dubois, 3
H S Anker 2	L Eichelberger, 2
E S G Barron, 2	E A Evans, Jr, 2
E P Benditt, 4	C F Failey, 2
S Black 2	R N Feinstein, 2
K Bloch, 2	N W Fugo, 3
W Bloom, 4	H Gaffron, 2
N R Brewer, 1	E M K. Geilung 1, 2, 3
M C H Brookes 5	R. W Gerard 1
W Burrows, 6	I Gersh, 4
P R Cannon, 4 6	G Gomori, 4
A J Carlson 1 5	W C Halstead, 1
J H Certhaml, 2	M E Hanke, 2
T B Coolidge 2	R W Harrison, 6
J M Coon 3	T R. Hogness 2
A Dorfman, 2	C B Huggins, 1

E M Humphreys, 4
J O Hutchens, 1
F E Kelsey, 3
F K O Kelsey 3
A T Kenyon 3
N Kletman, 1
H Kluver, 1
H G Kobrak, 1
H R Kraybill, 2
A L Lehninger, 2
J H Lewis, 4
R S Lille, 1R, 2
M A Lipton, 2
C G Loosli, 4
A B Luckhardt, 1
G H Marmont, 1
F C McLean 1, 2 3
C P Miller, 4 6
M Morse, 2

J W Moulder, 2
F J Mullin, 1
W D Neff, 1
H Oldham, 5
J F Perkins, Jr, 1
T Porter 5
F W Putnam, 2
H T Ricketts, 1
I Sandiford, 2, 5
B S Schweigert, 2, 5
R W Sperry, 1
P E Steiner, 4
W H Tahaferro, 6
J M Tobias, 1
B Vennesland, 2
P Weiss, 1
R. T. Woodyatt, 2
R E Zirkle, 1

UNIV OF ILLINOIS

D I Abramson 1	E R Kirch, 2
A Arkin, 1, 3	C A Krakower 4
A. Bachem, 1	A Leandorfier, 1
P Bailey, 1	S A. Levinson, 4
G A Bennett, 4	J P Marbarger, 1
O Bergeim 1, 2	W. S McCulloch, 1
O A Bessey 2, 5	H A McGuigan, 1R, 2, 3R
S B Binkley, 2	E E Painter,—
J E Bourque, 1	C C Pfeiffer, 3
G R Bucher, 1	C B Puestow, 1
P C Bucy 1	C I Reed 1
N O Calloway, 3	S R Rosenthal, 1 4
W J R Camp, 3	A. A Schiller, 1
H R Catchpole 1	R R Sonnenschein, 1
C W Darrow, 1	F Steigmann, 3
E R Grant, 1	K R W Unna 1, 3, 5
M I Grossman, 1	H. A. Waisman, 2
R C Ingraham, 1	G E Wakerlin, 1, 3
A. C Ivy 1, 5	W H Welker, 2
N R. Joseph 2	W V Whitehorn, 1
R W Keeton 1, 3	

WILSON LABS.

C E Graham, 2 S W Hier, 2

CHICAGO—UNCLASSIFIED

L Arnold, 4, 6	M T Hanke 2
F C Berg, 2, 5	K M Howell, 6
F A Gibbs 1	

Elgin

ELGIN STATE HOSP

M K. Horvitt, 2, 5

Evanston

NORTHWESTERN UNIV

F A. Brown, Jr, 1	S Malkoel 6
I M Klotz, 2	B Riegel 2
D B Lindsley 1	

Hines

VETERANS ADMINISTRATION HOSP

W R. Tweedy, 2 C C Wang, 2, 5

North Chicago

ABBOTT RESEARCH LABS

R. D Coghill, 2	A E Osterberg 2
G M Everett 3	R K. Richards 1, 3
D V A Frost, 2, 5	L. W Roth 1 3
D W Maccorquodale, 2	J E P Toman 1 3

Peoria

ALLIED MILLS, INC

J E Hunter, 5

USDA, NORTH REGIONAL RESEARCH LAB

R W Jackson, 2, 5

Quincy

QUINCY SPECIALTIES CO

J W Mull, 2

Rockford

ROCKFORD COLLEGE

D R. Stewart, 1

ROCKFORD MEM HOSP

S Natelson, 2

Urbana

UNIV OF ILLINOIS

H E Carter, 2, 5	H, H Mitchell, 2, 5
T K. Cureton, Jr, 1	C L Prosser, 1
G S Fraenkel, 5	W C Rose, 2, 5
I C Gunsalus, 2	J M Smith, 5
T S Hamilton, 2, 5	S Spiegelman, 1
L M Henderson, 2	F R Steggerda, 1
M Hinrichs, 1	C S Vestling, 2
B C Johnson, 2, 5	V J Wulff, 1
R E Johnson, 1, 2, 5	

Wilmette

AMERICAN INST OF BAKING

W B Bradley 1

Illinois—Unclassified

J I Farrell, 1	S E Owen, 1
H G Glass, 3	S B Raska, 1
H C Mason, 6	E G Weir 1

INDIANA

Bloomington

INDIANA UNIV

H G Day, 2, 5	S Robinson 1
P M Harmon, 1	H H Rostorfer, 1
F A Haurowitz, 2, 6	T J B Stier, 1
L S McClung 6	W J Van Wagtendonk 2
P A Nicoll 1	G E Wertenberger, 1

Elkhart

MILES LABS

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Evansville

MEAD JOHNSON AND CO

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O N Massengale 2	

Indianapolis

ELI LILLY AND CO

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K K Chen 1, 3	F G Henderson 3
G H A Clowes 2, 6	W A Jamieson 6
H A Dettwiler 6	H M Powell 6

INDIANA UNIV

D E Bowman, 2	R N Harger, 2, 3
V V Cole, 3	H R Hulpieu, 3
L W Freeman, 1	E W Shrigley, 6
R B Forney, 3	R L Thompson, 6

INDIANAPOLIS GENERAL HOSP

O M Helmer, 2	R E Shipley, 1
K G Kohlstaedt, 1	

PITMAN MOORE CO

B E Abreu, 3	C A Bunde, 1
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Lafayette

PURDUE UNIV

R C Corley, 2	F W Quackenbush, 2
S M Hauge, 5	C Schuck, 5
W A Hiestand, 1	M X Zarrow, 1
E T Mertz, 2	

Shelbyville

INLOW CLINIC

C C Scott, 3	V B Scott, 1
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Terre Haute

COMMERCIAL SOLVENTS CORP

F H Schultz, Jr, 3

Indiana—Unclassified

S Ansbacher, 2	F Neuwelt, 1
C E Bills, 2, 5	

IOWA

Ames

IOWA STATE COLLEGE

G Everson 5	R R Sealock, 2, 5
S W Fox, 2	P P Swanson, 5
E A Hewitt 1	B H Thomas 5
P M Nelson, 5	C H Werkman, 2
F Schlenk, 2	

Iowa City

STATE UNIV OF IOWA

S B Barker, 1	G Kalnitsky, 2
C P Berg 2, 5	G Marsh 1
J H Bodine, 1	H A. Mattill 1, 2, 5
S C Cullen, 3	A P McKee 6
T C Evans 1	W O Nelson, 1
R M. Featherstone, 3	I H Pierce 3
R B Gibson, 2	J R Porter 6
E G Gross, 1, 2, 3	J I Routh, 2
H M Hines, 1	F W Schueler, 3
S M Horvath, 1	G Stearns, 2, 5
W R Ingram 1	W W Tuttle, 1
R G Janes, 1	E D Warner 4
P C Jeans 5	T Winnick 2

Iowa—Unclassified

A L Daniels 2, 5R	W M Parkins 1R
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KANSAS

Kansas City and Lawrence

UNIV OF KANSAS

C M Downs 6	D J Mulford 2
T R Hamilton 4	C F Nelson 2
R M Lenberger 3	N P Sherwood 6
K Jochem 1	O O Stoland 1
G N Loofbourrow 1	

Manhattan

KANSAS STATE COLLEGE

L. Ascham 5
J S Hughes, 2, 5

M Kramer, 5

Wadsworth

VETERANS ADMINISTRATION HOSP

C J Weber, 2

Wichita

VETERANS ADMINISTRATION HOSP

N F Blau, 2

Kansas—Unclassified

V G Haury 3
M S Pittman, 5

O S Walters 1

KENTUCKY

Fort Knox

ARMY MED DEPT FIELD RESEARCH LAB

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R G Daggs 1, 5
H Jensen, 2

A D Keller, 1

W F Kocholaty, 2

G W Molnar, 1

Lexington

UNIV OF KENTUCKY

G D Buckner, 2
J C Humphries, 6

J S McHargue, 2

M Scherago, 6

USPHS

A J Eisenman, 2
H Isbell, 3

A. Wikler, 3

Louisville

UNIV OF LOUISVILLE

J T Bradbury, 1
P L Knoefel, 3
H Lawson 1

W S Rehm, Jr, 1

S M Walker, 1

LOUISVILLE GENERAL HOSP

A J Steigman, 6

Kentucky—Unclassified

W L Adams, 3

LOUISIANA

Baton Rouge and New Orleans

LOUISIANA STATE UNIV

R Ashman 1
R W Brauer 3
G J Buddingh 4
R L Holman, 4

G W McCoy, 6H

C Reynolds, 3

L L Rusoff 5

L A. Toth, 1

OCHSNER CLINIC

T Findley, Jr, 1
O Schales 2

A Segaloff, 4

SOUTHERN BAPTIST HOSP

T C Sherwood 1

TULANE UNIV

L M N Bach, 1
C E Dunlap 4
G A. Goldsmith, 5
W H Harris, 4
R Hodes, 1
H E King, 1
R Koster, 3
E Kun 3
F N Martin, Jr, 3

H S Mayerson, 1
H P Sarott 2, 5
M F Shaffer 4, 6
M H D Smith, 6
L Thomas, 6
W B Wendel, 2
A G C White, 2
W S Wilde, 1

USDA, SOUTHERN REGIONAL RESEARCH LAB

A M Altschul 2

U S MARINE HOSP

C H Steffee, 4

MAINE

F G Benedict, 1R, 2R

P Kyes, 6R

MARYLAND

Army Chemical Center

U S ARMY CHEMICAL CENTER

L E Chadwick 1
W H. Chambers, 1, 5
F N Craig, 1
D B Dill, 1, 2, 5
S H. Durlacher, 1
A. J. Dzieman, 1
E R Hart 3
C M Herget 1
H E Hinwich, 1
R G Horton, 1

B J Jandorf, 2
A S Marrazzi, 1 3
B P McNamara 3
H O Michel 2
F W Oberst 2, 3
W H Summerson, 2
H J Trurnit 1
C Tum Suden, 1
J H Wills, 1

Baltimore

BALTIMORE CITY HOSPITALS

M Landowne, 1
R. K. McDonald, 1

N W Shock, 1

CARNEGIE INSTITUTION OF WASHINGTON

L B Flexner 1, 2
S R M Reynolds, 1

D B Tyler, 1

HYNON WESTCOTT AND DUNNING

J H. Brewer 6

JOHNS HOPKINS HOSP

F B Bang, 4
R. J. Bing, 1
E A. Bliss, 6
A M Chesney, 4
W M Firor 1
J S Friedenwald, 1
W H Gantt 1
L N Gay 6
A M Harvey 1, 3
J E Howard 1
C C Hunt, 1
O R. Langworthy 1

J L Lilienthal, Jr, 1
J W Magladery, 1
M M Mayer, 6
V A Najjar, 2
E V Newinan 1
A. G. Osler 6
E A. Park, 4
A. R. Rich, 4
C P Richter 1
W W Scott 1
A C Woods, 6

JOHNS HOPKINS UNIV

E C Andrus, 1
A M Baetjer, 1
P Bard, 1
D Bodian, 6
F Brink Jr 1

R B Bromiley 1
D W Bronk, 1
C A Chandler 6
A Chapanus, 1
B F Chow 2 5 6

W M Clark, 2
B Cohen, 2
S P Colowick, 2
P W Davies 1
E H Dearborn 3
V G Dethier, 1
R. H. Follis, Jr 4
G O Gey, 1
R G Grenell, 1
H K Hartline, 1
L Hellerman, 2
R M Herriott, 2
W A. Himwich, 1
E Howard 1
H. A. Howe, 6
T P Hughes, 6
N O Kaplan, 2
S W Kuffler, 1
M. G. Larrabee, 1

D H K Lee, 1
P H Long, 3
T H Maron, 3
E K Marshall, Jr, 1, 2 3
E B McCollum, 5
E V McCollum, 2, 5
W D McElroy, 1
G S Mirick, 6
C T Morgan, 1
V B Mountcastle, 1
J E Rose, 1
M Rosenfeld, 3
E B Schoenbach,
C D Snyder, 1R
S A Talbot, 1
C B Thomas, 1
S S Tower, 1
A E Walker, 1
W La F Winkenwerder, 6

MARYLAND TUBERCULOSIS ASSN

G C Robinson, 1R

SINAI HOSP LABS

D I. Macht, 1R, 3

U S MARINE HOSP

L L Ashburn, 4

USPHS

J E Buren, 1

UNIV OF MARYLAND

W R Amberson, 1
M A. Andersch, 2
C J Carr, 3
C W Chapman, 3
F P Ferguson, 1

O G Harne, 1
J C Krantz, Jr, 3
G Lu 3
R H. Oster, 1
D C Smith, 1

Beltsville

USDA, NATL AGRICULTURE RESEARCH CENTER

M Adams, 2
H. G. Barott, 5R
E L Batchelder, 5
H. R. Bird, 5
E C Callison, 5
C A. Cary, 2
F A Csonka, 2
N R Ellis 2 5

T D Fontaine 2
R M. Fraps, 1
M W Kies, 2
E W Ligon, Jr 3
L A. Moore, 5
E Orent-Keiles, 2, 5
J F Sykes, 1

Bethesda

NATL INSTS OF HEALTH

G H Algure 4
P D Altland, 1
E M Anderson, 1
H B Andervont, 4
C B Anfinson Jr, 2
H D Baernstein, 2
J H Baxter 3
L V Beck 1
M Belkin 3
T Benzinger 1
W J Bowen 1
J Bozicovich 6
J H Bragdon 4
S E Branham, 6
G Brecher 4
B B Brodie 2 3
W R Bryan 1 4

D Burk, 2
H. W. Chalkley, 1
F S Daft, 2 5
A. J Dalton 4
I N Dubin 4
T B Dunn, 4
H M Dyer 2 5
H Eagle 3 4
W R. Earle 4
B E Eddy 6
N B Eddy 3
E W Emmart 3
K. M. Endicott 4
A. B. Eschenbrenner, 4
L D Felton 6
H Goldie 6
J P Greenstein 2

L A Heppel, 2
R Hertz, 1
B Highman, 4
B L Horecker, 2
W C Hueper, 4
J M Hundley, 5
L Karel, 3
J C Keresztesy, 2, 5
L Kilham, 6
A Kornberg, 2
C L Larson, 6
R D Lillie, 4
E Lorenz, 4
R C Maccardle, 4
L H Marshall, 1
W H Marshall, 1
H S Mason, 2
M E Maver, 2
A Meister, 2
H. P. Morris, 2, 5
B J Olson, 6
M E Reid, 5
S M Rosenthal, 3

R E Scantlebury, 1
W H Seabell, Jr, 2, 5
A Seidell, 2
A. M. Shanes, 1
M J Shear, 2
M Silverman, 2
M I Smith, 1R, 3R
W W Smith, 1
H Specht, 1
S S Spicer, 1
H L Stewart, 4
E F Stohlman, 3
A Szent-Gyorgyi, 2
H Tabor, 3
W T S Thorp, 4
S Udenfriend, 2
W F Von Oettingen, 3
F R White, 2
J White, 2
J G Wooley, 6
C I Wright, 1, 3
R. W G Wyckoff, 6

NAVAL MED RESEARCH INST

L A. Barnes, 6
K S Cole, 1
D Minard, 1

M F Morales, 1
J Sendroy, Jr, 2

College Park

UNIV OF MARYLAND

J C Shaw, 1

Fort Howard

VETERANS ADMINISTRATION HOSP

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Frederick

HOOD COLLEGE

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U S ARMY CHEMICAL CORPS, CAMP DETRICK

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A. K. Boor 2
C R Brewer, 2
L A. Chambers 1, 6
S S Elberg, 6

L D Fothergill, 6
F B Gordon, 4
J Victor, 4
O C Woolpert, 6
G G Wright, 6

Perry Point

VETERANS ADMINISTRATION HOSP

S F Howell 2

Rockville

CHESTNUT LODGE SANITARIUM

D M Rioch 1

USPHS

F Smith 1

Towson

GOUCHER COLLEGE

P J Crittenden 1 3

Maryland—Unclassified

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J L King 1R
R L Riley 1

J Steinhardt, 2
J Warren 6

MASSACHUSETTS

Amherst

AMHERST COLLEGE

V C Dewey, 2
O C Glaser 1R

G W Kidder, 2

UNIV OF MASSACHUSETTS

A D Holmes 2 5R
J O Holmes 2 5

H S Mitchell, 2, 5
A W Wertz, 5

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BOSTON CITY HOSP

W B Castle, 4
C S Davidson, 5
M Finland, 6

S Maddock, 4
G K Mallory, 4

BOSTON DISPENSARY

G Schmidt 2

BOSTON HEALTH DEPT

C Floyd, 6

BOSTON UNIV

E E Baker, 6
D L Belding, 4
G Edsall, 6
G P Fulton, 1
A. H. Hegnauer, 1
S B Hooker 6
E R Loew 1 3
F C Lowell 6

B R Lutz, 1
G L Maisson, 1, 3
W L Mendenhall, 1 3R
J W Stutzman, 1, 3
B S Walker, 2
L C Wyman, 1
A G Young 3

BETH ISRAEL HOSP

H L Blumgart, 1

M J Schlesinger, 4, 6

EVANS MEMORIAL

E M Follensby, 6

J F Ross 4

FORSYTH DENTAL INFIRMARY FOR CHILDREN

I G Wesson, 2, 3

HARVARD UNIV

O T Bailey, 4
E G Ball, 2
A C Barger, 1
G P Berry, 6
E S Castle 1
P O Chatfield, 1
H A Christian 4
F J Cohn, 1 2
M Cohn, 2
J B Conant, 2
A H Coons 6
M F Crawford, 1
W J Crozier 1
M D Eaton 6
I T Edsall 2
I F Enders, 6
S Farber, 4
C H Fiske, 2
A Forbes 1
H S Forbes 1R
W H Forbes, 1
R M Ferry, 2
E H Frieden 2
R Calambos 1

J L Gamble, 2, 5
O Gates, 4
R P Geyer 5
A Goldstein, 3
W T Goodale 1
R O Greep 1
R E Gross 4
J H Hanks 6
A B Hastings 1, 2, 5
I W Haynes, 1
D M Hegsted, 5
I L Hisaw, 1
M E Koshland,
O Krayner 3
E M Landis, 1
R A McFarland 1
R W McKee, 2
G A Miller 1
J H Mueller 2 4, 6
P L Munson 2
J P O'Hare 4
J R Pappenheimer, 1
G H Parker 1R
W C Quimby, 1R

D S Ruggs, 3
H F Root, 5
S J Sarnoff, 1
J H Shaw, 5
F F Snyder, 1
J C Snyder, 6
F J Stare, 2, 5
S S Stevens, 1
G W Thorn, 1
H C Trimble, 2, 5

C A Villee, Jr 2
G Wald 1, 2
C W Walter, 4
S Warren, 4
J H. Welsh 1
J L Whittenberger, 1
C M Williams, 1
G B Wislocki, 1
S B Wolbach, 4
J Wyman, Jr 1

LAHEY CLINIC

F N Allan, 4

ARTHUR D LITTLE, INC

M G Gray, 3

R C D Hickman, 2

MASSACHUSETTS GENERAL HOSP

J Aub, 1, 5
W Bauer, 1
H. K. Beecher, 3
M A. B. Brazier 1
L Dienes 6
L L Engel, 2
F Lipmann 2

T B Mallory, 4
B F Miller 2
I T Nathanson, 1
F M Rackemann, 6
B H Waksman, 6
P D White, 3
P Zamacnik, 2

MASSACHUSETTS INST OF TECH

R. S. Harris, 5
J C R Licklider, 1
F O Schmitt 1

I W Sizer, 1
D F Waugh, 1

NEW ENGLAND DEACONESS HOSP

E P Joslin, 5R
H B Kenton, 6

E H Tompkins, 4

NEW ENGLAND MED CENTER

J H Pratt, 1, 3, 4

TUFTS COLLEGE

E B Astwood, 1
A Bliss, 1
E A. Brown 6
A Canzanelli 1
B B Clark, 3
H N Christensen, 2

W H Fishman, 2
Z Hadidian, 1
D Rapport 1
J M Reiner, 1
S J Thannhauser, 2

VETERANS ADMINISTRATION REGIONAL OFFICE

J M Looney, 2

BOSTON AREA—UNCLASSIFIED

G P Grabfield 3
J W Mavor, 1R

Z B Miller 2
A M Pappenheimer 4

Lawrence

U S ARMY QMC CLIMATIC RESEARCH LAB

H S Belding 1

Lynn

METROPOLITAN STATE HOSP

S E Steiman, 1

Medford

TUFTS COLLEGE

L Carmichael, 1

K D Roeder 1

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FEDERATION NOTICE

Regulations for Submission of Abstracts

1951 Annual Meeting—April 29–May 3

Cleveland, Ohio

The 35th Annual Convention of the Federation will be held in Cleveland, Ohio, April 29–May 3, 1951. The headquarters hotels will be the Statler (Physiology, Pharmacology, Pathology) and the Cleveland (Biochemistry, Nutrition, Immunology). Scientific sessions will be held in the Cleveland Public Auditorium.

Deadline for Abstracts, January 13, 1951 In order to have the abstracts of papers to be presented on the program published and distributed to all members and subscribers to Federation Proceedings in advance of the Annual Meeting, the date of January 13 has been set as the deadline for the submission of abstracts to the Society Secretaries. This applies to both members and non-members who wish to present papers at the scientific sessions. The following regulations for the preparation of abstracts and titles, and for ordering reprints of abstracts, if desired, must be carefully followed to insure the appearance of the paper on the program.

1 Abstracts may not exceed 275 words or equivalent space, including title, authors' names, institutional connections, references and acknowledgments. The text of the abstract should consist of a single paragraph. Structural chemical formulae, figures or footnotes cannot be used. Tabular data are discouraged but, if used, must allow setting in a single column, and each line of a table and its heading must be counted as ten text words.

2 The Title heading must be arranged as follows *Line 1 Title*

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Examples See Federation Proceedings, March, 1950 (Vol. 9 No. 1, Part I) for examples of form and style to be followed.

3 The body of the abstract, typed double space, should follow the title heading. If more than one sheet must be used the sheets must be stapled together.

4 Abstracts must be submitted in quadruplicate to the Secretary of the Society of membership, to reach him not later than January 15, 1951.

5 Abstracts should be letter-perfect since there will be no opportunity for proof reading by authors. However, provision will be made for the correction of printer's errors in the June, 1951, issue of Federation Proceedings, if these are reported to Dr. M. O. Lee, 2101 Constitution Avenue, Washington 25, D. C., within two weeks after the Annual Meeting.

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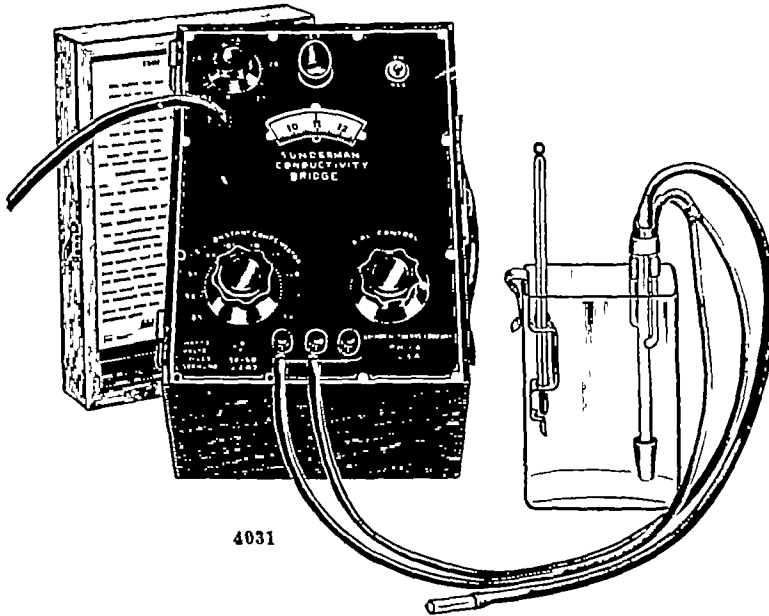


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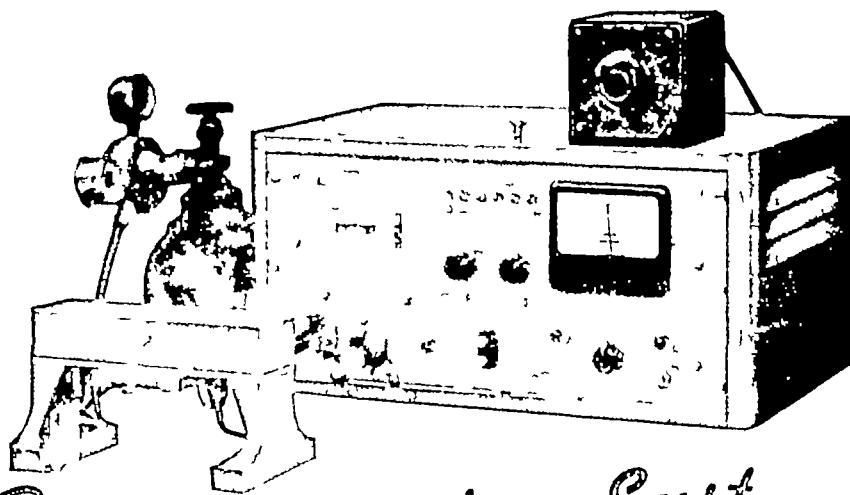
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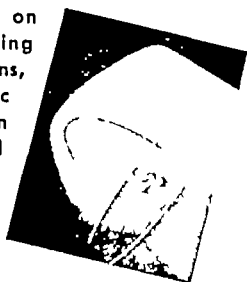
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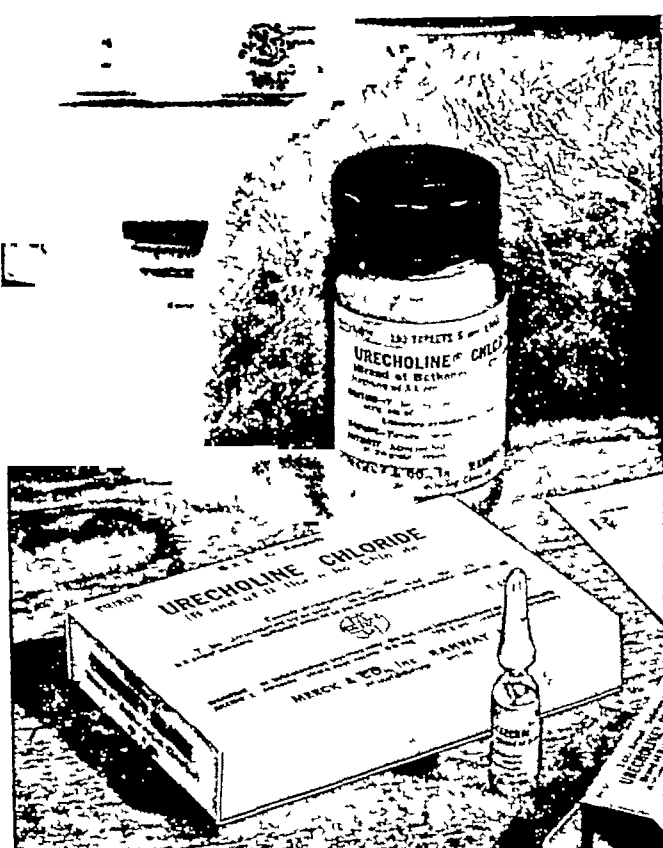
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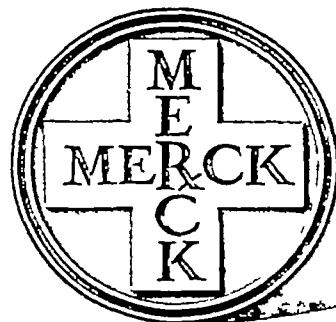
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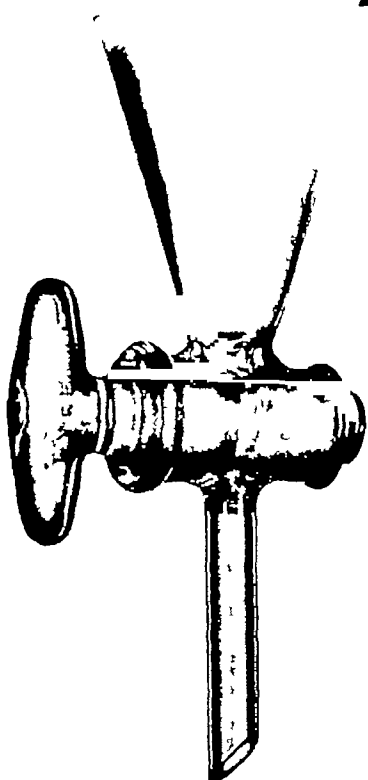
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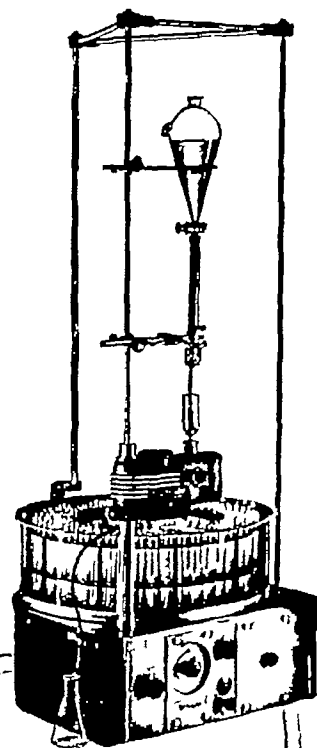
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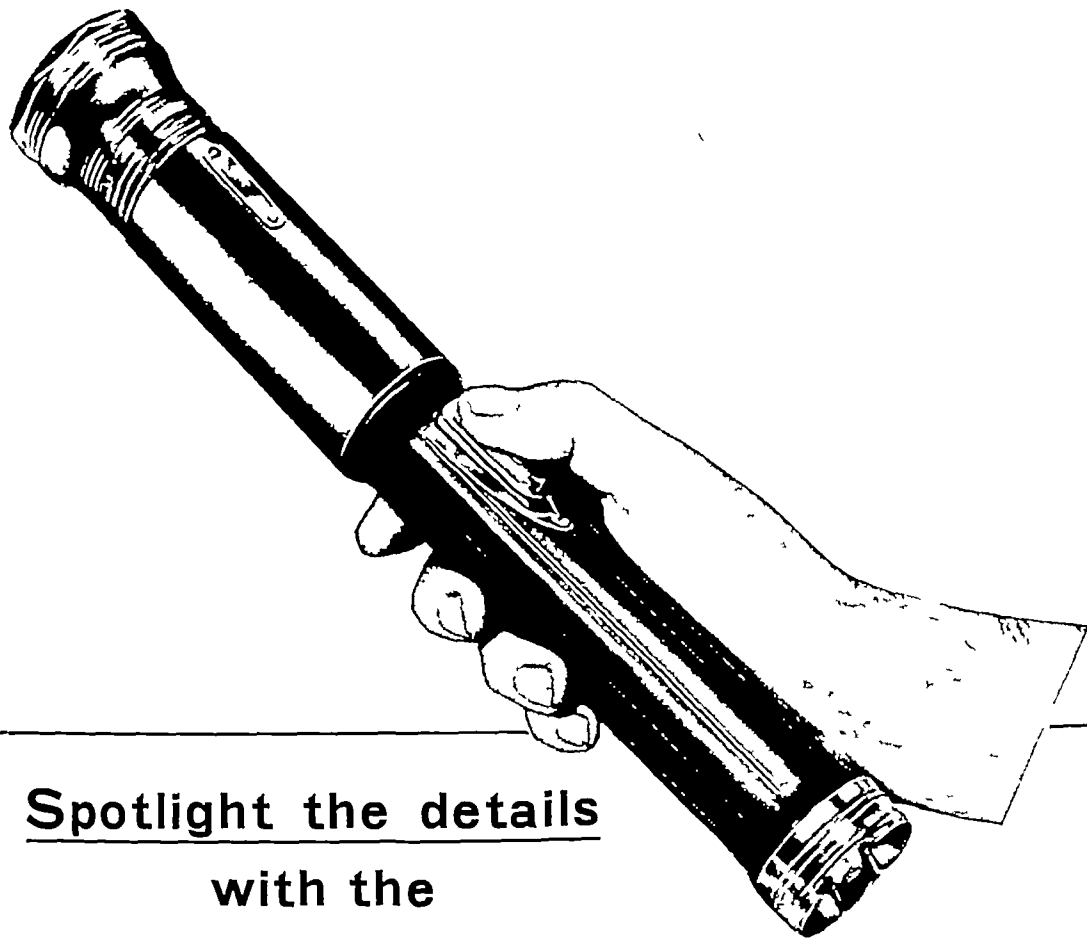
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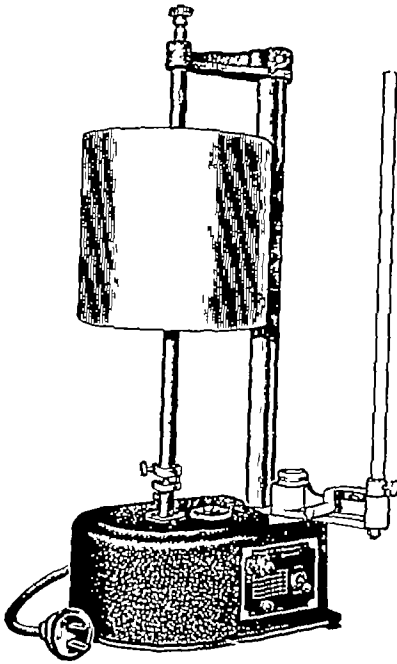
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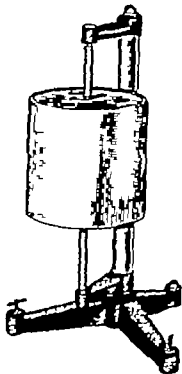


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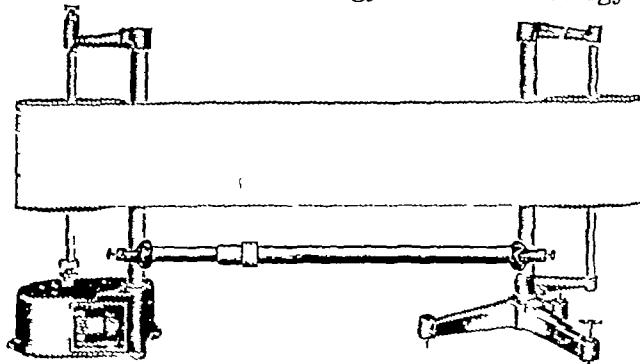
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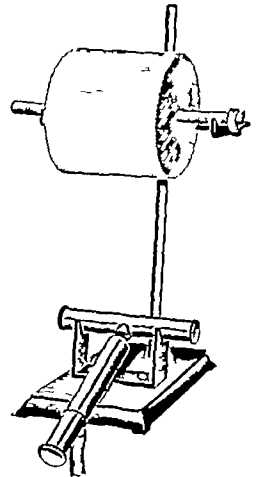
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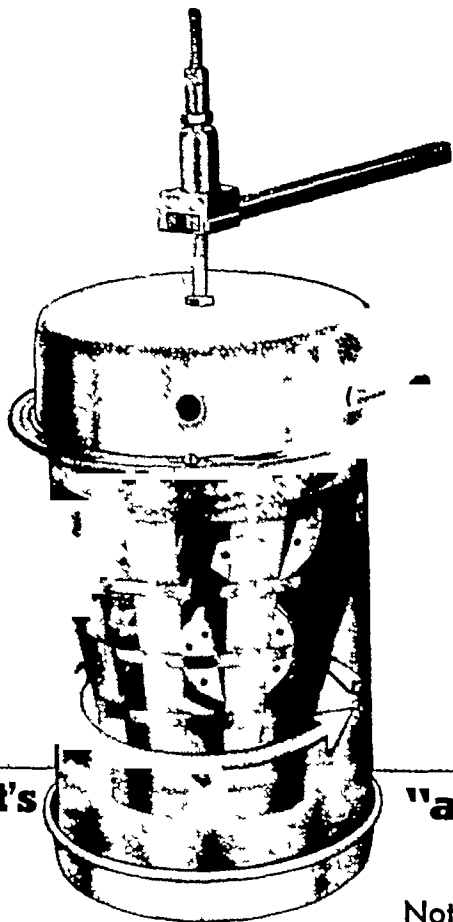
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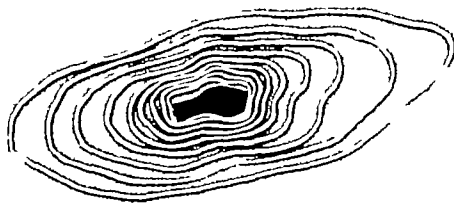
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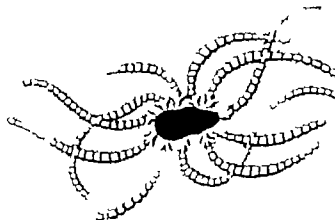
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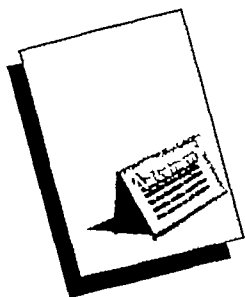
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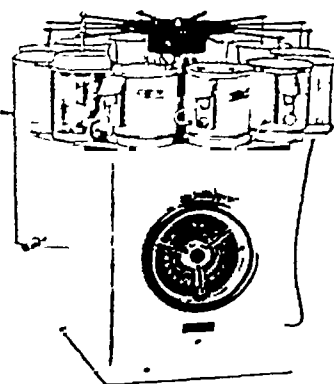
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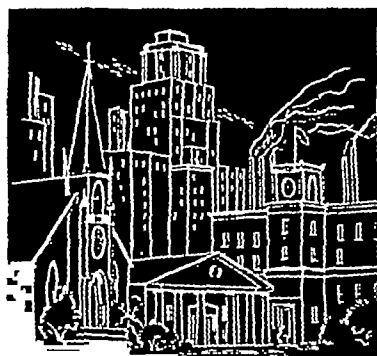
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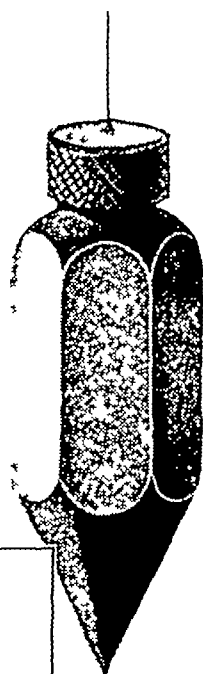
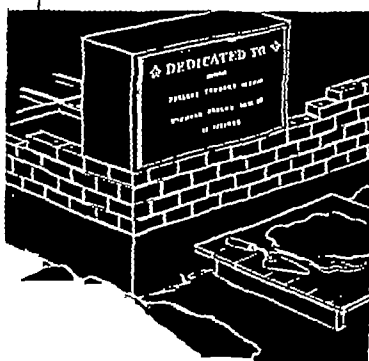


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CATALYTIC ACTION OF THE METAL PEPTIDASES²

EMIL L SMITH

From the Laboratory for the Study of Hereditary and Metabolic Disorders and the Departments of Biological Chemistry and Medicine, University of Utah School of Medicine

SALT LAKE CITY, UTAH

PROTEIN metabolism in the animal begins with proteolysis. The proteolytic enzymes of the gastro-intestinal tract and other tissues hydrolyze the peptide bonds of the proteins to form peptides of varying size and finally free amino acids. It is customary to classify the proteases in two groups: the true proteinases which can attack the high molecular weight proteins and the peptidases which can attack the smaller units, the peptides. Thanks to the work of Bergmann, Fruton and their collaborators (1) we know that this classification is not entirely valid. These investigators have shown that proteinases can hydrolyze simple peptide derivatives. Thus, trypsin attacks such a simple compound as α -benzoyl-L-argininamide at the amide linkage. A more valid classification was initiated by these investigators (1) in distinguishing between the endopeptidases and the exopeptidases. This means that the endopeptidases can attack at the interior of completely or partially substituted peptide chains while the exopeptidases generally attack peptides or their derivatives at bonds adjacent to a free α -amino or α -carboxyl group or both. Hence the terminology which distinguishes between aminopeptidases, carboxypeptidases and dipeptidases. Both the endo- and exopeptidases show a marked specificity toward peptide bonds involving particular amino acids. For example, pepsin attacks compounds like carbobenzoxyglutamylphenylalanine

or carbobenzoxyglutamyltyrosine. The only known compounds which pepsin can attack contain the aromatic rings of tyrosine or phenylalanine residues. Similarly, we speak of leucine aminopeptidase since this enzyme has a markedly rapid action on leucine peptides.

Most investigators agree that enzymes must form definite chemical compounds with their substrates. This thesis, first clearly stated by Michaelis and Menten (2), has been demonstrated by kinetic studies on a large number of enzymes. Keilin and Mann (3) found that peroxidase forms a complex with hydrogen peroxide, and, recently, Chance (4) has studied the spectroscopically well-defined compounds that are formed by catalase and peroxidase with their substrate, hydrogen peroxide. In 1926, Euler and Josephson (5) suggested that the polar groups which are required in the substrates of the peptidases indicate that the enzyme combines with its substrate not only at the sensitive peptide linkage but also with the free polar groups of the substrate, they spoke of this as the di-affinity concept. In other words, the enzyme has a two-point combination with its substrate.

Some years ago, Johnson and his co-workers (6) made the extremely important observation that many of the peptidase activities obtained from animal and plant tissues were greatly increased by the addition of certain metal ions. For example, it was found that leucine aminopeptidase is strongly activated by manganese or magnesium ions. It now appears that the role of the metals in the various peptidase systems is to form the complex between the proteins and the

¹ Detroit, Michigan, April 20, 1949.

² Much of the original work reported in this paper by the author and his collaborators was aided by grants from the United States Public Health Service.

substrates. Evidence of this comes in part from the essentiality of the metal. Leucine aminopeptidase and other peptidases are inhibited by such typical metal poisons as cyanide, sulfide etc. It is also demonstrated by the fact that when the enzyme is purified the activity decreases unless metal ions are added back to the system (7).

The metal does not act simply as an activator of the enzyme but appears to be linked in true chemical combination with the protein of the whole enzyme system. This may be illustrated by the studies on purified leucine aminopeptidase. When protein, metal and substrate are added simultaneously, the activity is, at first, quite low

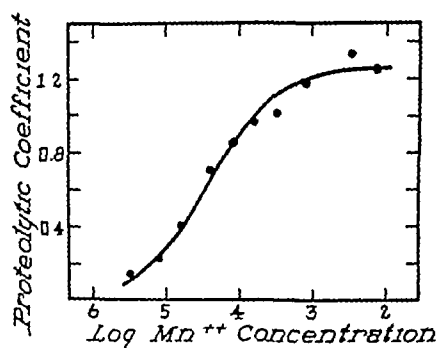


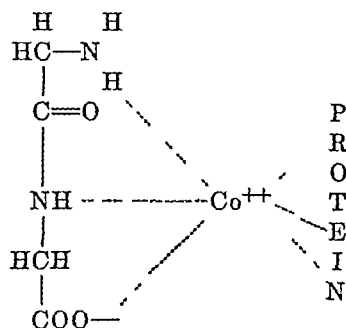
FIG 1 COMBINATION OF LEUCINE AMINOPEPTIDASE AND Mn^{++} . The curve is the theoretical one from the mass law equation for combination of one Mn^{++} per active group of enzyme (8)

with slow hydrolysis of the substrate, this is followed by a rapidly increasing rate of reaction. In contrast to this, if the metal is allowed to stand with the protein for some time before its addition to the substrate, then it is found that the hydrolysis of the substrate proceeds quite rapidly following the usual kinetics of a first-order reaction. The occurrence of such a time-reaction between the metal and the protein must be interpreted as due to formation of a new compound (7). Similar time-reactions have also been found with many other enzymes, e.g., various peptidases, phosphatase and arginase.

If a complex is formed, then the combination between metal and protein should follow the usual mass action law for such a dissociable complex. The effect of Mn^{++} on leucine aminopeptidase does show the type of curve to be expected for the mass law equilibrium (8). The theoretical curve which is drawn through the data in figure 1 indicates the requirement of one atom of manganese for each active group of the enzyme.

An example of how the metal is bound with its

substrate can be shown with the enzyme which has been called glycylglycine dipeptidase (9). From present information it appears that this enzyme is exceedingly specific, it is known to attack only this one peptide, glycylglycine. This enzyme is strongly activated by the addition of cobaltous ions and a definite spectroscopic complex is formed between glycylglycine and cobalt. This suggested that the enzyme-substrate complex might be formed by combination of the metal with the substrate on one hand and with the protein on the other. Therefore, many compounds were tested both for their ability to be hydrolyzed by the enzyme and for their ability to form complexes with cobalt. The structures are indicated in the following diagram.



If one hydrogen on the free amino group is replaced with a methyl group to form the compound sarcosylglycine, there is complex formation between the substrate and the metal and this compound is susceptible to hydrolysis. If, however, both hydrogens on the amino group are replaced to make N-dimethylglycylglycine, this compound is not hydrolyzed by the enzyme nor does it form a complex with cobalt. If the hydrogen at the sensitive peptide bond is substituted by a methyl group to make glycylsarcosine, there is no complex formation with cobalt and no hydrolysis by the enzyme. If the carboxyl group is altered to make glycylglycinamide, a spectroscopic compound is formed with cobalt but there is no hydrolysis by the enzyme. The complex formed between glycylglycinamide and cobalt has its maximal absorption position shifted about 40 $m\mu$ as compared to that given by cobalt and glycylglycine. It is clear that the chelation, the combination between cobalt and glycylglycinamide, must be different from the combination between cobalt and the substrate glycylglycine. This marked parallelism has also been found with a number of other compounds but they cannot all be mentioned here. Perhaps one more would be of

interest in this connection. The compound β -alanylglycine is not hydrolyzed by the enzyme nor does it form a spectroscopic complex with cobalt. It is well known that cobalt tends to make chelate compounds, that is ring compounds in which there are only five members, and that is the type of complex given by glycylglycine and cobalt. With β -alanylglycine a six-membered ring would be necessary but such compounds are very rare in the cobaltous series.

It appears from this, as a first approximation, that the ability of a compound to be susceptible to the action of this enzyme requires that the compound can form a complex with cobalt. Obviously, this is a necessary condition but not a sufficient condition since many peptides, for example, L-leucylglycine, can form complexes with cobalt but these compounds are not hydrolyzed by this enzyme. On the other hand, such a compound as glycyl-L-leucine, the isomeric peptide, does not form a strong complex with cobalt. Thus, there is exhibited the remarkable specificity of a metal ion in forming a strong complex with leucylglycine and a poor complex with glycylleucine. This specificity is indeed at a very low level. While we are all familiar with the concept that proteins are extremely specific in their combination with various substances, it is interesting to find such a specificity at the dipeptide level.

From the probable mode of combination of metal and substrate, we can begin to understand the differences between a dipeptidase and an aminopeptidase. Glycylglycine dipeptidase requires in its substrate a free amino group, a free hydrogen at the peptide nitrogen and a free carboxyl group; it appears that cobalt forms a complex with three valences bound to the substrate and probably with three valences bound to the protein.³ On the other hand, with leucine aminopeptidase it must be assumed that the metal, either manganese or magnesium, combines only with the free amino group and with the nitrogen at the peptide bond. A free carboxyl group is not required since the enzyme can hydrolyze leucinamide just as rapidly as it can the dipeptide, leucylglycine (7). Thus, we have an explanation in terms of the metal binding with

these substrates of the difference between an aminopeptidase and a dipeptidase.

If the general thesis is correct that the exopeptidases require free polar groups in their substrates because the metal combines with these polar groups, then we must examine carboxypeptidase. This enzyme was first isolated in crystalline form by Anson (10) some years ago, but no attempt appears to have been made to ascertain whether a metal is present. It was found (11) that carboxypeptidase is strongly inhibited by such typical metal poisons as cyanide, sulfide, pyrophosphate, orthophosphate, citrate, oxalate, etc. There is, therefore, a strong indication that a metal is present in carboxypeptidase. Examination, in a sensitive spectrograph, of the ash of a large amount of crystalline carboxypeptidase showed that the only metal present in significant amounts was magnesium. It was extremely gratifying that those metal ions that were used during the purification of the enzyme could not be detected in the final ashed material.

The first part of the thesis is confirmed in the sense that these exopeptidases, or the majority of them, appear to be metal proteins. Unlike the substrates for aminopeptidases and dipeptidases, the substrates for carboxypeptidase do not contain free amino groups. The best substrates for carboxypeptidase are those in which the amino group is blocked by an acylating group (12). For example, a typical substrate is carbobenzoxyglycyl-L-phenylalanine. The terminal carboxyl group is essential for the action of the enzyme. The peptide hydrogen is not essential for the action of this enzyme as is shown by the fact that the enzyme can hydrolyze such a compound as carbobenzoxytryptophylproline (13). Since proline is an imino acid, there is no hydrogen at the peptide bond. The unessential character of the peptide nitrogen is shown very strikingly by the discovery of Snoise, Schwert and Neurath (14) that carboxypeptidase can hydrolyze esters of the correct configuration, such a compound as hippurylphenyllactic acid is hydrolyzed by carboxypeptidase. Therefore, if it is assumed that the metal combines with the substrate in carboxypeptidase, the metal must bind with the free carboxyl group and with the keto group at the peptide bond and not with the nitrogen at all. An interesting correlation appears from this in that the chelate rings formed by the metal with the substrate in each case involve linkages at opposite sides of the sensitive peptide bond (15). It would

³ It had previously been assumed that Co^{++} possessed a coordination number of four in the enzyme-substrate complex (9). A more reasonable description of the data is given by assuming that Co^{++} is bound in a six-coordinate complex.

appear that the explanation of the distinction between the three kinds of exopeptidases that have been discussed is in terms of the nature of the groups bound by the metal in each enzyme

Since the metal in carboxypeptidase is tightly bound, it becomes possible to do quantitative inhibition studies in order to determine how many valences of the metal are available for combination with the substrate or, alternatively, for combination with an inhibitor which binds with the metal. It was found that each active group of carboxypeptidase is capable of binding two cyanide atoms or one orthophosphate or one sulfide

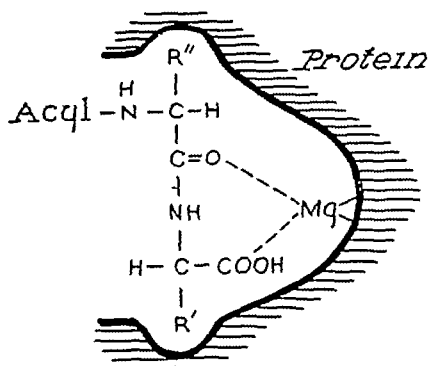


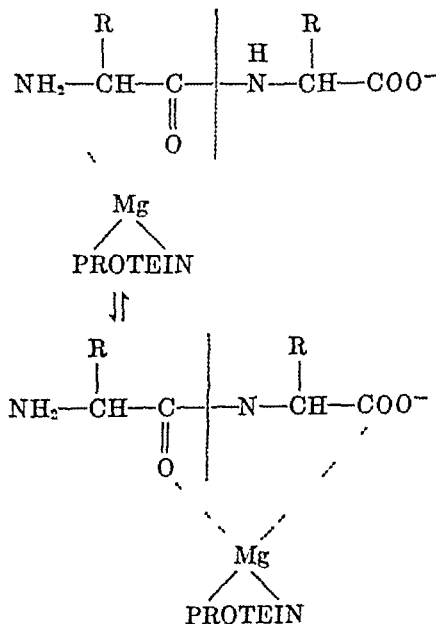
Fig 2 DIAGRAM INDICATES SPATIAL RELATIONSHIPS of the binding of an acylated dipeptide by carboxypeptidase

From these data it would appear that there are two free valences available for combination with the substrate or with an inhibitor (11, 15). This, then, is the true di-affinity as first defined by Euler and Josephson, but it may now be ascribed to the characteristic binding with the metal in the enzyme.

Obviously, in addition to the binding of the substrate by the metal through the polar groups, there are additional binding forces exerted by the protein moiety of the enzyme through the side chains of the substrate. For example, carbobenzyglycylglycine is a poor substrate for carboxypeptidase, but carbobenzyglycylphenylalanine is an excellent substrate (12). This indicates that the protein of carboxypeptidase binds very strongly with the terminal phenyl residue, and we can speak of this as the R group binding by the protein.

The polyaffinity concept first suggested by Bergmann and his co-workers (16) indicates that there must be a correct steric relationship between the polar groups of the substrate and the side chain branches of the substrate. Hence,

carboxypeptidase hydrolyzes only compounds in the L series and not in the D series (17, 18). A simple diagram (fig 2) may be drawn to indicate this polyaffinity relationship for carboxypeptidase. The polar groups are bound through the metal, presumably magnesium, and the R groups are bound by the protein. From the polyaffinity concept and the knowledge that the metal is concerned in binding the polar groups, a beginning may be made in explaining the substrate specificities and inhibition properties of this enzyme. It is known that compounds which have free amino groups are either not hydrolyzed at all or are acted upon extremely slowly by this enzyme (12). It may be supposed that the metal has a greater affinity for the amino group than it does for the terminal carboxyl group, and a different type of complex is formed than in the usual enzyme-substrate complex. Such a combination with a free dipeptide is not a fruitful combination in that the substrate is not hydrolyzed. An explanation for the lack of action is that the linkages are not on opposite sides of the peptide bond but are on the same side (15).



In the same way we can explain why an L-amino acid like L-phenylalanine is not an inhibitor for carboxypeptidase, the combination given by an L-amino acid with the enzyme would be a weak one since no chelate rings are formed. However, Elkins-Kaufman and Neurath (19) found that D-amino acids are strong competitive inhibitors of this enzyme. We must suppose that the reason for this is that in the D-amino acid the R

group binding is extremely strong and that with this orientation a carboxyl group is not presented to the enzyme, but instead, a free amino group (15)

The validity of this concept can be partially tested by inhibition studies of various kinds. For example, the enzyme may be inhibited by blocking the metal portion of the enzyme or by blocking the R group position or both. Ideally, we should expect that the strongest inhibitors would be those that would bind both with the metal and with the R group position of the protein. From the type of scheme that has been indicated, we can predict the type of substances which would be expected to inhibit this enzyme. The sensitivity of the known substrates indicates that the strongest binding at the R position of the protein is given by those compounds that contain aromatic residues (12, 13, 17). It is also to be expected that those compounds that contain carboxyl groups would bind with the metal. It was found that benzylmalonic acid is such a compound. It has two carboxyl groups and it has a side chain much like that of phenylalanine, and it is indeed a strong inhibitor of carboxypeptidase (20). It was found that one mole of benzylmalonic acid combines with each active group of the enzyme. Interestingly enough, the unsubstituted dicarboxylic acid, malonic acid, is only an extremely poor and doubtful inhibitor of carboxypeptidase. Malonic acid at 0.5 M gives only slight inhibition in contrast to the 50 per cent inhibition produced by benzylmalonic acid at 0.003 M. Similarly, other compounds which can block the R group position and which bind with the metal also proved to be strong inhibitors. Such a compound as benzoic acid is a weak inhibitor but phenylacetic acid is quite a strong inhibitor. Likewise, indolacetic acid and indolpropionic acid have been found to be strong inhibitors of carboxypeptidase. From a series of inhibitors of different configuration, we can begin to evaluate and understand the precise limits of the configurations required for binding with the R group and with the metal simultaneously. We can measure the distances in terms of the number of carbon atoms between the functional groups, we can vary the substituents on the aromatic ring, and we can vary the kinds of functional groups. Such studies are now in progress.

What further can be done with these ideas of metal binding with the substrate? As we have indicated the normal enzyme-substrate combination occurs through chelate ring formation of the

metal and the polar groups of the substrate. We must assume, as is known from many studies of inorganic and organic compounds, that the chelation is extremely strong indeed and that where ring formation is not possible, only weak combination occurs. How strong the chelation is between metals and amino acids or peptides is beautifully illustrated by an observation made by Neuberg and Mandl (21). They found that the metal ions known to be important in biological systems could not be precipitated as metal sulfides when amino acids or peptides were present. Moreover, they found that when an amino acid or peptide was added to an insoluble sulfide like zinc sulfide, the sulfide went back into solution. This indicates that the combination of the metal with amino acids or peptides is extremely strong indeed, and such a strong combination must be what is occurring in the enzyme-substrate complexes of the peptidases. The combination in the enzyme suggests that a strong electronic pull is exerted by the metal through the polar groups of the substrate to produce an electronic distortion or deformation at the sensitive peptide linkage (15). This deformation is presumably made still stronger through the combination of the metal with the protein, in other words, we must assume that the rôle of the protein, or part of the rôle of the protein in enzymes, is to make the metals even more electropositive than they normally are. The strong electropositive character increases the deformation at the peptide bond and that is what we mean, then, by the lowering of the free energy of the activation. In other words, the rôle of the enzyme as a catalyst is to lower the free energy of activation, and we can assume that this is caused in some way by the electronic pull through the metal. If this hypothesis is true, the real hydrolysis of the peptide bond is probably due to the acidic or basic catalysis of the hydrogen and hydroxyl ions of the aqueous solution. Hydrogen and hydroxyl ions can catalyze the hydrolysis of the peptide bonds. Normally, this hydrolysis occurs only in strongly acid or strongly alkaline solutions, but with a lower free energy of activation through the mediation of the enzyme, this catalysis now occurs in neutral solution.

As a consequence, the enzymatic hydrolysis of the peptides should follow many of the relationships which govern acid and base catalysis. For example, it is well known that with a series of related esters or amides the rate of the hydrolysis is a function of the strength of the acid which is

linked in the ester or amide bond (22) Examination of known substrates for carboxypeptidase shows this relationship to hold For example, chloroacetylphenylalanine is hydrolyzed very much more rapidly than acetylphenylalanine Obviously, chloroacetic acid is a far stronger acid than acetic acid Similarly, it was reported last year (23) that carbobenzoxy- β -alanylphenylalanine is hydrolyzed about a thousand times more slowly than carbobenzoxyglycylphenylalanine Again it has been found that carbobenzoxy- β -alanine is a much weaker acid than carbobenzoxyglycine (20) In other words, a substantial portion

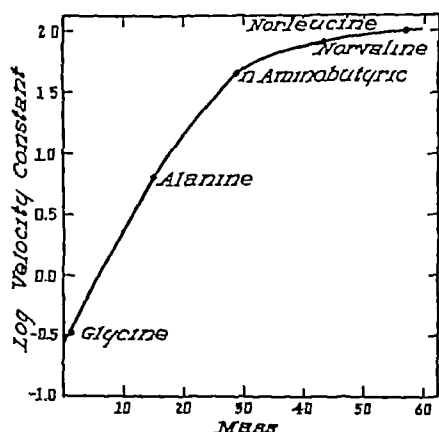


Fig 3 ACTION OF LEUCINE AMINOPEPTIDASE ON various aliphatic amino acid amides The logarithm of the relative first order velocity constant is plotted as a function of the mass (molecular weight) of the side chain bound to the alpha carbon The initial slope indicates that the rate of hydrolysis is directly proportional to the mass (24)

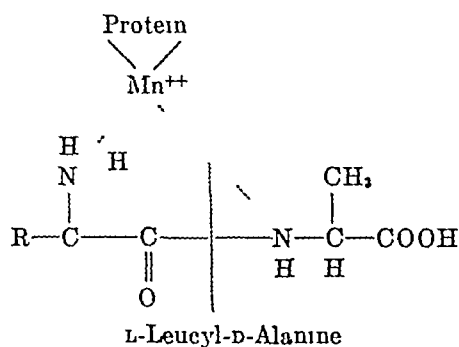
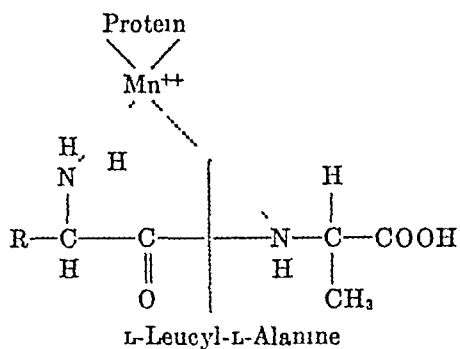
of what has been called the specificity of an enzyme is not specificity at all but is a function of the free energy of the bond which is exposed to the action of the enzyme

Thus, a beginning may be made in evaluating a number of distinct factors concerned in the specificity of these enzymes First of all, as already indicated, there is the di-affinity concept of Euler and Josephson We interpret this as the ability of the metal to form a chelate complex with the substrate Secondly, there is the polyaffinity concept of Bergmann and his collaborators The R groups must lie in a definite spatial position in relation to the polar groups This is a second factor in specificity A third factor, which was mentioned just a moment ago, is the free energy of the sensitive bond in terms of the acid strength of the carboxyl group which is linked in

the peptide bond Correspondingly, the energetic contribution of the amino group, imino group or ester group will also play a rôle in determining the rate of hydrolysis of such substrates

A fourth factor may be termed the true specificity as determined by the protein, namely, the binding of the R group by the protein itself and the contribution made by this binding in terms of lowering the free energy of activation The R group specificity has been measured very carefully with leucine aminopeptidase (24) Here we find, in contrast to former assumptions that this peptidase attacks only leucine compounds, that this enzyme hydrolyzes a variety of peptides containing different aliphatic residues The simplest compounds are the homologous aliphatic amino acid amides and strictly comparable data have been obtained for their hydrolysis It appears from these data (fig 3) that the binding by the protein of the side chain is a function of the mass of the side chain in terms of its molecular weight This suggests that the binding forces involved may perhaps be those of Van der Waal's forces since it is difficult to visualize other forces which are capable of binding aliphatic side chains Here is a fourth effect which determines the specificity of these enzymes

A fifth factor is one which appears to be a steric effect With leucine aminopeptidase we find that L-leucinamide is hydrolyzed very rapidly, on the other hand, D-leucinamide is hydrolyzed at less than one-tenth thousandth of the rate (7, 24) We cannot fix this in absolute terms since it is almost impossible to make absolute kinetic measurements involving such a slow hydrolysis The difference in rates between these L and D compounds is obviously due to the polyaffinity relationship, namely, the isobutyl side chain of the D-amino acid amide lies in such a position that it cannot combine with the protein at the same time as there is a combination of the amino group and the peptide nitrogen with the metal When the relative rate of hydrolysis of L-leucyl-L-alanine is compared to L-leucyl-D-alanine a different optical effect is observed Here the L-compound is hydrolyzed about 25 times more rapidly than its diastereoisomer, L-leucyl-D-alanine (24) Since the residue attached to the nitrogen of the sensitive peptide bond does not appear to have any affinity for the protein, this must be interpreted as a steric effect in which the methyl group of the D-alanine lies in such a position as to interfere with the combination of the metal with the peptide nitrogen



The residue attached to the peptide nitrogen does not seem to be bound by the protein because the rates of hydrolysis for a variety of compounds are almost identical, L-leucinamide, L-leucylglycine, L-leucyl-L-alanine, and L-leucyl- β -alanine are all hydrolyzed at the same rate so that the presence or absence of a carboxyl group or the change in position from an α -amino group to a β -amino group has no effect whatsoever. Thus, there is a fifth effect which is steric and which can influence the specificity of these enzymes.

To summarize, it may be remarked that while most earlier concepts of enzyme specificity were represented almost exclusively in terms of vague concepts such as lock and key mechanisms, we can now utilize the available information to evaluate separately at least five factors which determine the specificity of an enzyme. Undoubtedly, many other factors will also be discovered. The peptidases represent an extremely favorable group of enzymes in which to investigate these factors because the genius of Emil Fischer and Max Bergmann has given us the techniques for varying the potential substrates almost at will.

Our concepts of how enzymes work has been presented for the peptidases because we seem to be in a better position to do so with this group of enzymes. Obviously, two questions emerge in terms of the general theory which has been presented. First of all, do these concepts apply to other metal enzymes? Perhaps the simplest illustration is to how they apply may be shown with

carbonic anhydrase (15). Here is an enzyme which contains zinc as the active metal as shown by the work of Keilin and Mann (25). The substrate, bicarbonate ion, is extremely simple in character and there can be no great concern about R group binding with the protein. In fact, this factor may be neglected completely and we may suppose that zinc forms a chelate complex with the protein on one hand and with two of the oxygens of bicarbonate ion on the other. Since the bicarbonate ion has a resonating structure it doesn't matter which two are postulated. Hydrolysis is obviously a basic catalysis. After the splitting occurs, carbon dioxide is bound to one valence of the zinc and hydroxyl ion with the other. Since this enzyme is reversible in its action, these are the bindings that lead to the formation of bicarbonate ion. It should be emphasized that the types of linkages that have been postulated are not hypothetical. Zinc binds strongly with carbonate and zinc carbonate is an extremely insoluble substance with little tendency to dissociate. Likewise at pH 7.0 it is well-known that zinc forms subcarbonates which are mixed compounds containing both hydroxyl ions and carbonic acid. Thus, the types of linkages that have been assumed for the reversible action of carbonic anhydrase are identical with those known to exist for ordinary inorganic combinations of zinc.

Similarly, the β -keto-carboxylic acids like oxalosuccinic acid and oxaloacetic acid form beautiful spectroscopic chelate complexes with metal ions as reported by Kornberg, Ochoa and Mehler (26). In fact, with some of these compounds the combination with the metal is sufficient to lower the free energy of activation enough so that decarboxylation proceeds. With others, the protein is required in order to make the metal more electropositive and to lower the free energy of activation still further. Oxalosuccinic acid is rather unstable at pH 7.0 in the absence of any outside agents, adding a trace of metal which forms a chelate complex lowers the free energy of activation enough to allow the decarboxylation to proceed. On the other hand, acetoacetic acid is rather stable in aqueous solution and combination with metal alone does not lower the free energy of activation sufficiently. Here the specific protein is required. In a similar manner, these concepts of the chelate complexing of metals may be applied to various other metal enzymes such as the phosphatases, phosphorylases, etc.

A second question is, what about those enzymes

which do not contain metal ions. The discovery of the metal in carboxypeptidase represents an object lesson in our type of thinking. Perhaps other enzymes which are known to exist in fairly homogeneous form should be tested for the presence of metal ions. On the other hand, there are many enzymes like crystalline trypsin, which do not contain metal ions. We examined the action of various substances on crystalline trypsin and could not find any inhibition whatsoever by the usual metal poisons (11). Here our thinking must be different.

Since it is probable that all enzymes function by a fundamentally similar mechanism, let us assume that the chelate character of the enzyme-substrate combination is correct, and that the lowering of the free energy of activation is due in each instance to electronic deformation. Then, where there is no metal present, the rôle of the

metal ion must be taken over by other groups within the protein itself. There are extremely electropositive and electronegative groups in proteins. It would be useless at the present time to speculate as to the nature of these active groupings in the various enzymes. This is an experimental task for the future. However, it is well to recall at this point the extremely valuable and fundamental observations of Bergmann, Fruton and their collaborators (1) on the synthetic substrates of trypsin and other proteinases. Trypsin hydrolyzes synthetic peptide derivatives which contain free polar groupings, benzoylargininamide contains a free guanido group, benzoyllysineamide contains a free epsilon amino group. We must suppose that there is a combination between these polar groupings and groups at the peptide linkages with as yet unknown groups in the enzymes.

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UPTAKE OF LABELED AMINO ACIDS BY TISSUE PROTEINS IN VITRO¹

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THE uptake of labeled amino acids by tissue proteins has been construed by workers in this field to be the same as protein synthesis. We shall return to this topic later. Assuming, for the moment, this to be true, there can be no question that those who have entered the field were influenced by the work of Schoenheimer and his colleagues (1) who proved that the rate of protein degradation and resynthesis *in vivo* was much faster than was envisaged in the theory of endogenous and exogenous protein metabolism. We, in the Pasadena laboratory, had come to this conclusion some years earlier (2). Our evidence was mainly indirect, some additional evidence came from crude labeling experiments, and some was inferred from data on the free energy of formation of the peptide bond. We referred to the dynamic steady state of breakdown and synthesis of protein as the 'continuing metabolism' of nitrogen. The response to this view, generally, was unsympathetic. The direct and incontrovertible evidence came from the experiments of Schoenheimer and his colleagues on the feeding of N¹⁵ labeled amino acids and ammonia. This work and the clearing up of the problems of creatine (3-5) and of creatinine (6-8) formation cut away all the support of the theory of endogenous and exogenous metabolism of protein.

We were emboldened then to look with labeled amino acids for evidence of protein synthesis *in vitro*.

In vitro studies employing tracers appear to offer the best chance, at present, of gaining some insight into the mechanism of the biological synthesis of protein. The following considerations indicate the experimental problem of finding evi-

dence of protein synthesis *in vitro*. The minimum duration of the *in vivo* experiments with N¹⁵ labeled amino acids was 3 days, *in vitro* the maximum time is about 6 hours. On the basis that the rates *in vitro* would be the same as *in vivo* the concentrations of labeled amino acids likely to be in a tissue protein were, therefore, in the *in vitro* experiments 1/12 or roughly 10 per cent of those *in vivo*. Furthermore, whereas the whole organs or carcasses of several animals are available for analysis after an *in vivo* experiment, only 100 mg of protein or less may be available after *in vitro* experiments, especially where one wishes to make a series of comparisons. Roughly, then, the amount of a labeled amino acid to be measured after an *in vitro* experiment is about 1 per cent or less of that after an *in vivo* experiment. With radioactive isotopes such as C¹⁴ and S³⁵ this can be done easily. One tenth of a microgram of a labeled amino acid with a specific activity of 100,000 count/min/mg can be measured. We have been able to use leucine and lysine with specific activities as low as 6000 counts/min/mg.

C¹⁴ is the isotope which has been used most in *in vitro* work so far, it is especially convenient because of its long half life of 5100 years and ease of measurement. S³⁵ has a half life of 87 days. Tritium has been made available recently, its half life is 31 years.

Table 1 gives some figures on the amounts of different labeled amino acids taken up *in vitro* by intact cells of different animal tissues. Animal tissue slices or cells were suspended in a saline solution containing the labeled additive and incubated at 36 to 38°. The experimental time varied from 2 to 6 hours, the figures given represent approximately the maximum uptake and indicate the orders of magnitude in the longest time it is useful to run experiments of this kind.

The table shows that both indispensable and dispensable amino acids are taken *in vitro* by tissue proteins. Every tissue so far tested has

¹ The experimental data of the authors presented here were obtained in a project under the joint sponsorship of the United States Atomic Energy Commission and the Office of Naval Research.

taken up every labeled amino acid presented to it

Of the normal tissues so far investigated the uptake was fastest in bone marrow cells. The rates were slower and approximately equal in liver slices and in diaphragm. It is interesting that the CO_2 of bicarbonate is so rapidly taken up

TABLE 1 UPTAKE OF LABELED AMINO ACIDS BY INTACT CELLS IN VITRO

REFERENCE	LABELED ADDITIVE	AMT. OF LABELED AMINO ACID/GM. PROTEIN
<i>Rat liver slices</i>		
(9)	NaHCO_3	0.76 mg Aspartic + glutamic
(10)	DL Alanine	0.15 mg Alanine
(11)	DL Methionine	0.0069 mg Methionine + 0.0028 mg Cysteine
<i>Rat Diaphragm</i>		
(12)	L Leucine	0.23 mg Leucine
(12)	Glycine	0.15 mg Glycine
(12)	L-Lysine	0.054 mg Lysine
<i>Rabbit Bone Marrow Cells</i>		
(12)	L Leucine	1.15 mg Leucine
(12)	L Lysine	0.67 mg Lysine
(12)	Glycine	0.56 mg Glycine
<i>E. Coli, Non proliferating</i>		
(13)	DL-Methionine	0.58 mg Methionine

TABLE 2 UPTAKE OF AMINO ACIDS BY NORMAL, ADULT AND OTHER TISSUES

REFERENCE	LABELED ADDITIVE	AGE	COUNTS/MIN/MG PROTEIN
<i>Rat Liver Homogenate</i>			
(15)	Glycine	Fetal	375
		Adult	11
<i>Rat Liver Slices</i>			
(10)	DL-Alanine	Hepatoma	1.12 mg alanine
		Fetal	0.88 mg alanine
		Normal	0.15 mg alanine

into the dicarboxylic acids of proteins. The rapid uptake of labeled amino acids by bone marrow cells and diaphragm indicate that these tissues may be useful biological objects for a variety of physiological and pathological studies.

The figures on the amounts of labeled amino acids taken up have, as they stand, only an operational significance. From figures such as these one can calculate how many counts one is likely to find in the protein. A more relevant presentation of the results is as percentage of the labeled amino acids in the protein which had become

labeled, and to compare those figures with comparable ones obtained by Schoenheimer and his colleagues with N^{15} labeled amino acids *in vivo*. For example, the latter workers found that after feeding N^{15} labeled leucine to a rat for 3 days 8 per cent of the leucine in the liver proteins was labeled (1), we may say, therefore, that in 4 hours about 0.5 per cent was labeled. Our figure for bone marrow cell proteins *in vitro* in 4 hours is 1 per cent. In rat liver slices Melchior and Tarver

TABLE 3 UPTAKE OF AMINO ACIDS BY INTACT AND BROKEN CELLS

REFERENCE	LABELED ADDITIVE	HOW BROKEN	UPTAKE IN INTACT CELLS UPTAKE IN BROKEN CELLS RATIO
<i>Rat Liver</i>			
(11)	DL Methionine	Homogenized	4 to 41
(10)	Glycine	Homogenized	3
<i>Rat Diaphragm</i>			
(12)	Glycine	Homogenized	4
(12)	L-Leucine	Homogenized	4
(12)	L Lysine	Homogenized	5
<i>Rabbit Bone Marrow</i>			
(12)	Glycine	Cytolyzed with water	No uptake in broken cells
(14)	L-Leucine	Cytolyzed with water	No uptake in broken cells
(12)	L Lysine	Cytolyzed with water	No uptake in broken cells
(12)	Glycine	Lyophilized	No uptake in broken cells
(12)	L-Leucine	Lyophilized	No uptake in broken cells
(12)	L Lysine	Lyophilized	No uptake in broken cells
(12)	Glycine	Frozen and thawed 6 times	No uptake in broken cells
(12)	L-Leucine	Frozen and thawed 6 times	No uptake in broken cells
(12)	Glycine	Cytolyzed with ether	Almost no uptake
(12)	L-Leucine	Cytolyzed with ether	Almost no uptake

found 0.3 per cent of the methionine labeled in 4 hours, Zamecnik *et al.* found 0.23 per cent of the alanine labeled in 4 hours and 5 times this amount in hepatoma cells. The uptake of L-lysine by liver homogenate is about as fast as *in vivo* (14). These estimates, rough as they are, are sufficient to indicate that the uptake of labeled amino acids by tissue proteins is of the same order of magnitude *in vitro* as *in vivo*.

The uptake of labeled amino acids is much faster in embryonic and malignant than in normal adult tissues (table 2). Rabbit bone marrow cells which have the fastest uptake of any tissue in normal animals so far tested belong, of course, with embryonic tissues.

Mechanical damage to cells reduces the rate at which their proteins take up labeled amino acids

(tables 3 and 4) The important point is that liver homogenate (table 5) does take up glycine, leucine and lysine at a considerable rate. The rate of uptake of glycine by an homogenate is, to be sure, slower than in liver slices, but only one third slower, and the rate of uptake of lysine is as fast as *in vivo*. The uptake of lysine by liver homoge-

TABLE 4 UPTAKE OF AMINO ACIDS BY LIVER HOMOGENATES EFFECT OF VARIOUS TREATMENTS

REFER ENCE	LABELLED ADDITIVE	TREATMENT	RELATIVE ACTIVITY OF PROTEIN	
<i>Rat</i>				
(10)	Glycine	None	100	
		Homogenized with dis tilled water	19	
		Frozen and thawed 6 times prior to being homogenized	6	
<i>Guinea Pig</i>				
			WHOLE HOMOGENATE pH 6.2	SEDI- MENTED FRACTION pH 7.5
(12)	L Lysine	None	100	100
		Ether	78	72
		Frozen and thawed 6 times	110	90

TABLE 5 UPTAKE OF AMINO ACIDS BY WHOLE LIVER HOMOGENATE AND SEDIMENTED FRACTION

REFERENCE	LABELLED ADDITIVE	LIVER FRACTION	AMT. OF AMINO ACID*/GM. PROTEIN
<i>Rat</i>			
(16)	Glycine	Whole	0.15 mg Serine + glycine
	Glycine	Sedimented at 2500 g	0.25 mg Serine + 0.04 mg Glycine
<i>Guinea Pig</i>			
(14)	L Lysine	Whole	0.13 mg Lysine
	L-Lysine	Sedimented at 2500 g	0.33 mg Lysine
(12)	Glycine	Sedimented at 2500 g	0.13 mg Glycine + serine
	L-Leucine	Sedimented at 2500 g	0.11 mg Leucine

nate appears to be a special case of which we shall have more to say later.

But an homogenate is neither an enzyme solution nor a suspension of insoluble protein. A large fraction of the suspension consists of organized particles such as nuclei and mitochondria. When working with homogenates we are not free of organized structures, and the level of organization of the particles is much higher than even that of proteins.

Tissues differ in the rates at which they take

up different amino acids (table 6). The differences in the absolute rates are much greater than that of the relative rates. Thus bone marrow cells take up the 3 amino acids five times as fast as diaphragm although the order is the same.

In the one case tested so far, that of lysine, the L-isomer is taken up by liver homogenate protein much faster than the unnatural D-form (table 7). A part *certainly*, and possibly *all*, of the effect with the D-lysine may be ascribed to the small amount of the L-form in the D-preparation used.

In the course of an experimental run the rate of uptake of labeled amino acids by rabbit marrow cells and rat diaphragm is nearly linear up to 3

TABLE 6 RELATIVE UPTAKE OF GLYCINE, L-LEUCINE, AND L-LYSINE BY DIFFERENT TISSUES

RABBIT BONE MARROW CELLS		RAT DIAPHRAGM		GUINEA PIG LIVER SEDIMENTED FRACTION	
(12)	L Leucine 100	(12)	L Leucine 100	(12)	L Lysine 100
	L-Lysine 73		L-Lysine 79		Glycine 34
	Glycine 51		Glycine 34		L-Leucine 29

TABLE 7 UPTAKE OF L-LYSINE AND D-LYSINE BY GUINEA PIG LIVER HOMOGENATE

REFERENCE	LABELLED ADDITIVE	ACTIVITY OF PROTEIN COUNTS/MIN/MG. PROTEIN
(14)	L-Lysine	2.5
	D-Lysine ¹	0.6

¹ Contained 7-15% L-lysine

to 5 hours (fig. 1). In the case of glycine (fig. 2) with liver homogenate the rate is logarithmic up to 6 hours (when uptake is plotted against the logarithm of the time a straight line is obtained). In all of these cases whether the rate is linear or logarithmic it does not decline appreciably up to 3 to 4 hours. *Lysine* in liver homogenate again is an exception, the reaction comes to a stop in 2 hours (fig. 2).

The dependence of the rate of uptake on the initial concentration of labeled amino acid is in all cases but one up to a concentration of 50 mg per cent, (approximately 0.005 molar) logarithmic (figs. 3, 4 and 5). High concentrations may be somewhat inhibitory, as has been observed in other enzymatic reactions with amino acids. The exception again is the uptake of lysine by liver homogenate (fig. 5). The relation of uptake to initial concentration of labeled lysine is in contri-

distinction to all the other cases linear instead of logarithmic

The important point in the data of figures 3, 4 and 5 is that the concentrations of labeled amino acids from which tissues can incorporate them into their proteins are of the order of those in the blood. And the dependence of the rate of uptake on the concentration of labeled amino acid is greatest in the physiological range of concentration

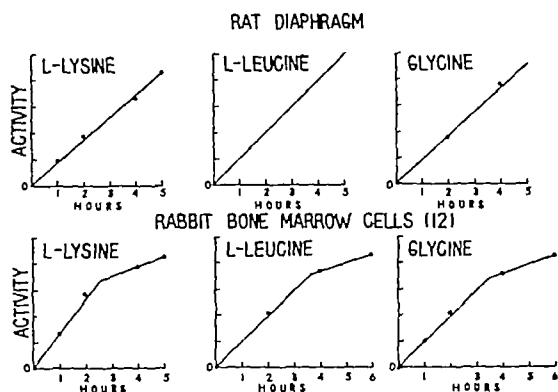


FIG 1 RATES of uptake of amino acids (12)

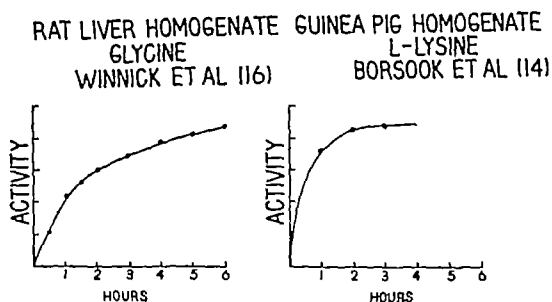


FIG 2 RATES of uptake of amino acids

Some years ago we determined the free energy of formation of the peptide bond in a number of simple di- and tri-peptides. It was clear that over the pH range from 4 to 8 and at any practical amino acid concentration the equilibrium position was beyond 99.9 per cent hydrolysis of the peptide. Ionization constants and solubilities were taken into account. Peptide synthesis, under physiological conditions, could not, therefore, be a simple mass action reversal of hydrolysis (2). The process must be coupled with an energy donating reaction. Most of the protein in the cell is unhydrolyzed, and not because the system is inert, Schoenheimer and his colleagues proved that the proteins of all tissues are continually being broken down and resynthesized. At thermodynamic equilibrium the protein of the body

would be practically all hydrolyzed if not coupled with energy donating reactions. The steady state with regard to proteins is maintained where it is by the respiration of the cell.

It was to be expected, therefore, that if the uptake of the labeled amino acids by tissue proteins

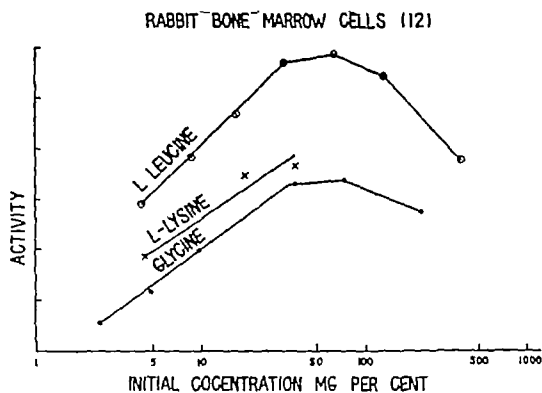


FIG 3 EFFECT ON UPTAKE of concentration of amino acids. The abscissa is on a logarithmic scale, ordinates for each curve are fractions of the maximum

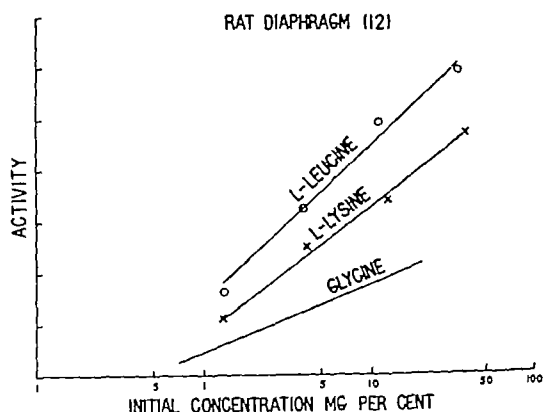


FIG 4 EFFECT ON UPTAKE of concentration of amino acids. The abscissa is on a logarithmic scale, ordinates for each curve are fractions of the maximum

is, indeed, peptide synthesis that this process would be inhibited by inhibition of respiration. Figure 6 shows that this is the case. The effects of dinitrophenol and of arsenate suggest that respiration promotes peptide synthesis by way of phosphorylation.

The foregoing observations are in accord with those of all other workers. Thus Melchior *et al* (13) found that the uptake of methionine by *E. coli* was inhibited by azide, fluoride and cyanide, Winnick *et al* (16) found that oxygen consumption and glycine uptake by rat liver homogenate went together and that the process was inhibited by

anaerobiosis Frantz *et al* found that anaerobiosis (17) and dinitrophenol (18) inhibited the uptake of alanine by rat liver slices

The exception, again, is the uptake of lysine by the proteins of guinea pig liver homogenate Table 8 summarizes some of the major differences in the process of lysine uptake by liver and that of all other amino acids in all tissues so far examined Lysine is taken up by at least two differ-

occur In the latter case most of the free energy of the bond is retained in a complex of the enzyme and one cleavage product Another cleavage product may then recombine with that on the enzyme and so reconstitute a peptide bond, and this process would be independent of respiration

We have surveyed the effects of metal ions such as cobalt, copper and manganese on the one hand because of the well known effects of cobalt and of copper on hemoglobin and red cell formation, and

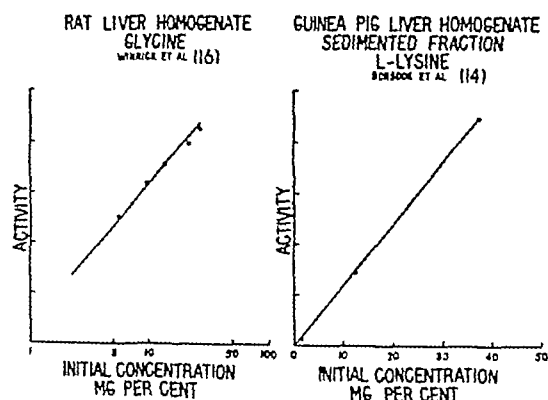


FIG 5 EFFECT ON UPTAKE OF CONCENTRATION OF amino acids The abscissae for the glycine and lysine curves are logarithmic and linear, respectively, ordinates are fractions of the maximum

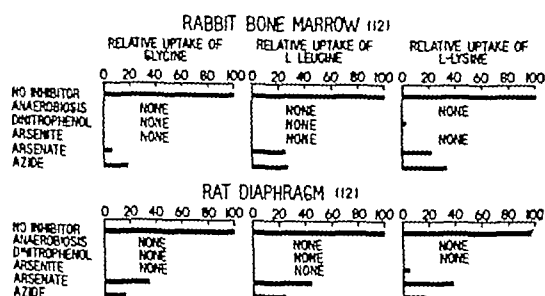


FIG 6 UPTAKE OF AMINO ACIDS IN presence of oxidation and phosphorylation inhibitors The concentration of the inhibitors was 0.001M

ent processes in liver homogenate, characterized by differences in pH optimum, dependence on calcium ions, sensitivity to fluoride and to oxidation and phosphorylation inhibitors (14)

The findings with lysine caution against generalization even with respect to so elementary a condition as dependence of amino acid uptake on respiration. Synthesis of peptides by coupled reactions between peptides and hydrolytic enzymes, as discovered by Bergmann and his collaborators (19), may play a part, and these would not depend on respiration. Or syntheses analogous to those of disaccharides by sucrose phosphorylase discovered by Doudoroff *et al* (20) may

TABLE 8. COMPARISON OF CONDITIONS AFFECTING THE UPTAKE OF AMINO ACIDS IN DIFFERENT TISSUES (12)

	GLYCINE, LEUCINE AND LYSINE IN BONE MARROW CELLS AND DIAPHRAGM AND GLYCINE IN LIVER HOMOGENATE	LYSINE Whole Homogenate	LYSINE Sedimented Fraction
Optimum pH	7.3-7.5	6.2	7.5
Frozen and thawed	100% inhibition	No inhibition	No inhibition
Dependence on calcium	None	Obligatory	Slight
Anaerobiosis	100% inhibition	No inhibition	Slight inhibition
Arsenite	100% inhibition	50% inhibition	No inhibition
Arsenate	80-95% inhibition	24% inhibition	No inhibition
Azide	65-75% inhibition	20% inhibition	8% inhibition
Dinitrophenol	100% inhibition	28% inhibition	No inhibition
Dependence on concentration of amino acid	Logarithmic	Linear	Linear

Concentration of inhibitors was 0.001 M

on the other because the activity of a number of peptidases depends on these ions

In bone marrow cells cobalt, copper and manganese were inhibitory with all 3 amino acids over a range of 0.05 to 0.001 molal. The final concentrations of the free metal ions in the reaction medium were certainly much less than these. Substantially the same effects were observed in diaphragm. Of the 3 metal ions copper was the most inhibitory and manganese the least. In liver, copper was inhibitory at an added concentration of 0.001M or higher. Manganese was not inhibitory and at concentrations below 0.001M there may have been a slightly increased rate of uptake. These findings argue against participation of peptidases in the uptake of labeled amino acids by tissue proteins.

The effects of cobalt in liver homogenate were especially interesting and point to the need of

caution in interpreting the effects of metal ions Table 9 shows that the addition of cobalt (Co Cl₂) increased the counts which could not be washed out of the protein after the homogenate had been incubated with labeled leucine or glycine But after the proteins (which had been exhaustively extracted with trichloroacetic acid) were treated with the ninhydrin reagent the CO₂ liberated gave a larger fraction of the counts originally in the protein As the ninhydrin reagent liberates CO₂ from the carboxyl groups of amino acids only when both their carboxyl and amino groups are free, we have interpreted this result as indicating

TABLE 9 EFFECT OF COBALT ON UPTAKE OF L-LEUCINE AND GLYCINE BY GUINEA PIG LIVER HOMOGENATE SEDIMENTED FRACTION (12)

CONCENTRA TION OF CoCl ₂ ADDED, MOLAL	COUNTS/MIN/ MG PROTEIN	AFTER NINHYDRIN TREATMENT OF PROTEIN COUNTS/MIN/MG PROTEIN	INHIBITION OF UPTAKE %	
		in CO ₂ liberated	Calculated remaining in protein	
<i>L-Leucine</i>				
0	3 0	0	3 0	
0 005	10 0	9 5	0 5	83
0 001	2 5	1 4	1 1	63
0 0005	2 6	0 7	1 9	36
0 0001	4 2	0 1	4 1	0
<i>Glycine</i>				
0	7 4	0	7 4	
0 005	9 3	6 3	3 0	59
0 001	4 1	1 8	2 3	68
0 0005	7 7	1 3	6 4	13
0 0001	8 5	0 6	7 9	0

the formation of a protein-cobalt-amino acid complex Similar results were obtained with lysine, but as the latter amino acid was labeled in the ϵ -position treatment with the ninhydrin reagent could not be used to demonstrate the protein-cobalt-amino acid complex Table 9 shows that as far as the actual incorporation of amino acids into the proteins is concerned, cobalt was inhibitory, as it was in marrow cells and in diaphragm

The degree of complex formation is greater the greater the concentration of added cobalt Not all proteins form this complex with cobalt to the same degree The most active we have found are those in the sedimented fraction of liver homogenate, diaphragm proteins come next, bone marrow proteins and egg albumin are the least active Amino acids also vary in their ability to partici-

pate in this complex formation Of the 3 amino acids we have tried the order of effectiveness is leucine, glycine and lysine The formation of this complex takes about 6 hours to reach completion at 38°, it does not proceed below pH 6.0, and increases progressively at pH values above that

Having several tissues each of which was capable of taking up glycine, leucine and lysine we were in a position to examine whether the amino acids mutually influence their uptake The results summarized in figure 7 show that glycine, leucine and lysine are taken up independently in the 3 tissues tested The counts in the protein when all 3 amino acids were added together was the sum of the 3 added separately

In other experiments addition of 2 radio-inactive amino acids together with one which was radio-active did not affect the uptake of the latter Nor did the addition of a mixture of amino

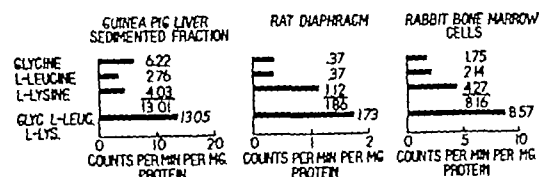


FIG 7 UPTAKE OF AMINO ACIDS added separately and together (12)

acids approximating the composition of casein or of hemoglobin affect the uptake of labeled glycine, leucine or lysine

This result needs to be correlated or reconciled with the observations in feeding experiments (21-25) It was found in those experiments that an indispensable amino acid is ineffective for growth or recovery from protein depletion unless it is fed within a few hours of other necessary amino acids In all of the *in vitro* experiments so far the tissues no doubt contained all the normal amino acids to some extent

A major assumption underlying all this work is that radioactivity in the protein after incubating a tissue with a radioactive amino acid means that the labeled amino acid has been incorporated into it by peptide bonds The whole structure of interpretation rests on this assumption It has not been proved

After it was found possible to identify with the ninhydrin reagent the binding of labeled amino acids to protein by cobalt all the crucial experiments with bone marrow cells, diaphragm and liver homogenate with glycine and leucine were repeated and the ninhydrin test applied to their

radioactivity The test is applicable to our glycine and leucine, which were labeled in their COOH groups, but not to the lysine, which was labeled in the ϵ -position The CO₂ liberated by ninhydrin from all the radioactive proteins, in those experiments in which no cobalt was added, gave no counts We may conclude, then, that the labeled amino acids were bound in such manner that either their COOH or their amino groups were masked, possibly by participation in peptide bonds but possibly also in bonds of some other kind

In many of the experiments by other workers and in all of ours the labeled amino acid was recovered as such after acid hydrolysis of the protein This showed that the radioactivity was carried into the protein by the labeled amino acid in such form that the amino acid could be recovered as such after complete acid hydrolysis

The results of this test and of the ninhydrin test were necessary but they are not sufficient evidence, they are not proof that the labeled amino acids were incorporated by peptide bond formation

It would be proof if a peptide could be isolated and identified among the partial hydrolysis products of a radioactive protein To obtain such evidence bone marrow protein was obtained radioactive by incubating the cells with labeled leucine An aliquot of the protein was treated with ninhydrin and the CO₂ evolved gave no counts The protein was partially hydrolyzed with a mixture of strong acetic and hydrochloric acids for 10 days at 38°, and the hydrolysate was then chromatographed on a starch column according to the method of Moore and Stein (26) The effluent fractions were analyzed with the ninhydrin reagent for amino nitrogen and also for radioactivity One region was found of coinciding ninhydrin color and of radioactivity The position of this region was in advance of the position of leucine, in fact 2 non-radioactive ninhydrin bands intervened between it and the leucine band Over 90 per cent of the radioactivity originally in the protein was in the one radioactive band

Fractions at the peak of the radioactive band were pooled and completely hydrolyzed with HCl, and the hydrolysate was again chromatographed The complete hydrolysate gave a starch chromatogram indicating 17 or more amino acids in the hydrolysate All of the radioactivity was confined to the leucine plus isoleucine band Carrier isolation was then applied to this radioactive

fraction and all of the radioactivity was identified as being in the leucine These results showed that partial hydrolyses of the radioactive protein liberated the incorporated radioactive leucine in a large peptide

The objection to this particular piece of evidence is that the radioactive fraction in the partial hydrolysate emerged from the starch column too soon for good resolution, and the peptide fraction in which the radioactivity was found was too complex for determination of its structure

We have repeated this experiment several times on bone marrow protein containing labeled glycine, leucine and lysine Among the partial hydrolyzed products a large peptide was found containing leucine and lysine

This kind of evidence brings us closer to rigorous proof that the labeled amino acids taken up

TABLE 10 SYNERGISTIC ACTION OF FRACTIONS OF GUINEA PIG LIVER HOMOGENATE ON UPTAKE OF GLYCINE (12)

INCUBATED WITH FRACTIONS SEDIMENTED AT 2500 g	FRACTIONS SEDIMENTED AT 30 000 g	RADIO- ACTIVITY OF PROTEIN Cts/min/mg	WEIGHT OF PROTEIN mg	TOTAL RADIO- ACTIVITY OF PROTEIN Cts/min
+	—	0 59	60	35
—	+	0 14	59	8
+	+	0 72	124	89

by the protein are incorporated by means of peptide bonds, but it is not yet proof It will be necessary to identify the peptides, isolate them and show that the labeled amino acids are constituent amino acids of the peptides

However the evidence at hand, as far as it goes, is in accord with the interpretation that the labeled amino acids are taken up by tissue proteins in peptide bonds There are no discrepancies and the evidence is quite varied in character

Multiple labeling of protein followed by partial hydrolysis and isolation of the peptides promises to be a new and useful method of determining the detailed amino acid pattern of proteins

Workers in the field of biological oxidations are finding that they have to deal with organized cytoplasmic particles We have found this in the methylation of guanidoacetic acid by methionine in liver homogenate We are now finding the same in this field Table 10 summarizes a result we have obtained with labeled glycine in guinea pig liver homogenate The fraction sedimented at 2500 g which consists largely of mitochondria, takes up glycine actively, the fraction sedimented

above 30,000 g, microsomes largely, take up much less, when the two fractions are incubated together the amount of glycine taken up is about double the sum of the two when incubated separately. There is clearly a synergistic action between the two sets of particles.

A summary of the situation in this field at present runs somewhat as follows. All the tissues so far tested can take up *in vitro* into their proteins every labeled amino acid which has been presented to them. The most active normal tissue in adult animals is bone marrow. The uptake of every amino acid in every tissue, with one exception, is immediately dependent on respiration.

The exception is the uptake of lysine by liver homogenate. The effective range of labeled amino acid concentration is within the order of that in the plasma, and the rate of uptake is of the order of that *in vivo*. Glycine, leucine and lysine are taken up independently of each other. The evidence indicates that the amino acids are taken up in peptide bonds, but this point is not yet proved. There is evidence in the case of liver homogenate, at least, of a synergistic action between two sets of intracellular particles.

A one sentence summary is that the field of the biological synthesis of protein is now wide open.

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MECHANISM OF PEPTIDE BOND FORMATION

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THE present understanding of the mechanism of peptide synthesis derives mainly from the study of relatively simple reactions. The animal body has fortunately supplied a number of model reactions for the study of synthesis of peptidic¹ links in two-component systems. Reactions of such a type are found among the so-called detoxication reaction, hippuric acid synthesis as well as acetylation of aromatic amine are relatively convenient model systems. The study of the acetylation of aromatic amines (1) in particular has relatively early furnished valuable detail which appears now rather generally applicable. It will, therefore, be advantageous to summarize first results obtained with this reaction.

ACETYLATION OF AROMATIC AMINES

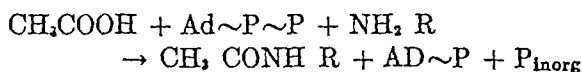
Our interest in this reaction had originally been aroused by the finding of acetyl phosphate as an intermediary in a reaction chain geared apparently on generation of energy-rich phosphate bonds (2). The emergence of this particular molecule, however, suggested that it might not only be a link, as it indeed can be, in a phosphate transfer chain. It was suggestive to imply that acetyl phosphate may, with the appropriate catalyst, also offer its organic part in a state of activation for synthesis (3). This expectation led rather early to the generalization that carboxyl activation by phosphorylation may be a major phase in biosynthesis (3).

After initial disappointments with the use of synthetic acetyl phosphate, it was observed that acetate plus adenylyl pyrophosphate (ATP) would serve in pigeon liver extract as acetyl donor to aromatic amines (1). Using a protein fraction of pigeon liver extract a near equivalence of ATP

¹ To simplify the following discussion, the term "peptidic link" is introduced as generic name for a $-NH-CO-$ link between any amino or carboxyl group. The term "peptide link" is then reserved for the 1-carboxyl, 2-amino-link between two α -amino acids as it occurs in protein.

breakdown and sulfonamide acetylation was obtained (4).

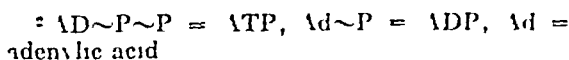
In table 1 a closely related reaction is included, namely, the activation of acetate by ATP to form hydroxamic acid in the presence of hydroxylamine. With a somewhat greater activity for hydroxamic acid formation in this extract, the stoichiometric relationship between acetylation and phosphate mineralization appears more clearly than in the case of sulfonamide acetylation. The overall condensation reaction is thus formulated:



R stands here for either aromatic residue or the hydroxyl group of hydroxylamine.

The hydroxamic acid reaction has proven a very helpful tool in the investigation of acyl activation. Some time ago, quite accidentally, we had become aware of an explosive, non-enzymatic liberation of inorganic phosphate from acetyl— or other acyl—phosphate on addition of hydroxylamine. The corresponding hydroxamic acid is formed which gives with ferric ion a deeply purple colored complex. This reaction is used now for a colorimetric determination of the acyl phosphate (5). Furthermore, if added to an enzymatic system, hydroxylamine may act as a trap for activated carboxyl groups which thereby become accessible to determination by the color reaction (6, 9, 10). Due to the great reactivity of hydroxylamine, the evaluation of such results requires well controlled conditions (cf. 7).

The further establishment of the carboxyl group as the site of activation in peptidic synthesis appeared important. In view of the existence of an energy-rich N-P phosphate bond in creatine phosphate, the possibility of a primary phosphorylation of the amino group appeared plausible. As mentioned previously, experiments



with synthetically prepared acetyl phosphate as acetyl donor for amines had proven unsuccessful. More recently, however, Dr Kaplan studied in our laboratory a compound formed by enzymatic phosphorylation of acetate with ATP, or phosphopyruvate plus ADP, in extracts of *E. coli*. He found it to differ in some respects from synthetic acetyl phosphate. This 'natural' ATP-acetate reaction product appeared active as acetyl donor, the compound was shown to condense with for-

if the two steps represent separable enzyme systems³.

GLUTAMINE

We now turn to the discussion of a very similar but more truly 'peptidic' reaction, the synthesis of glutamine. Speck found this reaction in acetone pigeon liver extract (9) prepared analogously to the one used in our sulfonamide acetylation experiments. In such an extract, glutamic acid, ATP and ammonia yield anaerobically glutamine when cysteine and magnesium ion are present. So far no indication for an involvement of coenzyme A in this reaction has been found. It is significant that hydroxylamine is practically as effective an acceptor as ammonia. This reaction is very rapid. In the experiment shown in table 3, 0.15 ml extract were used only and in 15 minutes nearly 2 micromoles of glutamine were formed.

TABLE 1 COMPARISON OF PHOSPHATE MINERALIZATION AND AMINE ACETYLATION OR HYDROXAMIC ACID FORMATION IN FRACTIONS OF PIGEON LIVER EXTRACT¹

ADDITIONS	P _{INORG}	ACETYL SULFANILAMIDE	HYDROXAMIC ACID
	μM formed	μM formed	μM formed
ATP	0.79	0	0
" + sulfanilamide	1.24	0.64	
" + hydroxylamine	1.83		1.4

¹ All samples contain acetate and coenzyme A

TABLE 2 SULFANILAMIDE ACETYLATION WITH 'NATURAL' ACETYL PHOSPHATE¹

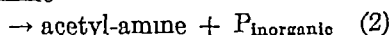
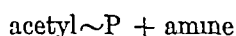
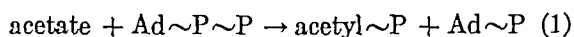
ACETYL PHOSPHATE	ATP	COENZYME A	SULFANILAMIDE ACETYLATED
μM	μM		μM
8, synthetic	1	+	0.15
	10 ²	+	0.85
6, natural		+	0.78
6, natural			0.23

¹ 1 ml pigeon liver extract incubated 2 hrs at 25°

² + acetate

mate in extracts of *E. coli* yielding pyruvate. It also acts, as shown in table 2, as acetyl donor for aromatic amines, significantly still requiring coenzyme A (8).

This observation then allows us to divide the overall reaction into two steps,



The intermediate formation of an acyl phosphate in the synthesis of a peptidic link is therewith established. It remains, however, still doubtful

TABLE 3 GLUTAMINE SYNTHESIS IN PIGEON LIVER EXTRACTS¹

SYSTEM	WITH 0.01 M NH ₄ Cl		WITH 0.3 M NH ₄ OH
	Amide formed	Inorganic phosphate formed	Hydroxamic acid formed
	μM	μM	μM
Complete	1.91	2.46	1.41
Without ATP	0.08		0.14
Without glutamate	0.13	0.54	0.11

¹ SPECK, J. F. *J. Biol. Chem.* 168: 402, 1947

With this rapid synthesis and a relatively low background ATP-decomposition the stoichiometric relationship between ATP breakdown and glutamic acid-ammonia condensation appears most clearly. Such rapid phosphate transfer followed immediately by condensation leading eventually to phosphate liberation is remarkable. It should make one very cautious not to mistake an ATP-decomposition of such a type for ATP hydrolysis. For instance, with some ammonium ion present, as is the rule in any crude tissue extract, an ATP breakdown with added glutamate, due to glutamine formation, could easily be mistaken for a 'glutamate-catalyzed ATP-ase' effect.

Simultaneously with Speck, Elliott in Cambridge, England, found in acetone brain extract (10) and in bacterial preparations (11) an analogous glutamine and glutamhydroxamic acid syn-

³ Recently a separation of the two enzymes, catalyzing steps 1 and 2, was accomplished by acetone fractionation of pigeon liver extract in the cold (14).

thesis (table 4) Although Speck as well as Elliott was not able to show an acyl phosphate accumulation in the absence of an acceptor, the reaction with hydroxylamine strongly indicates carboxyl activation as a primary reaction

HIPPURIC ACID

A third peptidic synthesis of this type was found to be hippuric acid condensation. The p-aminohippuric acid synthesis has been studied extensively by Cohen and his group (12) in rabbit liver homogenate. In this case the synthetic activity is particle-bound. The figure 1 from Cohen's paper (12) shows anaerobic synthesis due to addition of ATP. Because of the considerable

TABLE 4 GLUTAM-HYDROXAMIC ACID IN BRAIN EXTRACTS¹

SYSTEM	HYDROXYAMATE ²	PHOSPHATE ²
Complete	2.3	1.9
No glutamate	0	0.1
No ATP	0	0
No Mg	0.3	0.2

¹ ELLIOTT, W. H. *Nature* 161: 128, 1948

² Data for hydroxamate formation and phosphate liberation in $\mu\text{M}/\text{sample}$

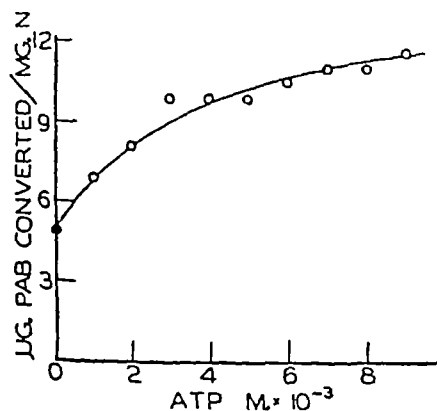


Fig 1 SUPPORT of anaerobic hippuric acid formation by ATP. Rat liver homogenate in nitrogen (12)

background decomposition of ATP, a large excess of this reagent has to be applied in such a particle suspension, which obviates experiments on the stoichiometry.

These three examples of peptide bond formation summarized in figure 2, we may conclude, present a thoroughly analogous situation: the energy-rich phosphate of ATP acts as immediate energy source, attaching itself primarily to the carboxyl component. Certainly this is so in the case of acetylation and presumably also in glut-

amine and hippurate synthesis. The inability to isolate the primary reaction products in tissue experiments at least, may be due to the presence there of a very active acyl phosphatase in such preparations (13). It should be pointed out, however, that in Speck's experiments glutamic acid alone causes only little if any specific ATP breakdown. And, conversely, no ATP breakdown occurs with ammonia and hydroxylamine alone. This may be due to a special construction of the enzyme which permits reaction only if both the primary phosphate acceptor and the second participant in the condensation reaction are present. Or, the equilibrium conditions or the transfer from ATP allow no appreciable accumulation of the reaction product. The definite answer to such rather important questions of finer reaction mechanism may be obtainable by fractionation of pigeon liver extract and such experiments are in

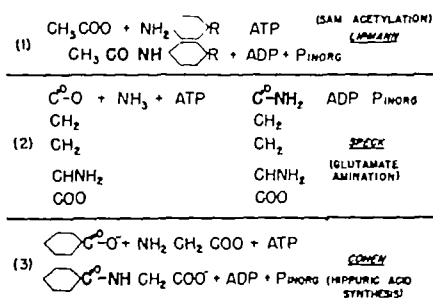


Fig 2 SYNOPSIS of ATP-dependent peptidic syntheses. Two-component systems

progress. Presently, we have only found that glutamine synthesis and acetylation may quite easily be separated on fractionation with ammonium sulfate (14).

GLUTATHIONE

So far we have considered only two component systems and it is not too surprising that at this still exploratory stage best information on the mechanism is available with these relatively simple reactions. Promising progress, however, was made recently with the study of the mechanism of synthesis of a tripeptide, namely glutathione.

After the use of isotopes as a rapid turnover rate with this abundant cell constituent, Bloch reported to have obtained in liver homogenate support of anaerobic incorporation of radioactive glycine when ATP was added (15). No exchange occurred in the absence of a phosphate donor. He now has been able to obtain the reaction in acetone pigeon liver extract, which seems to be an almost inexhaustible source of ATP.

clear that a single energy-rich phosphate bond is used to effect a single peptide link. This is evidenced by the one to one relationship between synthesis and phosphate liberation. Judging plainly from the energy data, the possibility would

the limits of 2500 and 3000 calories per peptide bond.

On the other hand, the pyrophosphate link in adenylyl pyrophosphate represents 12,000¹ calories (3). This means that excess energy is spilling

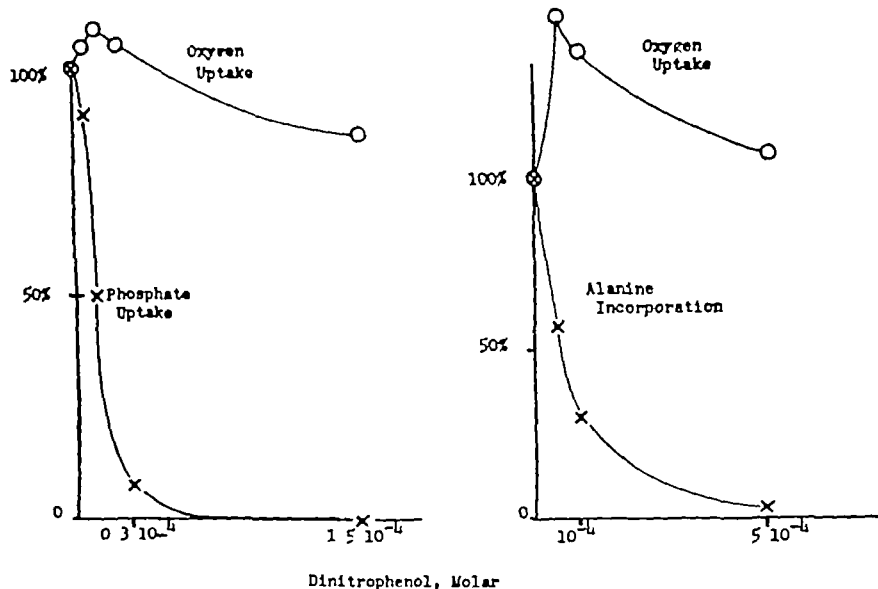


Fig 4 *m*-DINITROPHENOL INHIBITION. Comparison of the inhibition by dinitrophenol of phosphorylation and protein synthesis. The inhibition of phosphorylation was measured in kidney homogenate as described by Loomis and Lipmann (20). Protein synthesis was measured in liver slices. The data reproduced are taken from the paper by Frantz *et al* (21). For better comparison the data are given in percentages of normal oxygen uptake, phosphorylation and alanine incorporation.

TABLE 6 FREE ENERGY CHANGE WITH PEPTIDE SYNTHESIS¹ IN WATER, AT 310.5°K

COMPOUND	ΔF_0 CAL.	κ (SYN)
Glycyl-glycine	3070	0.0076
Alanyl-glycine	2590	0.016
Leucyl-glycine	2795	0.012
Glycero-phosphate ²	2280	0.025

¹ Measurements by Henry Borsook (private communication)

² LIPMANN, F. *Advances in Enzymology* 1:99, 1941

exist that an energy-rich phosphate bond could cover two or even more peptide links. Thus, however, would anyway be unlikely for reasons of reaction mechanism.

The energy requirement for peptide synthesis has been determined by Borsook (23). His earlier data were recently revised and extended and Dr Borsook has kindly permitted me to use his most recent and as yet unpublished results. This and related data are listed in table 6. The energy figures for a number of typical peptides show no great deviation from the average and fall inside

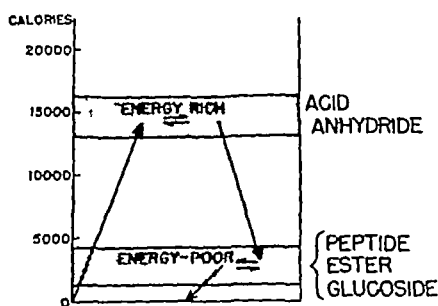


Fig 5 CHARTING OF RELATIONSHIP between the two energy levels dominant in cellular chemistry and corresponding to energy-rich and energy-poor phosphate bond levels.

over with a net utilization of around 20 to 25 per cent in the formation of the new bond. The scheme of figure 5 indicates such correlation between energy donor and acceptor system to be a very general occurrence in cellular synthesis.

As has been earlier pointed out elsewhere (3), there is a distinct grouping around two energy levels, a higher one at 12,000 calories and a lower at 3000 calories. The energy-rich phosphate bond carries excess energy if performing condensation

reactions on the lower level, as, e g, ester, peptide and glucoside syntheses. In such rather frequent instances then not more than about a quarter of the available energy is utilized.

This appears, however, not too surprising. The

more and more realized applicability in biosynthesis of a standard form of energy for a great variety of purposes has to entail in some instances a more or less pronounced loss of energy, in exchange for a simplification of procedure.

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MECHANISM OF UREA SYNTHESIS

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IN 1932 Krebs succeeded, for the first time, in demonstrating the *in vitro* formation of urea in mammalian liver. He showed in further detail that the synthesis proceeds by a stepwise mechanism, the ornithine cycle. It is of interest to recent developments in this field that the rapid formation of urea in rat liver slices, as carried out by Krebs and Henseleit (1), required the presence of lactate and oxygen in addition to ornithine, bicarbonate and NH_3 .

More recently Cohen and Hayano established the conditions for obtaining arginine synthesis (2) in rat liver homogenates starting with citrulline (Step II of the ornithine cycle), and for obtaining citrulline synthesis (2) from ornithine (Step I). According to their studies of Step II, glutamic acid, rather than NH_3 , appeared to be the nitrogen donor in arginine formation from citrulline. Mg ion, catalytic amounts of adenosine triphosphate (ATP) and oxygen were required in addition to the amino acid substrates.

Similar results had been obtained in kidney slices with glutamic and aspartic acid by Borsook and Dubnoff (3) who proposed that the transfer of the $-\text{NH}_2$ group occurred by a transamination accompanied by the simultaneous oxidative removal of 2 H atoms (transimination). An α -keto acid, thought to be the second product of the reaction would, of course, escape detection in respiring preparations.

Under the conditions of Borsook and Dubnoff, or of Cohen and Hayano, neither slices nor homogenates can form arginine in the absence of oxygen. Moreover, since the latter group found glutamic acid to be much more effective than aspartic acid in liver homogenates, they considered glutamic acid to be the specific $-\text{NH}_2$ donor in the transamination reaction. Aspartic acid was thought to be active only in so far as it could be converted to glutamic acid. We hoped that further insight into the nature of this mechanism might be gained from a study of the isolated system.

By separation and partial purification of the enzymes involved in the citrulline to arginine conversion, it can be shown that aspartic acid is the specific $-\text{NH}_2$ donor and that the transfer of nitrogen does not involve an oxidative step. The enzymes concerned are quite soluble and may be readily obtained from extracts of acetone powder mammalian liver. They withstand exposure to alcohol and ammonium sulfate fractionation procedures at low temperature and keep moderately well (4).

Mechanism of Nitrogen Transfer In the reaction, aspartic acid and citrulline are converted to arginine and malic acid, anaerobically, in the presence of Mg ions. ATP participates directly as a reactant.

As shown in table 1, line 1, 14.4 μM of arginine (estimated as urea) and an equivalent amount of malic acid were formed from aspartic acid and citrulline in 20 minutes by an extract of beef liver acetone powder, subjected to one alcohol fractionation. When either citrulline or aspartic acid was omitted, neither arginine nor malic acid was formed.

Although ATP is specifically required, maximum rates cannot be reached with ATP alone, owing to competition by contaminating ATP-ase and to an inhibition caused by high concentrations of ATP. The difficulty can be overcome by employing 3-phosphoglyceric acid, in excess, as a source of high energy phosphate ($\sim\text{pH}$), and ATP in catalytic amounts, to effect the phosphate transfer. The glycolytic enzymes catalyzing the transfer of $\sim\text{pH}$ through phosphopyruvic acid and ADP are present in the preparation. When necessary their concentration was augmented by addition of a fraction from rabbit muscle extract. The dependence of arginine synthesis upon $\sim\text{pH}$ is shown in table 1, line 3, when phosphoglyceric acid was omitted, neither arginine nor malic acid was formed.

Glutamic acid could not replace aspartic acid (line 5) nor could any of some 20 other amino acids tried. With glutamic and oxaloacetic acids

(line 4) 7 μ M of arginine and malate were formed. The activity of this combination is due to the formation of aspartic acid by transamination. Assay of the glutamic-aspartic transaminase content of various preparations shows that the extent to which the combination of glutamic and oxalacetic acids will replace aspartic acid is proportional to the transaminase content of the preparation.

Aspartic acid cannot be replaced by NH_3 or by the combination of oxalacetic acid and NH_3 . It is

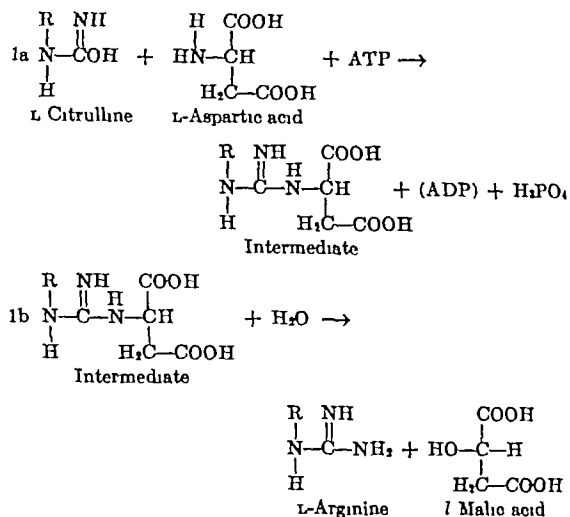
TABLE 1 SYNTHESIS OF ARGININE FROM CITRULLINE IN ALCOHOL FRACTIONATED EXTRACT OF OX LIVER ACETONE POWDER

Each tube contained in addition 4 μ M ATP, 13 μ M MgSO_4 , 0.4 ml phosphate buffer, pH 7.5, enzyme preparation containing 31 mg protein and 8 mg muscle extract in a final volume of 4 ml. Time 20 min, 38°.

SUBSTRATE ADDED					FOUND	
L-Aspartate 20 μ M	L-Glutamate 20 μ M	Oxalacetate 30 μ M	L-Citrulline 20 μ M	3 Phosphoglycerate 50 μ M	Arginine μ M	L-Malic acid μ M
+			+	+	14.4	14.5
+				+	0.0	0.0
+			+		0.9	1.0
	+	+	+	+	7.0	6.8
	+		+	+	0.0	0.0
	+	+		+	0.0	0.1
		+	+	+	0.0	0.2

particularly significant that in the absence of citrulline, no combination including oxalacetic acid gave rise to malic acid. For example, with oxalacetic and glutamic acids (line 6), malic acid would certainly have been formed by a glutamic dehydrogenase-malic dehydrogenase catalyzed dismutation (5) had diphosphopyridine nucleotide (DPN) been present. Under the given experimental conditions, malic acid cannot be formed independently of arginine.

The simultaneous formation of arginine and malic acid, by interaction of the two substrates, strongly suggests that the mechanism of nitrogen transfer involves a preliminary condensation of the amino group of aspartic acid with the ureido group of citrulline to form a C—NH—C linkage, followed by hydrolytic cleavage on the second side of the nitrogen, as shown in reactions 1a and 1b.



Regarding optical specificity of the overall reaction, only L-citrulline and L-aspartic acid react, while L-malic acid and L-arginine are formed. Identification of the products of the reaction has been established by isolation from a large-scale enzymatic run. L-Malic acid was isolated as the cinchonine salt and estimated quantitatively with the highly purified 'malic enzyme' described by Ochoa, Kornberg and Mehler (6). Since arginase was always present in excess, urea was estimated by the colorimetric method of Archibald (7) and L-ornithine was isolated as the dibenzoyl derivative.

Intermediary Condensation Product. The intermediary condensation product shown in reaction 1a is quite different from that postulated in keto-amino acid transamination. In the latter case, the reaction, however formulated (8-10), supposedly occurs through transient Schiff base formation, and the whole reaction is catalyzed by a single enzyme. Here the condensation product is depicted as an N,N' disubstituted guanidine and at least two enzymatically distinct steps are involved in the overall synthesis of arginine.

The two enzymes have been separated by ammonium sulfate fractionation of extracts of ox liver acetone powder, thus permitting a study of each step separately. In the first step (reaction 1a), ATP and Mg ion are specifically required. Urea formation from CO_2 and NH_3 is known to be endergonic. It may therefore be reasonably assumed that both citrulline and arginine synthesis are each endergonic, and that as far as arginine synthesis is concerned, it is precisely in the condensation reaction, that energy, supplied by ATP, is actually utilized.

Presumably, condensation occurs via a pre-

linumary phosphorylation. Although much information regarding the phosphorylation step is still lacking, phosphorylation of the isourea form of citrulline rather than of aspartic acid would appear more likely, for it would provide a means of shifting the citrulline equilibrium toward the more reactive isourea form.

Table 2 shows some of the experimental evidence for the stepwise reaction. Since phosphoglyceric acid was used as the source of \sim pH in the presence of condensing enzyme A, the amount of pyruvic acid formed, as well as the amount of inorganic phosphate, is a measure of the extent of \sim pH transfer. Citrulline disappears

TABLE 2 STEPWISE ENZYMIC FORMATION OF ARGININE

The conditions are as in table 1, line 1, except that muscle extract was omitted and glycylglycine buffer was substituted for phosphate. In the first step a solution of enzyme A containing 25 mg of protein was added to each tube. 38°, 20 min. After heat inactivation, enzyme B (18 mg protein per tube) was added. 38°, 20 min. The values are corrected for the small amounts of pyruvate and inorganic phosphate formed in the absence of citrulline.

	ENZYME A μ M	ENZYME B μ M
Citrulline removed	11.0	
Pyruvate formed	15.5	
Inorganic P formed	17.4	
Arginine formed	0.2	6.1
Malate formed	0.3	6.3

ance was found to be roughly equivalent to the amount of pyruvate and inorganic phosphate formed. The excess of these two over the amount of citrulline removed is undoubtedly due to competition with some contaminating ATP-ase. Neither arginine nor malic acid was formed, but the condensation product appeared to accumulate, for upon addition of the hydrolyzing enzyme B to the deproteinized reaction mixture, arginine and malic acid were formed in equivalent amounts.

About 50 mg of the intermediate have been accumulated in a large scale enzymatic run with enzyme A. By fractionation of the barium salt with alcohol, the intermediate has been freed of organic and inorganic phosphorous compounds originally present in the medium, and of citrulline, though traces of aspartic and pyruvic acids

remain. At this stage of purification, enzymatic cleavage to arginine and malic acid requires no further additions beyond enzyme B and the intermediate, thus confirming the formulation of reaction 1b as being purely hydrolytic.

Comparison of Arginine Synthesis in Slices, Homogenates, and Extracts. A comparison of the specific activity, expressed as Q_{urea} (μ l arginine or urea formed per mg protein per hour), of various liver preparations is given in table 3. The activity of crude extracts of ox liver acetone powder varies with the age of the powder. A preparation made from a powder after 4 weeks'

TABLE 3 SPECIFIC ACTIVITY OF VARIOUS LIVER PREPARATIONS

SPECIES	PREPARATION	CONDITIONS	Q_{urea}
Rat	Slice	NH ₄ , Lactate	18
	Homogenate	Glutamate ²	14
	Homogenate	Aspartate, pyruvate ²	16
	Acetone powder extract	Aspartate, \sim pH	18.8
	Acetone powder extract	Aspartate, \sim pH + muscle extr	20.2
Ox	Acetone powder extract	Aspartate, \sim pH	7.4
	Acetone powder extract	Aspartate, \sim pH + muscle extr	11.2
	EthOH fraction 3	Aspartate, \sim pH	7.8
	EthOH fraction 3	Aspartate, \sim pH + muscle extr	20.0
	EthOH fraction 4	Aspartate, \sim pH	17.9
	EthOH fraction 4	Aspartate, \sim pH + muscle extr	33.6

¹ Q_{urea} recalculated from the data of Krebs and Henseleit assuming 85% of the dry weight to be protein. ² Calculated from the rate of arginine synthesis per mg N given by Cohen and Hayano. ³ Calculated from the data in table 4, assuming 85% of the dry weight to be protein.

storage had a Q_{urea} of 7 which was increased to 11 by the addition of an extract of rabbit muscle, thus accelerating the rate of arginine synthesis to the point where only arginine synthesizing enzymes were limiting. On alcohol fractionation, a three-fold purification was reached in fraction 4 (Q_{urea} 33 with added muscle extract) corresponding to about 30 per cent alcohol.

Though much less stable, extracts of rat liver acetone powder have a higher specific activity. Under optimal conditions a Q_{urea} of 20 was found as compared to a Q_{urea} of 18 obtained by Krebs for rat liver slices, and a Q_{urea} of 14 obtained by Cohen and Hayano for rat liver homogenate in the presence of glutamic acid. In the presence of aspartic acid, supplemented with pyruvic acid

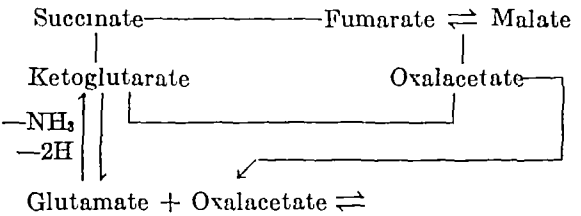
rat liver homogenates gave a somewhat higher Q_{urea} value of 16

On calculating the total activity of an extract of rat liver acetone powder, it appears that about 50 per cent of the original arginine synthesizing activity of the whole tissue can immediately be accounted for in the extract, without correction for destruction by acetone treatment or incompleteness of extraction, suggesting that the same enzyme system is involved regardless of the substrates offered or of other variations in experimental conditions

RELATIONSHIP OF THE TRICARBOXYLIC
CYCLE AND OF TRANSAMINASE TO
ARGININE SYNTHESIS

If, as studies on the anaerobic isolated system indicate, aspartic acid is the specific $-NH_2$ donor in arginine synthesis, then a number of questions are raised in relation to published observations concerning the following main points 1) Glutamic acid is four times as effective as aspartic acid in homogenates, aerobically (2, 11) 2) Both glutamic acid and aspartic acid are poor precursors in liver slices (1, 2) 3) The general mechanism of nitrogen transfer from amino acids to form aspartic acid, prior to arginine synthesis, is not immediately apparent

With regard to glutamic acid, an outline of the proposed enzymatic pathway is shown below where the rectangle is meant to represent the Krebs tricarboxylic cycle in highly schematized form, including only those intermediates of the cycle pertinent to the present discussion



In homogenates, aerobically, when glutamate is the $-NH_2$ donor, some of the glutamic acid is oxidized through glutamic dehydrogenase and the tricarboxylic cycle, to form oxalacetate. The latter is then converted to aspartate by transamination with a further amount of glutamate. ATP, generated by phosphorylations coupled with oxidation, is formed during these steps. From a quantitative point of view, it has been

shown by Ochoa (12) that the ratio of $\sim pH$ generated per atom of oxygen is 3.1, thus more than an ample supply would be provided for arginine synthesis. The oxygen requirement in liver homogenates, when glutamate is the NH_2 donor, must then be ascribed to dependence upon a) ATP generation and b) oxalacetate formation.

When aspartate is the NH_2 donor, in homogenates, arginine synthesis is poor because as-

TABLE 4. SYNTHESIS OF ARGININE FROM CITRULLINE IN RAT LIVER HOMOGENATES UNDER AEROBIC CONDITIONS IN THE ABSENCE AND PRESENCE OF MALONATE

In addition each vessel contained 20 μM L-citrulline, 5 μM ATP, 10 μM $MgSO_4$, 0.3 ml of 0.25M potassium phosphate, pH 7.5, 0.5 ml of 25% homogenate. Final volume 3.0 ml, 38° time 40 minutes. Calculated for 31 mg dry weight of tissue. The values are given in μM .

SUBSTRATE ADDED 20 μM	WITH L-ASPARTATE				WITH L-GLUTAMATE			
	Arginine		O ₂ uptake		Arginine		O ₂ uptake	
	av	a.d.	av	a.d.	av	a.d.	av	a.d.
None	3.5	± 0.8	14.9	± 0.2	9.6	± 1.1	30.5	± 2.7
Pyruvate ¹	12.8	± 0.7	29.4	± 1.2	6.0	± 1.0	30.5	± 1.8
3-phosphoglycerate	12.9	± 0.9	28.9	± 1.2	6.2	± 1.1	22.1	± 5.5
Oxalacetate	14.4	± 0.4	30.1	± 1.9	10.9	± 1.2	28.8	± 5.0
Fumarate	13.4	± 1.0	27.4	± 1.6	12.6	± 0.8	30.8	± 2.4
α -ketoglutarate	11.1	± 0.4	23.7	± 3.9	4.4	± 0.7	23.1	± 3.1

With 60 μM of malonate per vessel

None	3.3	± 1.0	10.8	± 2.0	2.9	± 0.0	18.9	± 0.6
Pyruvate	11.4	± 0.2	19.9	± 1.3	2.6	± 0.4	19.1	± 1.4
2-phosphoglycerate	11.8	± 1.3	21.5	± 2.3	2.4	± 0.3	17.7	± 2.0
Oxalacetate	13.3	± 0.4	24.1	± 1.9	13.3	± 0.3	27.4	± 2.6
Fumarate	13.3	± 0.5	25.4	± 1.6	12.8	± 1.0	28.1	± 3.7
α -ketoglutarate	11.8	± 0.1	19.8	± 2.7	1.5	± 0.1	13.8	± 1.9

¹ Average deviation. ² 2.5 μM fumaric acid was added as a primer. Good respiration was often obtained without it, but the addition insured uniformly high values. Fumarate was not added to pyruvate when glutamate was employed nor was it added in any malonate experiment.

partic acid is oxidized very slowly and little, if any, oxalacetate is released to enter the tricarboxylic cycle. Sufficient ATP for arginine synthesis is therefore not made available, it being supplied directly only in catalytic amounts. If aspartic acid is supplemented by pyruvate, α -ketoglutarate, or any other member of the tricarboxylic cycle, arginine synthesis is increased to a high value. The values for oxygen uptake and arginine synthesis from aspartic acid, with and without the addition of various respiratory substrates, are given in table 4.

If when glutamate is offered aspartate must be formed by transamination, prior to arginine

synthesis, then it may be anticipated that whatever affects the concentration of aspartic acid, either directly through the transaminase equilibrium or secondarily, will affect the rate of arginine synthesis

Certain inhibitory effects reported from the laboratories of Borsook, Cohen, Krebs and Leuthardt may be explained on this basis. For example, the addition of α -ketoglutarate to glutamate (2, 11, 13) inhibits the rate of arginine synthesis about 40 to 60 per cent by pushing the transaminase equilibrium to the left, thus lowering the aspartate concentration (table 4)

Pyruvate exerts a similar inhibition (2, 3, 11, 13), by transamination with glutamic acid, some glutamate is removed and α -ketoglutarate formed. Both of these effects tend to diminish the aspartic acid concentration (table 4)

When aspartate is offered directly as the $-\text{NH}_2$ donor, the aspartate concentration is in excess, as far as enzyme saturation is concerned. Even if lowered from 20 to 15 μM by the addition of 20 μM of α -ketoglutarate, arginine synthesis will still be quite rapid (table 4)

Malonate also inhibits arginine synthesis from glutamate (2, 11, 14). This can readily be explained by the well-known block of α -ketoglutarate oxidation at the succinic dehydrogenase step, thus preventing the formation of oxalacetate. This explains why the addition of the latter or of a closely related precursor, such as fumaric or malic acids (14), specifically removes the inhibition (table 4). As is to be expected, the addition of malonate has practically no effect on arginine synthesis from aspartate under optimal conditions (*i.e.* in the presence of a respiratory substrate), for the rate of ATP generation, while excessive in the absence of malonate (see values for O_2 uptake in table 4) is sufficiently rapid even in the presence of malonate, to maintain a high rate of arginine synthesis.

Anaerobic Synthesis of Arginine in Homogenates
Once the requirements of the system are known, it is possible to obtain arginine synthesis in homogenates under anaerobic conditions by utilizing glycolytic reactions to generate $\sim\text{pH}$. Table 5 shows the amount of arginine formed in the presence of citrulline, aspartic acid and phosphoglyceric acid or hexose diphosphate. None was formed in the absence of a supply of $\sim\text{pH}$. The addition of phosphoglyceric acid, or of hexose diphosphate, caused no arginine formation when glutamate was substituted for aspartate, unless oxalacetate was also present.

Owing to the decreased efficiency of anaerobic, as compared to aerobic phosphorylation, competition with ATP-ase is less successful anaerobically. The rates of arginine synthesis were therefore much lower, anaerobically, than those obtained with an equal amount of homogenate in oxygen. A considerable portion of the ATP-ase present is associated with the suspended particles of the homogenate, and can be removed by high speed centrifugation. The last column of table 5 gives the values obtained in an equivalent

TABLE 5 SYNTHESIS OF ARGININE FROM CITRULLINE IN RAT LIVER HOMOGENATES UNDER ANAEROBIC CONDITIONS

Further additions and other conditions as in table 4 except that N_2 replaced O_2 in the gas space and 4 mg DPN was added to each vessel containing hexosediphosphate. The dry weight of 0.5 ml of homogenate and 0.4 ml of supernatant respectively were 34.7 mg and 17.1 mg. The values are given in μM .

SUBSTRATE ADDED					ARGININE FOUND	
L. Aspartate 20 μM	L. Glutamate 20 μM	Oxalacetate 30 μM	3 Phosphoglycerate 40 μM	Hexose diphosphate 25 μM	Whole homogenate	Supernatant
+			+		3.9	7.7
+				+	5.7	8.0
+					0.6	1.2
	+		+		0.1	0.0
	+			+	0.5	0.4
	+	+	+		4.6	6.8
	+	+		+	4.2	4.9

amount of supernatant, after removing the particles. Arginine when formed was found to be higher than in the whole homogenate in each case.

These experiments parallel the behavior of extracts of acetone powder. From the data in table 5, it may be seen that both the arginine synthesizing enzymes and transaminase are present in the supernate. This further supports the view that the particles were required only for the generation of ATP and of oxalacetate, when glutamate was the $-\text{NH}_2$ donor in the aerobic experiments.

Transfer of Amino Nitrogen to Oxalacetate
As to the general problem of $-\text{NH}_2$ transfer to form aspartic acid, tissue slice, isolated enzyme and isotope evidence (15-17) indicate that a good part of amino acid nitrogen probably goes through NH . However, there appears to be no known en-

zyme which oxidizes aspartic acid rapidly, comparable for example to glutamic dehydrogenase in activity and reversibility. Nevertheless rapid nitrogen transfer is possible by means of a combination of glutamic dehydrogenase and glutamic-aspartic transaminase, particularly under conditions where the newly formed aspartate can be continuously removed.

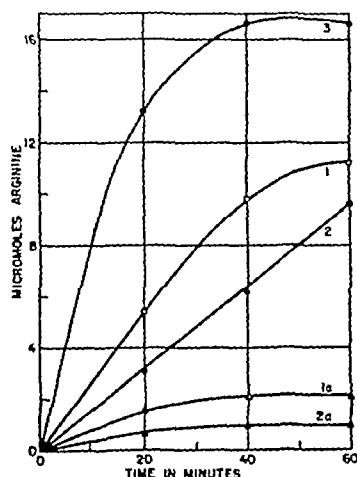


FIG 1 TRANSFER OF NH_3 to citrulline through aspartic acid by anaerobic dismutation and transamination in ox liver acetone powder extracts. Curve 1 malate, α -ketoglutarate dismutation. Curve 1a same as curve 1 in the absence of either DPN, NH_3 or malate. Curve 2 triosephosphate, α -ketoglutarate dismutation. Curve 2a same as curve 2 in the absence of either DPN, NH_3 or oxalacetate. Curve 3 arginine formation with aspartate in the same amount of extract. All tubes contained 4 μM ATP, 20 μM L-citrulline, 13 μM MgSO_4 , 0.4 ml of 0.25 M potassium phosphate pH 7.5 and 1.0 ml acetone powder extract in a final volume of 4 ml, temperature 38°. Further additions were: curve 1 20 μM each NH_4Cl and L-malate, 10 μM α -ketoglutarate, 50 μM 3-phosphoglyceric acid and 2 mg DPN. Curve 2 20 μM NH_4Cl , 10 μM α -ketoglutarate, 30 μM oxalacetate, 25 μM hexosediphosphate and 2 mg DPN. Curve 3 20 μM L-aspartate, 50 μM 3-phosphoglycerate.

The transfer of NH_3 to form aspartic acid has been carried out in extracts of ox liver acetone powder which contain these enzymes by means of dismutation reactions coupled with transamination.

- 1 Malic acid + $\text{DPN}_{ox} \rightleftharpoons$
oxalacetic acid + DPN_{red}
- 1a Triosephosphate + $\text{H}_2\text{PO}_4 + \text{DPN}_{ox} \rightleftharpoons$
diphosphoglyceric acid + DPN_{red}
- 2 $\text{NH}_3 + \alpha$ -ketoglutaric acid + $\text{DPN}_{red} \rightleftharpoons$
glutamic + DPN_{ox}

- 3 Glutamic acid + oxalacetic acid \rightleftharpoons
 α -ketoglutaric acid + aspartic acid
- 4 Malic acid + $\text{NH}_3 \rightleftharpoons$ aspartic acid
- 4a Triose + $\text{H}_2\text{PO}_4 + \text{NH}_3 + \text{oxalacetic acid} \rightleftharpoons$
phosphate acid
diphospho + aspartic
glyceric acid

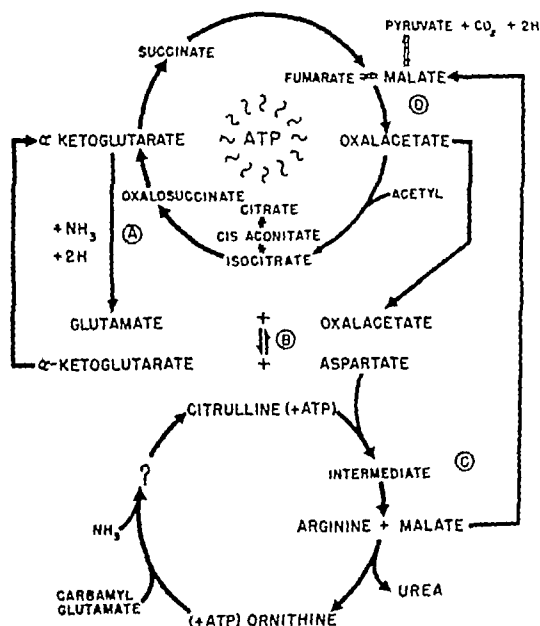


FIG 2 PATHWAY OF AMINO NITROGEN TRANSFER to citrulline in urea synthesis and the relationship of the ornithine cycle to the tricarboxylic cycle.

In the first dismutation, malic acid undergoes a DPN linked dismutation with NH_3 and α -ketoglutarate, catalyzed by malic and glutamic dehydrogenases (reactions 1 and 2) resulting in the formation of oxalacetate and glutamate, which then transaminate (reaction 3) resulting in aspartate formation (reaction 4). In the presence of phosphoglycerate and citrulline, the amount of arginine formed becomes a measure of the rate of aspartate synthesis (fig 1, curve 1).

In the second dismutation, triosephosphate dehydrogenase (reaction 1a) replaced malic dehydrogenase. Starting with NH_3 , α -ketoglutarate, oxalacetate and hexosediphosphate (as the source of triosephosphate), aspartate will be formed as shown by reaction 4a. With the resulting diphosphoglycerate as the source of $\sim\text{pH}$, arginine was formed as shown in curve 2. The rate of arginine

formation in an equal amount of extract with aspartate and phosphoglycerate is shown in curve 3. Curves 1a and 2a represent blank values for the dismutations in the absence of NH_2 or DPN

PHYSIOLOGICAL PATHWAY OF AMINO NITROGEN TRANSFER IN UREA FORMATION

The individual steps in the aerobic transfer of nitrogen to form urea and then relationship to transaminase and to the tricarboxylic cycle of Krebs are summarized in figure 2

Citrulline formation from ornithine (Step E), which appears to require ATP generated by the tricarboxylic cycle, proceeds with carbamyl glutamate as the source of the ureido carbon, according to the most recently published work of Cohen and Grisolia (18). Citrulline is then converted to arginine (Step C) by a two-step reaction involving ATP (generated oxidatively) and aspartate, in the formation of an intermediate, which is then split to form arginine and malic acid.

In homogenates with glutamate as the $-\text{NH}_2$ source, the transfer of nitrogen will start by

transamination (Step B) with oxalacetate supplied by respiration.

In slices, NH_2 transfer will occur at Step A (glutamic dehydrogenase) and Step E. It has become apparent from recent studies on the permeability of liver slices that the poor activity of aspartic and glutamic acids is due to their slow rate of permeability. In the intact animal, NH_2 , arising from amino acid deamination will enter the cycle as with slices.

The scheme represents an expansion of the ornithine cycle of Krebs, connected with the Krebs tricarboxylic cycle by transaminase and by two simpler cycles, one which permits the catalytic turnover of α -ketoglutaric acid as a carrier of NH_2 by means of glutamic dehydrogenase, and the other which permits the malic acid (formed as the second product in arginine synthesis) to function, catalytically, in aspartic acid formation by means of malic dehydrogenase.

From studies on the isolated system and on homogenates, the scheme shown here appears to represent a major pathway of urea formation and accounts for most of the observations on slices and homogenates reported from other laboratories.

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NUTRITION, RENAL LESIONS AND HYPERTENSION¹

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PREVENTION of the accumulation of excess fat in the liver by dietary choline was demonstrated in our laboratories in 1932 using depancreatized dogs and normal rats (1). In 1939, Griffith and Wade (2) reported that in addition to fatty livers, weanling rats exhibited a fulminating, hemorrhagic lesion of the kidneys (fig 6). The occurrence of this syndrome was subsequently confirmed by Gyorgy and Goldblatt (3), by Engel and Salmon (4) and by Handler (5), and it has also been extensively studied in our laboratory. In 1947, Sobin and Landis (6) demonstrated that the blood pressure remained normal in young rats maintained on diets deficient in choline for as long as 7 months. We have confirmed these results by direct measurements in weanling rats exhibiting the syndrome of acute choline deficiency, and also in older animals which were given similar diets for longer periods. As an extension of these investigations, we have also studied the blood pressures in a third group of rats. These were fed the diet low in choline for 6 days at ages of 3 to 4 weeks, and this was then replaced by a fully normal food mixture for the remainder of the experimental period of 4 to 7 months. Of these animals, more than one-third developed a moderate or severe degree of hypertension during the period of observation.

RENAL LESION PRODUCED IN WEANLING RATS BY DIETARY CHOLINE DEFICIENCY

A brief outline of the changes which occur in the kidneys of weanling rats during the acute

stage of choline deficiency will be presented, for these lesions are presumably responsible for the elevation in the level of blood pressure which later develops in many of those animals which survive this initial period. Christensen (7) working with Griffith and Wade reported the pathology of the hemorrhagic renal syndrome. On gross examination, the kidneys are swollen and red and may attain weights twice the normal (fig 6). Christensen's histological investigations demonstrated the presence of a widespread tubular lesion throughout the renal cortices, associated with hemorrhage into and beneath the capsule (fig 7). Tubules in the outer portion of the cortex were necrotic and those lying more deeply were degenerated (fig 8). Hyaline casts filled the tubules at the cortico-medullary junction (fig 9). Stainable fat droplets were present in some of the cortical tubules. These observations were confirmed by Gyorgy and Goldblatt (3) who also emphasized the primary nature of the tubular lesions, for glomerular degeneration always followed the tubular necrosis as a secondary event.

The pathogenesis of these lesions has been the subject of detailed investigations in our laboratories (8). It was shown that the initial lesion demonstrable in the kidneys of weanling rats fed diets low in choline consists of the formation of small droplets of stainable fat within the epithelial cells of the proximal convoluted tubules of the cortex (figs 10 and 11). These droplets appear within 2 to 5 days of dietary choline deficiency. This is associated with generalized swelling of the affected nephrons. Evidence has been presented (8) which indicates that tubular swelling produces obstruction of the intervening cortical capillary plexus with resultant tubular ischemia and necrosis (figs

¹ Presented as part of the Symposium on Nutrition in Preventive Medicine, Detroit, Mich., April 21, 1949.

1 and 2) This mechanism has been termed tubular obstruction of capillaries (TOC) Proximal to the sites of capillary obstruction, the cortical vessels become engorged and rupture into and beneath the renal capsule (fig 7) This combination of parenchymal swelling and necrosis with associated capsular hemorrhage is responsible for the gross appearance of the kidneys The development of this lesion is necessarily accompanied by a reduction of the volume and extent of the renal vascular plexus in the cortex As glomeruli are destroyed in the later stages, their total numbers are accordingly reduced

PRODUCTION OF HYPERTENSION IN SURVIVORS OF THE HEMORRHAGIC RENAL SYNDROME

Methods Weanling albino rats of the Wistar strain (35-42 gm) were fed a diet very low in choline and its precursors² The object at this stage of the experiment was to produce the greatest degree of renal damage compatible with life A series of preliminary trials indicated that the optimum duration of dietary choline deficiency for this purpose was only 5 or 6 days Pair-fed control animals received the same basal diet supplemented with 0.35 per cent choline chloride At the end of this short period of choline deficiency,

the experimental group and also the controls were given a good stock diet which allowed excellent growth and development throughout the remaining 4 to 7 months of the experiment During this period, indirect determinations of the blood pressures of all animals were made at intervals by the method of Sobin (9) But the final assessment of arterial tension was based on direct measurement of blood pressure as indicated by a fine-bore mercury manometer connected with a 24-gauge needle inserted into the animal's femoral artery Following exsanguination and death, the heart was weighed to the nearest 1/100 gram in each case For comparative purposes, heart weight was expressed as a percentage of the total body weight Paraffin sections of kidneys, heart and other viscera were stained by a variety of methods On an histological basis, the animals were tabulated in three groups according to the amount of residual renal damage observed in microsections *slight*, *moderate* or *severe* The series included 62 animals which had survived varying degrees of the renal hemorrhagic syndrome and 36 controls of similar age and weight The controls had received the same diet as the test animals throughout the entire experiment, but were given a choline supplement during the initial 5- to 6-day period when the experimental groups were deprived of lipotropic factors

Results These are presented in graphic form (fig 5) Ten of the 62 experimental animals had suffered *severe* residual renal damage as demonstrated by microscopic examination The average blood pressure of this group was 195 mm Hg (systolic-diastolic mean) and their heart weights were nearly double the normal Thirteen rats had *moderate* degrees of renal damage, and of these, 11 had blood pressures of 150 mm Hg or over and were thus considered hypertensive, the average for the group was 165 mm Hg The average weight of the hearts of the animals which comprised this second group was approximately one-third greater than that of the controls (expressed as percentage of body weight) The third group, which consisted of the remaining 39 experimental rats, had suffered only *slight* degrees of residual renal damage, and the average of the levels of blood pressure in these animals was only a little higher than for the controls The blood pressure of all these control animals, with one exception, were lower than 140 mm Hg The average figure was 118 mm Hg

The microscopic appearance of kidneys with

Arachin, 12%	Salts, 5%	Beef fat, 10%
Gelatin, 6%	Celluloflour, 2%	Corn oil, 2%
Casein, 3%	Sucrose, 57.5%	Cod liver oil concentrate,***
Fibrin, 1%	Vitamin powder,** 1%	0.015%
		Cystine,
		0.50%

* Salts composition/100 gm	MgSO ₄ (anhydrous), 7.18 gm
Calcium lactate, 35.00 gm	Fe citrate, 3.20 gm
Calcium carbonate, 5.06 gm	and trace salts
Ca(H ₂ PO ₄) ₂ · H ₂ O 14.60 gm	MnSO ₄ · 4H ₂ O, 0.33 gm
K ₂ HPO ₄ , 6.46 gm	ZnSO ₄ · 7H ₂ O, 0.035 gm
NaH ₂ PO ₄ · H ₂ O, 18.76 gm	CuSO ₄ · 3H ₂ O, 0.039 gm
NaCl, 9.34 gm	KI, 0.00039 gm

**Vitamin powder/100 gm mixture

Thiamin, 500 mg
Riboflavin, 250 mg
Pyridoxine, 200 mg
Calcium pantothenate, 1 gm
Nicotinic acid, 1 gm

Added to powdered sugar, 997.05 gm

***Cod liver oil concentrate used, contains 50,000 I.U. of Vit D and 200,000 I.U. of Vit A/gram
Supplied by Messrs. Averst, McKenna and Harrison, Montreal, Canada

severe degrees of damage indicated extensive loss of both tubules and glomeruli (fig 12) Those which had escaped destruction were often greatly enlarged Casts filled most of the distended tubules (fig 13) Hyalinization in varying degrees was encountered in many glomeruli (figs 16 and 17) Preparations injected with india ink demonstrated that the glomerular capillary bed was most severely reduced in those animals which had developed the greatest elevation in blood pressure (figs 3 and 4) Arterial lesions were present which were very similar in nature to those which are associated with both benign and malignant phases of human hypertension (figs 18 to 21) In two of 10 rats with arteriolar necrosis, the level of non-protein nitrogen in the blood was determined and found in both instances to exceed 350 mg per cent No evidence of inflammatory disease could be demonstrated in sections of any of the hypertrophied hearts of the hypertensive animals

Discussion The renal lesions of the hypertensive animals were the result not only of the acute period of choline deficiency in early life, but probably also of the hypertension sustained during the last few weeks before death Such features as hyalinization of glomerular loops and thickening of Bowman's capsule are, in our opinion, most likely the result of high levels of intravascular pressure On the other hand, parenchymal loss is probably attributable to the preliminary period of acute dietary deficiency Experiments are now in progress, the results of which should help us to obtain a better understanding of the pathogenesis of these renal lesions

It has been suggested by Dr Handler (10) that fibrotic organization in the areas of capsular and subcapsular hemorrhages, which occur in the acute initial period of deficiency, might be a factor in the production of hypertension later in these animals Such a mechanism would be analogous to that which operates in rats which develop high levels of blood pressure after the kidneys have been wrapped with cellophane or silk (11) (fig 14) In a few of our animals, very slight proliferation of fibrous tissue had occurred in capsular areas (fig 12), but in most cases there was no evidence of abnormal amounts (fig 15) In the acute period of renal damage, however, the site of greatest necrosis is just beneath the capsule It is possible that this necrotic tissue may act like a thickened capsule, but until the entire problem of the pathogenesis of these kidney changes has been more intensively studied, fur-

ther discussion along these lines will not be attempted

The reduction of the renal cortical vasculature which has been demonstrated is probably a factor of prime importance in the development of hypertension in these experiments This would be comparable to that type of hypertension which develops in rats which have been subtotally nephrectomized (12) We have no evidence regarding the hormonal mechanisms involved, but presumably they would be similar to those operating in animals rendered hypertensive by the Goldblatt technique (13)*

Initial attempts to produce hypertension in rats by allowing them to survive the hemorrhagic renal syndrome of choline deficiency met with little success Large numbers of weanling animals were fed the deficient diet for periods as long as 10 days Only a few which were very resistant to this type of renal lesion survived Irreversible damage to the kidneys of a degree sufficient to influence the level of blood pressure in later life had apparently not been produced in these few hardy survivors, for hypertension did not develop and histological examination of the kidneys revealed few abnormalities In later experiments a greater measure of success was obtained by restricting the length of time the young animals were fed the low-choline diet to periods of only 5 or 6 days The greater number of animals which then survived included many with extensive renal damage A significant proportion of these survivors later developed hypertension when fed the normal food mixture

In the experiments conducted by Sobin and Landis (6) in which animals were deprived of dietary choline for long periods, the experimental conditions would also automatically tend to eliminate those rats which were most susceptible to this type of kidney damage Furthermore, the growth of survivors of the acute period of deficiency when left on low-choline diets, is less than if they are transferred to normal rations such as we have used Slower growth rats of survivors would be associated with lesser demands on the cardiovascular-renal systems in these animals, and thus taken together with only slight degrees of initial kidney damage would explain the apparent

* We have seen the hemorrhagic renal syndrome in an adult rat which after a period of undernutrition was placed on a choline-deficient diet We have not yet learned how to produce these lesions consistently in adult rats

discrepancies between our results and those of Sobin and Landis Dr Handler who has con-

which contains a greater percentage of protein than is contained in most choline-deficient food mixtures Our stock diets employed in the second phase of the experiments contained a normal amount of protein, which was of course a higher



FIG 1 (upper) APPEARANCE OF CORTICAL CAPILLARY PLEXUS in kidney of a normal weanling rat used as control The vascular system of the rat was injected with a filtered dilution of india ink introduced under physiological pressure into heparinized, anesthetized animal via left ventricle Slices of formalin-fixed kidney, 100 μ in thickness, were dehydrated in ascending strengths of alcohol and cleared in benzyl benzoate The dense plexus is so well injected that glomeruli are obscured

FIG 2 (lower) APPEARANCE OF CORTICAL CAPILLARY PLEXUS in kidney of a weanling rat fed basal diet deficient in choline for a period of 5 days The technique of demonstration was identical with that described above for fig 1 Note failure of injection mass to penetrate terminal portions of plexus which supply the periphery of each renal lobule In stained sections, the tubules in the ischemic regions were necrotic The mechanism by which this type of regional capillary ischemia is produced has been designated TOC—tubular obstruction of the capillaries The evidence, which has been presented in detail elsewhere (8), indicates that initial lesion in development of hemorrhagic renal syndrome consists of appearance of striable fat in epithelium of the tubules which, at the same time, become swollen Pressure is thus exerted on capillaries which lie between affected tubules and results in vascular occlusion—TOC

firmed our findings (10) has suggested a further explanation He feels that to produce hypertension, it is necessary to feed the survivors a diet

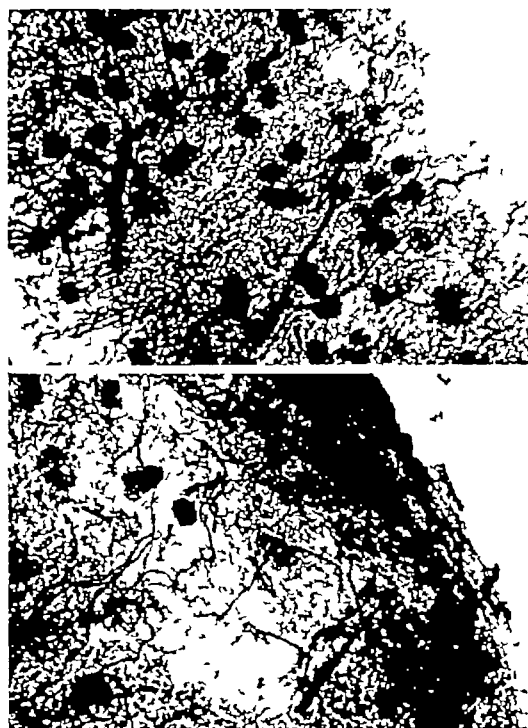


FIG 3 (upper) APPEARANCE OF CORTICAL CAPILLARY PLEXUS in kidney of an adult rat which belonged to the control group With age, tubules have increased in size so that capillary plexus is separated and not sufficiently dense to obscure glomeruli which also are larger than those in weanling rat illustrated in fig 1 Techniques employed for preparing and photographing the specimen are the same as those outlined for fig 1

FIG 4 (lower) APPEARANCE OF CORTICAL CAPILLARY PLEXUS in kidney of an adult rat which developed a malignant type of hypertension The animal belonged to the experimental group which was fed choline-deficient diet for 6 days followed by a full normal diet until time of death several months later Note obvious reduction in number of glomeruli Technical details are the same as described for fig 1

level than that in the basal choline-deficient ration

Selye (14) Krakower and Hemo (15) and Lenel *et al* (16) have demonstrated the inter-related roles of the adrenal cortex, the kidney and of dietary salt in the production of hypertension in chickens Deane and Olson (17) have shown that there is hypertrophy of the adrenal cortex in weanling rats which exhibit the syndrome of

hemorrhagic kidney. It is possible that this hypertrophy may contribute in some manner to the development of hypertension in animals surviving the syndrome. A normal amount of salt was present in the diets we employed, but further investigations along these lines are possibly indicated.

Calder (18a, 18b) has reported experiments in which a slight rise in the level of arterial pressure apparently occurred in rats which had been fed diets partially or completely deficient in the heat-

weeks on low-choline diets. As previously pointed out, Sobin and Landis (6) were unable to demonstrate alterations in the blood pressures of such

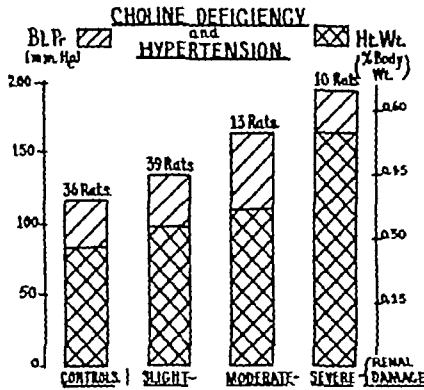


FIG 5 COMPARISON OF HEART WEIGHTS AND SYSTOLIC-DIASTOLIC LEVELS of blood pressure (direct method of measurement) in the 4 groups of rats which totalled 98 in number. The upper limits of the bars indicate average levels of blood pressure for each group according to the scale on the left. The shorter, cross hatched portion of each bar indicates for each group the average heart weight expressed as a percentage of body weight, according to the scale on the right. The number of rats in various groups is stated above each column. The animals were selected according to degree of parenchymal damage indicated at base of each column after assessment of microsections of kidneys.

stable fractions of the vitamin B complex. He found an average difference in blood pressure of 23 mm Hg between the rats in his experimental and control groups, as determined by direct measurements taken by cannulation of the abdominal aorta. The levels of blood pressure of his experimental animals (direct readings) averaged only 144 mm Hg. Calder did not report the cardiac weights of the animals in these experiments. Although he describes lesions of the renal parenchyma and thickening of both the arteries and arterioles with hyaline degeneration of the latter, his photomicrographs suggest that these changes were very slight. The parenchymal lesions resemble minor degrees of those which have been produced (8) in young adult rats maintained for several



FIG 6 APPEARANCE ON GROSS EXAMINATION of kidneys and liver (below) of a weanling rat fed the basal diet low in choline for a period of 7 days. Similar organs from a control rat pair fed the basal diet supplemented with choline during the same period are included (above) for comparison. The scale at right is in centimeters. Note increase in size of these organs of the experimental animal, the pale appearance of its fatty liver and the dark hue of its kidneys which were dusky red.

FIG 7 NECROTIC TUBULES IN SUBCAPSULAR REGION are widely separated by extravasated blood in this kidney section from a weanling rat fed a diet low in choline for 7 days. Hematoxylin and eosin stain $\times 150$.

FIG 8 SWOLLEN TUBULES SURROUND A GLOMERULUS in the kidney of a weanling rat fed a diet low in choline for 5 days. Tubules further away from the glomerulus and nearer the periphery of the renal lobule (upper right) are necrotic. Hematoxylin and eosin stain $\times 250$.

FIG 9 THE EPITHELIUM OF MEDULLARY TUBULES which are swollen with eosinophilic colloid is thinned and stretched. From the same section illustrated in preceding figure $\times 250$.

animals, and our investigations have confirmed theirs. The degree of hypertension reported by Calder is comparable perhaps to that which developed in the animals in our experiments which

comprised the group with only slight renal damage. Few of these animals were grouped as hyper-

it will require further work to decide whether or not choline deficiency is the responsible factor. In the meantime, it cannot be considered established that a significant hypertension can be produced by Calder's procedure.

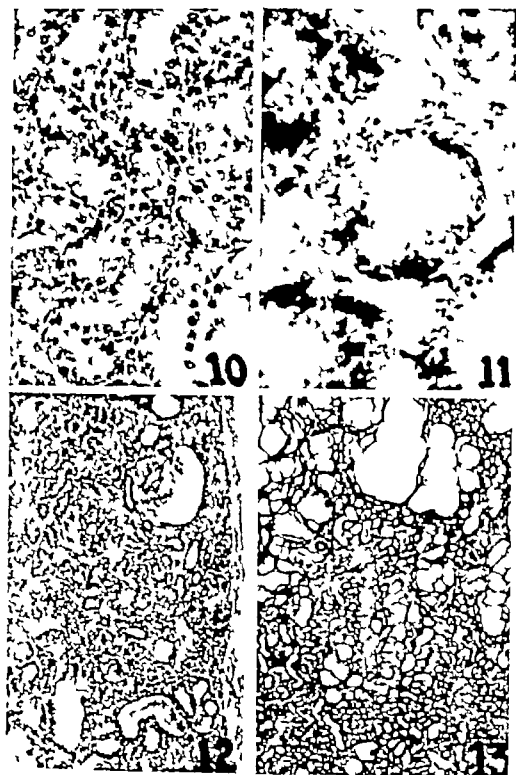


FIG 10 THE LESION which is initially demonstrable in course of development of renal hemorrhagic syndrome of choline-deficient weanling rats consists of appearance of small sudanophilic droplets in epithelium of proximal convoluted tubules which become swollen and compress intervening capillaries. The fat droplets appear as small black dots in the photograph. Frozen section of renal cortex from a weanling rat fed the choline-deficient diet for 2 days, Sudan IV and hematoxylin, Wratten G and H filters, $\times 100$.

FIG 11 RELATIVELY LARGE AMOUNTS OF STAINABLE LIPID in tubules of renal cortex of a weanling rat fed the basal diet for 6 days. Frozen section, Sudan IV and hematoxylin, Wratten G and H filters, $\times 425$.

FIG 12 SEVERE PARENCHYMAL DAMAGE IN KIDNEY of a rat surviving the hemorrhagic renal syndrome and attaining maturity on a normal diet. Note glomerular fibrosis and subcapsular scarring. The capsule itself is slightly thickened. Hematoxylin and eosin stain, $\times 70$.

FIG 13 PARENCHYMAL DAMAGE IN KIDNEY of a rat in the same group as the animal from which the section illustrated in fig 12 was prepared. In this case note extreme degree of tubular distension. Hematoxylin and eosin stain, $\times 25$.



FIG 14 EXTREME THICKENING OF RENAL CAPSULE of a rat which developed hypertension 2 months after kidney had been wrapped with gauze. A small area of underlying parenchyma is included in lower right of the field. For comparison with fig 15. Hematoxylin and eosin stain, $\times 20$.

FIG 15 COMPLETE ABSENCE OF FIBROSIS OF RENAL CAPSULE of a rat developing hypertension subsequent to a brief period of dietary choline deficiency in early life. Note thickening of glomerular capsule, tubular distension and cellular hyperplasia of small artery in lower left of the field. Compare with preceding figure. Hematoxylin and eosin stain, $\times 50$.

FIG 16 HYALINIZATION OF A GLOMERULUS AND ARTERIOLE in kidney of a rat with hypertension subsequent to dietary choline deficiency. Gomori's chromium hematoxylin and phloxine stain, $\times 250$.

FIG 17 DIFFUSE THICKENING OF CAPILLARY LOOPS in a glomerulus of kidney of a rat with hypertension associated with choline deficiency in early life. Azocarmine, anilin blue and orange G stain, Wratten B and L filters, $\times 250$.

tensive, as in our experiments only those with levels of blood pressure (systolic-diastolic averages) of 150 mm Hg or more were so designated. If Calder's findings are confirmed and extended,

The method of producing experimental hypertension in rats by short periods of dietary choline deficiency in early life is one which may introduce less complications than do procedures such as injection of nephrotoxic sera, or those involving

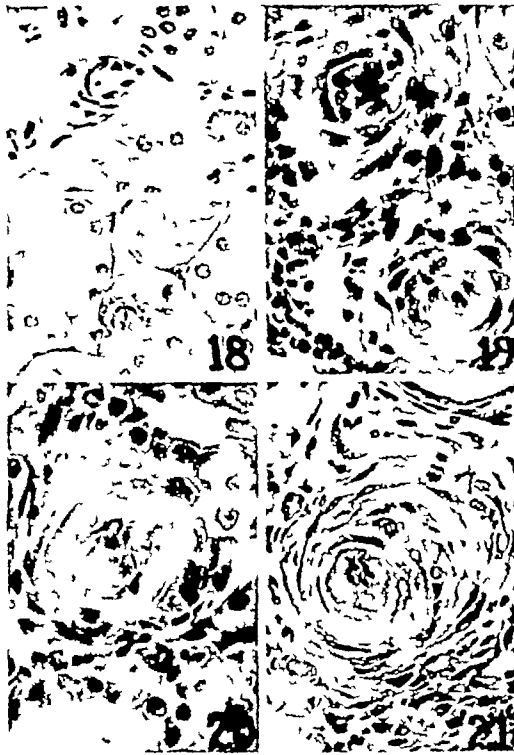


FIG 18 EXAMPLES OF 2 NORMAL ARTERIOLES IN renal cortices of kidneys of rats belonging to the control group Compare with figs 19, 20 and 21 Hematoxylin and eosin stain, $\times 300$

FIG 19 EARLY CELLULAR HYPERPLASIA OF ARTERIOLES in kidney of a rat surviving the hemorrhagic renal syndrome with only a *slight* degree of parenchymal damage This animal's blood pressure (systolic/diastolic average measured by a direct method) at the time it was killed was 150 mm Hg Hematoxylin and eosin stain, $\times 300$

FIG 20 HYALINE DEGENERATION WITH A SUGGESTION OF EARLY NECROSIS in a cortical arteriole in kidney of rat surviving the hemorrhagic renal syndrome with a *moderate* degree of parenchymal damage Blood pressure measured 170 mm Hg (systolic/diastolic average) Hematoxylin and eosin stain, $\times 300$

FIG 21 ADVANCED FIBRINOID NECROSIS OF AN ARTERIOLE in the renal cortex of a rat surviving a brief period of acute dietary choline deficiency in early life with a *severe* degree of parenchymal damage Blood pressure measured 210 mm Hg (systolic/diastolic average) Hematoxylin and eosin stain, $\times 300$

operations such as laparotomy The only difference in the treatment of the animals of our control

series and those in which hypertension was produced lay in the withholding of less than 0.02 grams of choline chloride from the diets of the latter for a brief period of 5 or 6 days

Our findings and those of Sobin and Landis have indicated that if young rats which have survived the hemorrhagic renal syndrome are maintained on diets deficient in choline throughout the entire experimental period, hypertension will not be produced If however, choline and its precursors (including protein) are restored to the diet after kidney damage has occurred, malignant hypertension may develop in later life We have already suggested that an important factor in explaining the absence of hypertension in the first instance may be the slow rate of growth which occurs in this group of animals The demands on the damaged cardiovascular system in these rats would be correspondingly lessened and renal metabolism would presumably be at a lower level because of the low percentage of protein in the deficient type of diet The relative importance of these various factors in the etiology of the hypertension we have produced is a subject of investigations we are planning to pursue

An important outcome of this work is the proof that extensive, and often fatal, pathological changes during adult life may have their origin in a very short period of dietary abnormality in infancy Correction of the abnormality during adult life, under certain conditions, may lead to pathological changes (hypertension in the present instance) which would otherwise not have occurred These findings suggest that the later effects of lesions produced by other dietary deficiencies in very young animals may prove a fruitful field of investigation which may yield the investigator a rich harvest

The nutritional aspects of this work have been made possible by a grant from the Nutrition Foundation, New York The histological studies were supported by the National Research Council of Canada

We are indebted to Dr Jessie H Ridout for a great deal of help with the diets The technical assistance of D H Beaton and W D Wilson in the histological work is gratefully acknowledged

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DISTRIBUTION OF ANTIBODY TO POLIOMYELITIS IN VACCINATED AND PARALYTIC MONKEYS¹

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THE study of immunity to poliomyelitis has taken on new interest since finding that, by intramuscular vaccination with active poliomyelitis virus, the majority of monkeys can be rendered immune to intracerebral challenge with large doses of active virus (1). This method has been effective in differentiating types of poliomyelitis virus (2, 3) as well as instrumental in studying immunogenesis of poliomyelitis. I plan to discuss the immune response to poliomyelitis in monkeys induced by a variety of exposures to virus.

The distribution of antibody in and out of the central nervous system in vaccinated animals has already been contrasted with that in paralyzed animals (4). These data will be reviewed and more recent observations will be presented on antibody distribution in vaccinated animals which have been challenged intracerebrally with various infectious and non-infectious nervous tissue suspensions.

Table 1 gives the distribution of antibody in fluids and CNS tissues of monkeys vaccinated with or paralyzed by Lansing virus.

The rhesus monkeys in the first group were vaccinated 4 to 6 times intramuscularly with 1 cc doses of 10 per cent Lansing infected monkey spinal cord. At 6 weeks, spinal fluid and blood were taken. After perfusion with sterile saline solution, the monkeys were killed. The thalamus, cervical and lumbar enlargements of the spinal cord and the visual cortex were saved. The thalamus was of interest as the region of inoculation,

and, like the anterior horn, a susceptible area. The visual cortex was selected as a non-susceptible area of gray matter. The spinal cord enlargements frozen on dry ice were quartered longitudinally and from the anterior quadrants the white matter was scraped away leaving sticks of gray matter. A single layer of gray matter was obtained from a flattened, frozen piece of visual cortex by scraping away the adjacent layers of white and gray matter. Suspensions of these tissues at a 1/32 dilution in saline were made in a TenBroeck tissue grinder. Further 10-fold dilutions were made from these. Each tissue and fluid was titrated by intracerebral mouse neutralization test, in the presence of 10 LD₅₀ of Lansing virus.

From table 1 it can be seen that in 8 vaccinated monkeys, serum titers arranged in decreasing order ranged from high to moderate titers (titers of 30-38 are maximum in our experience). Yet no antibody was demonstrable in spinal fluid or the parts of the CNS saved. The titers recorded as <1 indicate that from 3 to 5 mice were spared of the group of 8 which received the 1/32 dilution of nervous tissue and virus. The sparing of a few mice is inadequate to establish either a true neutralization or a negative result and is therefore recorded as <1. Tests with many of these tissues which yielded borderline results have been repeated and again yielded an indefinite result.

The next group in table 1 of 11 monkeys which were paralyzed as a result of intracerebral injection of Lansing virus were also studied for antibody distribution in the same tissues (except for thalamus). A marked contrast to the vaccinated group is apparent. The highest titers appeared in

¹ Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

the anterior horn of these convalescents, killed from 16 days to 5 months after onset of paralysis. Serum titers approximated those of the spinal fluid (suggesting a spilling over of antibody from the affected regions of the CNS) whereas no antibody was demonstrable in the visual cortex. The levels in the anterior horn were in most instances 10- to 100-fold greater than those in the fluids

TABLE 1 DISTRIBUTION OF ANTI-LANSING NEUTRALIZING ANTIBODY IN VACCINATED AND PARALYZED MONKEYS

	RHESUS NO	SERUM	SPINAL FLUID	THALAMUS	ANTERIOR HORN	VISUAL CORTEX
Lansing Vaccinated	B726	2 8 ¹	0		0	0
	B724	2 7	0		0	<1
	D926	2 6	0	0	0	
	B725	2 5	0		0	0
	D928	2 2	0	0	0	
	D927	2 1	0	<1	<1	
	B727	1 9	0		0	0
	D929	1 6	0	<1	0	
Lansing Paralyzed	B431	2 4	1 5+		2 9	0
	B429	2 2			3 1	0
	B739	1 9	1 9		2 9	0
	B736	1 6	1 0		2 6	0
	B737	1 2	1 0		2 3	0
	B444	1 2	1 5		3 3	0
	B738	<1	1 0		2 7	0
	B734	0	1 0		2 1	0
	B741	0	0		2 0	0
	B742	0	0		2 0	0
	B740	0	0		0	0
Brunhilde Paralyzed	D312				0	
	D374				0	
	D437				0	
	D438				0	

¹Neg log 50% endpoint by mouse neutralization test

The last group of 4 individuals in table 1 were killed 3-7 weeks after onset of paralysis incited by Brunhilde virus. No antibody to Lansing virus was demonstrable in the anterior horn of the spinal cord. The Brunhilde strain of virus is known to be immunologically distinct from Lansing virus as shown by reciprocal immunity tests in vaccinated animals. However, a cross-relationship among poliomyelitis viruses is demonstrable by reinoculation of paralytic convalescent monkeys. Such animals are 100 per cent immune to the

same virus as that with which they were vaccinated or to another poliomyelitis virus of the same immunological type. On the other hand, they are not fully susceptible to poliomyelitis virus of a different immunological type. For example, about half of a Brunhilde-convalescent group resists an intracerebral injection of a large dose of Lansing virus and vice versa (5). Just what is the basis of this relationship we do not know. The negative result presented here fails to throw light on the question.

TABLE 2 DISTRIBUTION OF NEUTRALIZING ANTIBODY IN LANSING-VACCINATED MONKEYS AFTER INTRACEREBRAL CHALLENGE

I C CHALLENGE	RHESUS NO	OUT-COME	SERUM	SPINAL FLUID	ANTERIOR HORN	VISUAL CORTEX
Lansing	D920	N	3 4 ¹	2 5		2 0
	E422	NP	3 3	1 5+	3 5+	1 5 1 2
	D921	N	3 0	2 3	3 0	2 0
	E423	N	2 6	0 5	1 9	1 1 1 0
	D922	N	2 3	<1 0	1 0	0
	E425	N	2 2	1 5+	2 2	0 1 5
Lansing, Minnesota	D210	P	3 4			3 4
	D218	P	3 3			1 9
	D221	P	3 2			2 5
	D220	P	2 9			1 5
	D217	P	2 7			2 0
Minnesota	D924	P	2 5	1 4	2 8	2 9
	D925	P	2 4	1 6	2 0	2 2
	D923	P	<1	0	<1	0
Normal CNS	E433	N	2 7	0	0	1 2 <1
	E430	N	2 6	0	0	0 0
	E431	N	2 4	0	<1	0 0
	E428	N	1 9	0	<1	0 <1
	E429	N	1 8	0	0	0 <1

N = no paralysis NP = non-paralytic poliomyelitis P = paralysis

¹Neg log 50% endpoint by mouse neutralization test

Having found that in vaccinated animals antibody is found primarily in the blood stream, and that with sufficiently high level of antibody such individuals are immune to intracerebral injection of large doses of homologous virus, I was interested to find what the distribution of antibody would be after such intracerebral challenge. Accordingly, 6 Lansing-vaccinated monkeys which proved immune to intracerebral injection of 10,000 LD₅₀ of Lansing virus were bled, tapped for spinal fluid, perfused with sterile saline and killed for study.

They appear as the first group in table 2. It

can be seen that they had moderate to high levels of serum antibody. The majority had antibody in the spinal fluid at a somewhat lower level. Within the CNS, antibody levels of the thalamus approximated those of the serum, but what is more remarkable was the finding of antibody in the majority of individuals in distant parts of the CNS, viz., the anterior horn and visual cortex. (No antibody was demonstrable in the anterior horn of the 2 individuals with the lowest serum antibody levels.) This finding immediately raises the question of whether the virus incited infection. In three individuals (E422 series) the spinal cord was examined histologically at levels T₂ and L₁ and one (E422) was found to have had inapparent infection. However, the pattern of antibody in this individual was no different from that in the others (with the possible exception of an outstanding concentration in the thalamus). In a larger series of 23 monkeys, vaccinated with and immune to intracerebral injection of Lansing virus, from which the medulla and spinal cord at levels C7 and L7 were examined, this individual alone showed evidence of infection. Thus the immunity of the vaccinated animal as a rule does not depend on CNS infection. It is fascinating to speculate on what may account for this antibody response at remote parts of the CNS. Either it is simply an immune response to introduction of antigen, perhaps as a secondary stimulus, or possibly the antigen as active virus exerts some immunogenic effect beyond what an inactivated preparation would, in spite of no histological evidence of infection. Against the latter hypothesis is the fact that antibody response seems to be universal through the CNS, and not confined to the susceptible areas. The effect of injection of inactivated virus intracerebrally has not been studied. Another control has been done, however, on the effect of intracerebral injection of normal CNS tissue suspension in vaccinated animals. This group of 5 monkeys appears at the bottom of table 2. With moderately high serum antibody levels, they had no antibody definitely demonstrable in the CNS, with the possible exception of the anterior horn of one individual (no material was available to repeat this test). This control serves to establish that the injection of CNS tissue (with the hemorrhage and ensuing local inflammatory reaction) does not in itself introduce anti-poliomyelitic neutralizing substance into the CNS.

Another line of attack is the effect of heterotypic poliomyelitic virus infection in Lansing-

vaccinated animals. This approach was thought of in connection with the question of whether the antibody in the affected areas of CNS in a Lansing paralyzed animal was developed locally or represented an accumulation of antibody produced elsewhere. The second and third groups of animals in table 2 bear on this point. The monkeys in both groups were vaccinated intramuscularly with Lansing virus, and challenged subsequently with Minnesota virus. In one group, they received an intervening Lansing challenge. In the group which failed to have this homologous challenge, one individual (D923) had such low titer of circulating antibody that it probably would not have passed such a challenge and is therefore not comparable to the others. The other 7 individuals showed moderate to high levels of antibody in the anterior horn of the spinal cord and in this regard are comparable to Lansing paralytic animals. The two individuals in which thalamus was tested showed equally high levels of antibody there, with lower levels in the spinal fluid. These findings support the contention that antibody may accumulate in the CNS in response to an inflammation reaction. This is perhaps opposed to the hypothesis which I have proposed of local formation of antibody in the affected parts of the CNS. There is one factor, however, which must be looked into further, and that is the question of possible antigenic relationship of these two poliomyelitis viruses. We now know they are sufficiently distinct immunologically to be considered two types, but to what degree there is a relationship we have not yet sufficient quantitative data to know. This problem recalls the resistance of approximately 50 per cent paralytic convalescent animals to reinfection with heterotypic virus. But as yet there is no immunological evidence to account for this resistance.

To summarize, Lansing paralytic convalescent monkeys have highest antibody levels in the affected parts of the CNS, in contrast to vaccinated animals in which antibody is demonstrable in the serum but not in the CNS. If circulating antibody is sufficiently high in such vaccinated individuals, they are immune to intracerebral injection of a large dose of homologous virus. In response to such injection of virus, antibody appears throughout the nervous system, even in distant, insusceptible areas.

Monkeys paralyzed with one poliomyelitis virus show no demonstrable antibody in the anterior horn of the spinal cord to a heterotypic virus. Nevertheless, monkeys vaccinated with one virus

and paralyzed with a heterotypic polomyelitis virus acquire in the anterior horn high levels of neutralizing antibody to the virus with which they were vaccinated

Does this suggest that antigenic stimulus with any polomyelitis virus within the CNS in an

animal already vaccinated with a polomyelitis virus renders the entire CNS immune? Since this stimulus has not yet been given in the form of inactivated virus, it is all too obvious how many steps will have to be taken before dreaming of any practical application of such an idea to man

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LOCAL IMMUNE RESPONSE AND VIRAL GROWTH RATE AS FACTORS IN IMMUNITY TO EQUINE ENCEPHALOMYELITIS VIRUS

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THIS discussion will be limited to a brief review of recent studies on the mechanism of acquired active immunity of mice to intracerebral challenge doses of equine encephalomyelitis (E E) virus (1, 2). These studies were the outgrowth of earlier work on the possible significance of humoral factors, specifically neutralizing antibody, in relation to resistance to infection.

Previous efforts to establish or rule out the existence of a quantitative correlation between antibody titers and resistance to infection with E E or other viruses were limited, by and large, to the antibody present at the time of exposure to the infecting dose of virus. This approach has yielded inconsistent or inconclusive results. It was suggested that the relatively high titers of neutralizing antibody in the serum, which appeared to be associated with resistance to intracerebral challenge doses of E E virus (3, 4), reflected the presence within the central nervous system of antibody in adequate concentration. This suggestion was based on the finding (4, 5) that antibody in actively or passively immunized animals was distributed between serum and the central nervous system at a ratio of 100/1 to 300/1. Two observations made it clear, however, that such a concept of immunity as a static function of serum antibody titer was untenable. a) In immunized guinea pigs which had survived an intracerebral challenge dose the serum/brain ratio was altered as a result of a disproportionate increase in neutralizing titer of brain tissue (6). b) The degree of resistance of mice vaccinated with formalinized Western E E virus was found to vary with the use of different strains of this virus as challenge inoculum (7). In particular, mice which following vaccination were resistant to certain strains were not or only partially protected against the 'R I' strain. This was true even for mice immunized

with vaccines prepared from the R I strain itself. Since it was not possible to show serological differences between the R I and the other strains, it was of interest to investigate the nature of this phenomenon.

One point of distinction between the R I and the other strains was that the former had undergone numerous brain-to-brain passages in mice. In the course of this procedure it had acquired the ability to kill mice in about one half of the time required by comparable amounts of the less well adapted strains.

Comparison of the rates of multiplication in the mouse brain of the 'fast' R I strain and a representative 'slow' strain (Kelser) has revealed differences corresponding to their relative rapidity of action. The belief that increase in the rate of viral multiplication is responsible for the difference in response of immunized mice to the R I and the Kelser strains has been strengthened by the finding that the latter, after 37 additional rapid brain-to-brain passages, simulated the R I strain in rapidity of action, and that vaccinated mice were significantly more resistant to the 'slow' parent strain than to its 'fast' derivative.

The difference in response to the two strains was not demonstrable in mice vaccinated with large doses of inactivated virus. By reducing the dosage of vaccine, a level of immunity could be induced at which all of the mice challenged with 10^5 LD₅₀ of the 'slow' strain would survive, while those challenged with the same amount of the 'fast' strain would succumb.

When the fate of the two virus strains in the brains of mice so treated was studied, the following difference was found: the R I strain multiplied at about the same rate in vaccinated as in normal mice. The Kelser strain, to which the vaccinated mice were immune, also multiplied, but at a slower rate than in normal mice. The maxi-

imum increase was about 100-fold over the amount found at 3 hours after inoculation, and virus persisted at the higher level for as long as 4 days after inoculation. No virus was demonstrable under these conditions in brains harvested on or after the 5th day.

Further tests were predicated on the assumption that a local immune response, stimulated by the challenge inoculum, could compete successfully with the slowly multiplying strain but not with the fast R I strain. It was found that brains in which virus was no longer detectable (i.e., in the experiment just described, those harvested from the 5th day on) were capable of neutralizing virus. The neutralizing titer of brain tissue increased markedly, reaching a maximum level after about 10 days and maintaining it for at least 4 months. As a result, the serum/brain ratio of neutralizing titers was reduced from a 'physiological' value of about 200/1 to less than 10/1.

The magnitude of this shift in serum/brain ratio depended inversely on the degree of pre-challenge immunity. One may assume that intensive vaccination by itself stimulated a primary immune response sufficient to render part of the intracerebral inoculum inert as antigen. This fits in with the observation already mentioned that the difference in response to challenge with 'slow' and 'fast' strains was not demonstrable in mice vaccinated with large doses of inactive virus.

The view that the increase in neutralizing titer of brain tissue is due to the antigenic booster action of the intracerebral inoculum finds indirect support in the observation that mice vaccinated with the smallest immunogenic amounts of vaccine were protected against large amounts, as much as 10^5 LD₅₀, but only partially resistant to about 10 to 100 LD₅₀ of the 'slow' strain. Comparative studies on the rate of viral multiplication after intracerebral inoculation of varying amounts have yielded converging curves for large seed inocula and more closely parallel curves for small amounts. This has been interpreted as indicating that after inoculation of large amounts only a fraction of the inoculum is utilized in the initiation of infection, leaving the bulk free to act as antigen. Small inocula, on the other hand, are presumably taken up quantitatively by susceptible cells, leaving no virus available as free antigen. Hence, while in animals 'sensitized' by extremely low-grade immunization there is no adequate primary immune response to overcome small challenge doses, large doses exert an anam-

netic booster effect resulting in the animal's survival. This type of 'paradoxical response' has also been described for other infections, notably in the case of rabies virus where standard potency tests on vaccines involve intracerebral challenge inoculations in immunized mice (8, 9). It may be responsible for misleading results, especially when the challenge test does not include the entire range of effective virus dilutions.

The conclusion that the virus-inactivating substance present in brain tissue is neutralizing antibody is based on the following evidence: a) The reduction in serum/brain ratio of neutralizing titers following challenge is paralleled by similar changes in the ratio of titers of complement-fixing antibody. b) It is specific. In animals vaccinated with a mixture of inactive Western and Eastern E E viruses and challenged with active virus of either type, the relative concentration of the homologous, but not of the heterologous, antibody in brain tissue rises. c) Like antibody in the serum, it follows the 'percentage law' on dilution of underneutralized mixtures.

It may be said, then, that the fate of a vaccinated animal after intracerebral challenge inoculation of E E virus depends on a variety of factors. Death or complete protection are not the only alternatives. Even in the immune, surviving animal, the challenge virus may multiply and persist at high levels for several days. Except for hyperimmunized mice, the decisive factor appears to be the more effective force in a competition between rate of viral multiplication and intensity of local immune response. The latter, in turn, is determined not only by the degree of 'sensitization' acquired with vaccination but also by the size of the challenge inoculum. The term 'sensitization' is used in this connection as denoting an increase in the potential ability of the immunized animal to respond locally to the antigenic stimulus of the intracerebral inoculum. It is chiefly this increase by which mice vaccinated with relatively small doses of inactive virus differ from normal animals.

It is not intended to suggest that mechanisms as complex as the one postulated here apply to other virus-host systems. It must be remembered that the artefact of an intracerebral inoculation is associated with extensive trauma in the immediate vicinity of susceptible cells. The inoculum, moreover, presumably is diffused throughout the closed system of the central nervous system. Thus, if it contains a large excess of virus which

does not participate in initiating infection, this excess is free to act as antigen wherever cellular elements involved in the immune response are exposed to it. It is conceivable that the mechanism of immunity may not involve all of the

factors mentioned to the same extent in those infections, for example, in which the virus acts directly on exposed surface cells or in which the site of inoculation is removed from the susceptible tissue.

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REMARKS ON CELLULAR RESISTANCE TO MAMMALIAN VIRUSES¹

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INTEREST arose in the theory of cellular immunity as applied to viral infections when it became apparent that this class of pathogens could multiply only in close association with living cells—indeed probably only within the confines of the cell membrane itself. With the establishment of this fact the obvious deduction was drawn that these pathogens were particularly fitted to elicit an immune response dependent exclusively upon the development of intracellular antiviral factors. This inference has led to numerous experimental attempts to obtain evidence of acquired cellular immunity to viruses. To a few of the more significant of these I shall refer toward the end of this paper. It may be stated at once, however, that they have not provided an unequivocal answer to the problem. At present, the available evidence permits merely the assessment of the probability that this phenomenon may occur.

In contrast to the doubtful status of acquired cellular immunity, the evidence for the existence of natural or innate resistance of cells to infection by viruses is convincing. I shall therefore review this evidence in some detail, especially that which has been gained through studies in tissue cultures. Before doing so, I shall define the concept of cellular resistance which I have followed in the selection of the material.

Cellular resistance depends upon an intrinsic or acquired property or properties of the cell, which independent of factors in the body fluids may prevent the multiplication of a virus or destroy its activity. Obviously this definition is extremely broad and would include such non-specific and non-immunologic properties as an impermeable cell membrane or the absence of an enzyme system. To embrace, however, the phenomena of natural immunity to viruses such breadth of definition is clearly essential.

NATURAL OR INNATE CELLULAR RESISTANCE

Factors other than the activity of cells which might be responsible for natural resistance. At the outset we may appropriately consider the possibility that certain non-cellular factors could be responsible for the natural resistance of certain species. To do so will serve to reveal the dominant position of the cell in this type of immunity. Such factors may be grouped under three headings: 1) non-specific antiviral effects of plasma, or other body fluids which are destroyed by moderate heating, 2) thermostable virus-neutralizing substances, 3) physical conditions incompatible with survival or multiplication of the virus.

In respect to the first of these, data exist which indicate clearly that fresh unheated serum from normal susceptible animals may inactivate or restrict the multiplication of a virus if mixed with it and then inoculated. For example, Douglas and Smith (1) found that fresh rabbit serum mixed with vaccinia virus and then inoculated intradermally into rabbits definitely modified the development of the lesion. We have also observed, in unpublished experiments, that fresh rabbit and cat sera added to small amounts of vaccinia virus reduce the size and number of pocks which subsequently develop on the chorioallantoic membrane of chick embryos. Recently Leymaster and Ward (2) in a study of the augmenting effect of complement on mumps neutralizing antibody found that undiluted or slightly diluted unheated sera of normal monkeys and presumably susceptible human beings were capable of preventing infection of chick embryos by the mumps virus.

Although such observations are worthy of note for those engaged in the demonstration and titration of virus neutralizing antibodies, one cannot regard the antiviral effect *in vitro* of unheated normal serum as playing a significant part in natural resistance. For it is obvious, as in the case of anthrax, that there is no correlation be-

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tween host susceptibility and the capacity of the serum *in vitro* to inhibit the virus

A purely physical factor, the normal temperature of the body, may render some species naturally resistant to attack by certain viruses. Dr. Pearson and I (3) a few years ago obtained evidence that the striking difference between the high susceptibility of the chick embryo and the complete resistance of the newly hatched chick to infection with the Melbourne strain of influenza A virus was adequately accounted for by the difference in temperature of the two hosts. Subsequently McLean and his co-workers (4) likewise found that the multiplication of a strain of influenza B virus was markedly inhibited at temperatures corresponding to the body temperature of birds. I am not aware that similar studies have been made with other viruses such as those causing equine encephalomyelitis which are highly virulent for the chick embryo but to which the fowl is almost or completely resistant. Such investigations, though, would be of value in assessing the general importance of a high body temperature in the natural resistance of the fowl to many mammalian viruses as compared with that of its embryo.

In addition to these three classes of non-cellular factors, one can conjecture that other conditions such as an unusually high hydrogen-ion concentration of the saliva or the presence of antagonistic micro-organisms in the flora of the nose, the throat or the gastrointestinal tract might arrest or destroy certain viruses at their portal of entry. In general, however, it would seem, that with the exception of differences in normal body temperature, non-cellular factors are of little significance.

Evidence that cellular resistance is the principal factor in natural immunity. By exclusion, then, one arrives at the tissue cells as the major factor in natural resistance to viruses. Moreover, a great deal of evidence—far too much to be reviewed here—of a more direct character has been accumulated through studies of the behavior of viruses in living animals especially in regard to the type of cell which is injured and the organ or tissue in which multiplication occurs. From certain points of view even more convincing than these observations made *in vivo* are the results obtained by tissue culture techniques. Since I have been interested for some time in these methods and since the time is limited, I shall confine my remarks mainly to a review of the

results so obtained in respect to the problems under discussion. In their evaluation, however, one should bear in mind, that, as Beard and Rous (5) have said, the physiological isolation inevitable to all cultivation *in vitro* is not always an advantage from the experimental point of view.

When directed to the investigation of natural cellular immunity the tissue culture technique has shown that in most cases in which adult tissues have been employed the growth or failure of growth of a given virus *in vitro* is correlated with susceptibility or resistance *in vivo*. So far, I believe, lack of such correlation has been observed only when the resistance *in vitro* of embryonic tissues has been compared with that of the living adult of the same or another species. In these instances where embryonic tissues have proved susceptible and the living adult relatively resistant, it is possible that differences in the properties of the cells may be involved. But before this conclusion can be accepted, the effect of non-cellular factors such as a more elevated body temperature of the adult must be eliminated.

Examples of correlation between natural resistance *in vivo* and *in vitro* have been described by Andrewes (6) and by Ivanovics and Hyde (7) in the case of Virus III infections of rabbits. The same relationship has been demonstrated by Andrewes (8) for the salivary gland virus of guinea pigs. Hallauer (9) who has reviewed the literature up to 1939 cites experiments of Frenkel and van Waveren who found that the virus of foot and mouth disease can only be cultivated in the tissues of susceptible species such as the cow, sheep and the guinea pig. Findlay (10) was able to obtain multiplication of fowl pox only in tissues of susceptible birds. A particularly good instance of agreement between host resistance and the *in vitro* behavior of the cells is to be found in the studies of Hallauer (9) on different strains of fowl plague virus. He was unable to propagate in mouse embryonic tissues a strain to which mice were entirely refractory, although he succeeded easily when he used chick embryonic tissue. On the other hand, working with two strains which induced infection in mice as well as in fowl, he was able to cultivate both agents in cultures of mouse tissue as well as in those composed of chicken cells. Sabin and Olitsky (12) succeeded in growing a strain of human poliomyelitis only in cultures consisting of human embryonic brain

fragments. The virus rapidly disappeared from cultures of chicken or rabbit nervous tissues. Employing the Lansing strain of poliomyelitis virus we have recently confirmed the findings of Sabin and Olitsky in respect to growth in human nervous tissues (13). As yet, however, we have been unable to obtain evidence of the multiplication of this virus in fragments of mouse brain. Since the Lansing strain is pathogenic for mice, this unexpected finding might appear as an exception to the general correlation between *in vivo* and *in vitro* susceptibility. But there may well be other reasons for this discrepancy.

The difference in resistance to a number of viruses of various types of cells derived from the same host species has also been investigated by means of tissue cultures. Unfortunately there have been few studies in which pure cultures of cells have been employed. They are sufficient, however, to establish the fact that some viruses can multiply only in certain cell types. Thus Carrel (14) as early as 1926 showed that the Rous sarcoma virus failed to proliferate in cultures of chick fibroblasts but actively increased in cultures of monocytes. The agents of foot and mouth disease and fowl plague according to Köbe and Fertig (15) and to Hallauer (11) multiply only in epithelium. Findings of this sort serve to demonstrate even more clearly than in the living animal the strict cytotropism of some viruses. They also forcefully reveal how closely adapted these viruses are to subtle differences in metabolism or other properties which must distinguish one cell type from another. In contrast to such agents the cytotropism of certain other viruses appears to be much broader. The virus of vaccinia has been grown in pure cultures of Kupffer cells by Beard and Rous (5) and in cultures of blood monocytes by Florman and Enders (16) as well as in corneal epithelium by Rivers and his co-workers (17).

With Feller and Weller (18) we have obtained evidence that this virus will also increase in fibroblasts although pure cell cultures were not used. If cell injury and death be criteria for the multiplication of a virus, the agent of equine encephalomyelitis is pancytotropic in respect to the cells of the chick embryo as revealed by unpublished experiments of Relova and myself and by the more recent observations of Huang (19). In experiments carried out during the past year with the Lansing strain of poliomyelitis virus we have, in collaboration with Robbins and Weller (13), determined that multiplication takes

place in cultures not only of nervous tissue but in those composed of fragments of intestine as well as in those consisting mainly of skin and striated muscle. Since no intact nerve cells were present in the cultures of skin and muscle it is highly probable that cells other than those of the nervous system are susceptible to this strain of the virus.

Varying degree of resistance of cells to different viruses. We have so far been mainly concerned with observations which show that certain cells may be completely resistant while others are susceptible. There are many data to suggest, however, that between these two extremes different types of cells may exhibit variable degrees of susceptibility to different viruses. Variation in susceptibility may be expressed in two ways: first, by differences in the quantity of the virus produced by different cells within a fixed period, second, by the extent of the injury the cell undergoes as a result of the presence of the virus.

That different tissues, at least, vary in their capacity to support the multiplication of a virus is indicated by numerous experiments in which the virus content of tissues removed from infected animals has been measured. As illustrations, it will be sufficient to mention two investigations in which determinations of the concentration of virus present in different parts of the infected chick embryo have been carried out. Pearson (20) employing the Melbourne strain of influenza A virus found it present in largest quantities in the allantoic fluid and yolk sac. Relatively low concentrations were observed in the amniotic fluid and in the embryo itself. Similarly in unpublished experiments we have observed that with an egg-adapted strain of mumps virus the highest yields were obtained in the embryonic fluids and membranes. In the embryo itself small amounts were encountered. Very little has been done with tissue cultures to determine whether isolated cells vary in their capacity to support the growth of virus. I can only refer to some observations made in my laboratory a few years ago when it was noted that in roller tube cultures of fibroblasts developing from fragments of chick embryonic heart muscle the production of vaccinia virus appeared to be somewhat greater than in comparable cultures of blood monocytes.

As I have said, the extent of injury the cell undergoes as a result of the presence of a virus may also be interpreted as measure of its resistance or susceptibility to the pathogenic effect of the infecting agent. Thus it has long been apparent that the nerve cells are particularly sus-

ceptible to certain neurotropic viruses, the parenchyma cells of the liver to yellow fever virus and so on. Indeed so little affected are other types of cells in such diseases that it has often been held that the responsible agents multiply only in those cells which exhibit gross manifestations of injury. Evidence obtained by a variety of techniques during the past 15 or 20 years shows, however, that obvious cell injury is not the inevitable consequence of virus multiplication. Some of the most conclusive information on this important point has been gained through measurements of virus increase in tissue cultures correlated with the histological examination of the infected tissues. Thus the virus of equine encephalomyelitis not only increases very rapidly in cultures of mixed chicken embryonic tissues but within 24 to 48 hours brings about the death of all or nearly all the cells as indicated by morphological appearance and cessation of metabolism (19). In sharp contrast the virus of mumps, as we have recently found, increases relatively slowly in cultures of chick amniotic membrane and continues to multiply for several weeks without inducing any recognizable change in cell morphology or metabolism as measured by acid production (21). Similarly Florman and I (16) could detect no pathologic changes in cultures of blood monocytes in which vaccinia virus continued to multiply for several weeks. Vaccinia virus, however, induces the formation of cytoplasmic inclusions in fibroblasts growing out of fragments of chick embryonic tissues in roller tube cultures (18). Nevertheless the death of these cells does not appear to ensue immediately. Indeed such cultures may continue to metabolize and cell growth take place for many weeks while multiplication of the virus continues at a fairly constant rate. It is also probable that certain other viruses exert an injurious effect on cells only after prolonged contact. Thus we have lately repeatedly observed in cultures of various human embryonic tissues infected with the Lansing strain of poliomyelitis virus degenerative changes in most of the cells after 25 to 32 days of cultivation although it was shown that the agent had undergone active multiplication at a much earlier period when no cytologic abnormalities attributable to the effect of the virus could be discerned (13). Dr. Herbert Morgan has observed an analogous series of events in tissue cultures infected with psittacosis virus (22).

I have dwelt at this length upon the variation in the pathogenicity of various viruses for different cell types not only because it illustrates

a difference in resistance between cells but to re-emphasize the fact that the multiplication of some mammalian viruses may occur, and often does occur, quite independently of any demonstrable injurious effect on the cells with which they are associated. This fact, as we shall see, may be important in considering the problem of acquired cellular immunity which I shall now very briefly discuss.

PROBLEM OF ACQUIRED CELLULAR IMMUNITY TO VIRUSES

Indications that cells acquire specific resistance as a result of infection. The possibility that tissue cells may acquire a specific immunity to viruses is suggested mainly by the failure in numerous instances to demonstrate a constant correlation between the presence or concentration of virus-neutralizing antibodies in the body fluids and the resistance to reinoculation of an animal that has been previously infected or vaccinated.

Many examples of this phenomenon could be cited. It will suffice here to recall only one. Hodes and Webster (23) found that mice which had been vaccinated with St. Louis encephalitis virus developed a very considerable resistance to reinfection which lasted for 6 or 7 weeks. During most of this period, however, virus neutralizing antibody could not be demonstrated in the serum. Later when such antibody had appeared, resistance to reinfection had diminished. At the end of 20 weeks when the animals again became entirely susceptible to infection, the antibody was still present in high titer in the blood.

Findings of this sort are indeed difficult to explain entirely on the grounds of humoral immunity. In some cases as Vieuchange and Galli (24) have shown in dermal infections with vaccinia virus the neutralizing antibody first emerges locally in the area of the infected tissues and only appears later in the blood stream. But the interval of time between these two events is usually short, not exceeding a few days. It is difficult therefore to explain such phenomena as described by Webster and Hodes on the basis of local antibody retention.

The theory of course has long been current that under certain circumstances the antibody produced at the site of infection remains permanently fixed to the cells and does not enter the circulation in appreciable quantities. Lépine (25), just before the war, again discussed this hypothesis and presented some evidence to support it. On the whole,

though, in spite of many attempts to demonstrate antibodies exclusively associated with the cells no convincing evidence that this occurs has been presented

In other instances interference between the inactive virus originally introduced and that employed for reinoculation might be responsible for the observed resistance. The effects of interference, however, would not be expected to be very durable—at least when inactivated virus is employed as an immunizing agent

Failure to demonstrate the development of cellular mechanisms which prevent the multiplication of virus If, then, neither virus neutralizing antibody nor the interference phenomenon adequately explain such manifestations of acquired resistance, can it be shown that the cells of immunized animals removed from the influence of neutralizing antibody or other inhibiting factors in the serum fail to support the multiplication of the homologous virus?

The bulk of experimental results would seem to leave little doubt that the answer to this question is in the negative. When adequate precautions have been taken to eliminate absorbed antibody, fixed tissue cells derived from immune animals have been shown by the tissue culture method to be as susceptible as those obtained from normal individuals. Moreover, various types of phagocytic cells removed from immune donors when freed of antibody by repeated washing have not been found to inhibit the multiplication of virus to a degree exceeding that exhibited by comparable cells from normal animals. In support of these statements it is only necessary to refer to the work of Andrewes on the propagation of herpes simplex virus (26) and Virus III (27) in fragments of rabbit testicle, the experiments of Rous, Hudack and MacMaster (28) with rabbit embryonic tissues and vaccinia virus, those of Beard and Rous (5) with isolated Kupffer cells and vaccinia and those of Smorodinseff and Shushkina (29) with polymorphonuclear leucocytes and macrophages from mice immunized against influenza A virus

Possibly unrecognized mechanisms dependent upon increased resistance of cells In conclusion we shall inquire whether there may be any other possible mechanisms of acquired immunity to viruses associated with or developed by the cells. Relatively recent observations suggest that perhaps two hitherto unrecognized responses of this sort may occur. Various workers such as Grabar

and Schoen (30) in France as cited by Lépine (25) and Gard (31) in Sweden have obtained from extracts of infected tissue removed during the acute stage of certain virus diseases, factors which when mixed with the homologous virus inhibit its activity. Whether or not these antiviral factors are to be identified with serum antibodies or whether they are distinct products of the cell or possibly the virus itself has not yet been conclusively determined. It is probable, however, that they do not represent the usual type of antibody. In a personal communication Dr. Gard has informed me that the factor he has obtained from mouse brain infected with Theiler's virus is more heat resistant than the known antibodies and also resists digestion by such active proteolytic enzymes as ficin. Grabar and Schoen prepared their material from the brains and lungs of mice infected with the virus of lymphogranuloma venereum. Mice infected with this virus do not develop in the blood appreciable quantities of neutralizing antibodies. It is unlikely therefore, that the antiviral activity of these extracts can be attributed to the usual type of neutralizing antibody. In this connection the recent experiments of Bodian (32) are also relevant. He has shown that monkeys suffering from acute poliomyelitis caused by one serologic type of virus are usually refractory to intracerebral inoculation with a heterologous strain. In contrast, convalescent monkeys are not immune to heterologous strains. Bodian does not believe that the resistance exhibited by monkeys in the acute phase of the disease depends upon the interference phenomenon.

The possibility, then, that under the impact of primary infections certain cells may respond by the production of antiviral factors which have hitherto not been recognized must obviously be further investigated.

Another approach to the problem may have been opened by observations by Galli and Cipollone (33) which were reported in 1941, but have not yet been confirmed. Following repeated unilateral intratesticular inoculation of formalin-inactivated vaccinia virus in rabbits, these workers noted that a high concentration of virus neutralizing antibodies developed in the blood serum. In such animals, in spite of the presence of the antibody, active virus introduced into the skin or into the untreated testis produced reactions which were only moderately or slightly less severe than those observed in unvaccinated rabbits. In contrast, the injection of the same quantity of

virus into the vaccinated testicle did not induce an inflammatory reaction. Yet titration of the virus content of each testicle showed that the agent had increased greatly and to the same degree in both. Failure of the vaccinated testicle to react could be demonstrated at least 37 days after inoculation of the last dose of vaccine. If these experiments can be confirmed, it would seem that

resistance to the pathogenic effect of a virus may be induced without increasing the capacity of the cell to prevent the multiplication of the agent. The thought that the response of a cell may be altered artificially in this manner, is perhaps rendered more acceptable by the fact that in normal cells the response to injury is not an inevitable consequence of virus proliferation.

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INHIBITION OF SOME HEMAGGLUTINATING VIRUSES

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THE polysaccharides have been studied more intensively than several other agents (1-3) which are known to inhibit viruses. This study of the polysaccharides has been integrated with research on the relation of virus to host cell. I will first briefly mention this relation as it applies to the viruses of the mumps-influenza group, and later will describe inhibitors of these viruses found in serum, ovarian cyst contents and other mucous secretions, allantoic fluid, vegetable polysaccharides and certain bacterial extracts.

You are all familiar with the phenomenon of the attachment of the hemagglutinating viruses to red cells (4), and know that with many strains there is a subsequent elution of apparently unchanged virus and a permanent alteration of the cell. Elution is believed to be due to enzymic alteration of cellular receptors.

Several types of bacteria, notably the *cholera vibrio*, produce soluble products which cause receptor alteration much more rapidly and completely than do the viruses of mumps, Newcastle disease or influenza (5).

The attachment of virus to red cell has been considered to be a model of the early stages of the infection of living cells. The comparison may be only partly valid (6).

Polysaccharide inhibitors may be conceived to act in two ways: they may prevent the attachment of virus to cells (red cells, or living or dead tissue cells) or may modify the multiplication of virus within living cells.

In normal animal serum, there are at least two types of inhibitor. One is possibly a globulin, it is destroyed by heat at 65° C. in less than 30 minutes (7). It is slowly destroyed by cholera filtrates, but apparently is not altered by incubation with live virus. It inhibits hemagglutination by living virus, but only to low titer, this titer varies between strains, being in general higher with the more recently isolated strains. The titer also varies considerably according to the particular chicken which supplies the red cells for the Hirst inhibition test (8).

A second class of inhibitor we believe to be a

mucoid (9) and I will assume this to be so during the rest of this paper. It inhibits hemagglutination by virus which has been heated to 56° C. for 30 minutes and it is the substance responsible for the phenomenon described by Francis (10) in 1947. It is thermostable, in fact the apparent titer of the substance increases up to 8-fold when a 1:10 dilution of serum in saline is heated to 100° C. for one minute. It is rapidly destroyed by cholera filtrates, and also by live virus at 37° C. (11). In the latter case the system reaches an equilibrium, at which the amount of inhibitor inactivated is characteristic of the virus used (12). The inhibitor is destroyed by large amounts of periodate (12), a reagent which is particularly active against carbohydrates.

A similar mucoid inhibitor has been isolated from human ovarian cysts (12), and the action of periodate on this mucoid has been particularly studied (13). The raw mucoid will inhibit hemagglutination by heated WSE influenza virus, but not by living WSE, and will reduce the infective titer of WSE 10-100 fold. A large amount of periodate will destroy this inhibitory power completely, but mucoid treated with small amounts of periodate will inhibit hemagglutination by living WSE virus, and will reduce the infectivity of WSE about 1000-fold as tested in the chick embryo. You will see that mild periodate treatment of mucoid has magnified its inhibitory power against live virus, such treated mucoid can no longer be destroyed by virus, or by choleia enzyme.

Essentially the same properties are shared by inhibitors from a wide variety of sources, such as egg white (14), allantoic fluid (15, 16) and aqueous extracts of many tissues (17), particularly of certain salivary glands of ferrets, men, mice and sheep. It is possible that blood group A substance should be included in this list (12). A similar inhibitor can be extracted from red cells of certain species (17, 18), partly because of this, there has been commonly accepted the working hypothesis that red cell receptors and soluble inhibitors are closely related.

Probably all these inhibitors act by competing with the red cell for the virus surface. It seems likely that this competition gives rise to a state of dynamic equilibrium between the various possible combinations of virus, cell and inhibitor. For example the estimated titer of either inhibitor in serum is greatly influenced by the type of chicken cells used in the system (11). When cells with a low affinity for heated virus are employed, the titer of mucoid inhibitor in a serum may be, say 5000, if cells with a high affinity are added to the system, the inhibitory titer will be in the region of 100 with the same serum. This suggests that the union of virus with inhibitor is reversible, and that, in the circumstances I have described, the dissociation of virus and inhibitor is not due primarily to enzymic action of the virus. This is supported by the finding of Hardy and Horsfall (19) that virus in the presence of a certain inhibitor in allantoic fluid is equally as infectious as virus in the absence of inhibitor, though the virus cannot free itself from this inhibitor by enzymic action.

We have seen that in this competition for the virus, the advantage is given to the mucoid rather than to the red cells, either when the mucoid is partially treated with periodate or when the virus is heated. Either procedure prevents subsequent action of the virus enzyme. It has been concluded that these mucoids act as inhibitors because their attraction for the heated virus is not reduced by enzymic activity, and conversely that live virus is not inhibited because it can inactivate the inhibitor (19-21). There appear to be two difficulties to this explanation. a) Although at 0° C enzymic action of virus on mucoid inhibitor is minimal, live Lee virus in our hands is not inhibited to significant titer even at this temperature. b) When live virus agglutinates red cells at 0° C in the presence of inhibitor, the thermostable agent which can be recovered from the system is still highly potent in the inhibition of heated virus. Of course, there can be no doubt that live virus will react with inhibitor and destroy it at 37° C and that heated virus cannot react in this way. But is it safe to assume that this functional difference is the basis of the inhibition of heated virus by mucoid? It may be, for example, that the greater effect of the mucoid inhibitor on heated virus is due to a thermal alteration of the adsorptive surface of the virus rather than to the loss of enzymic activity per se (22).

Svedmyr reports (16) that the inhibitory agent

for PR8 hemagglutinin, present in normal allantoic fluid, is in the main connected with particles with a sedimentation constant of 200 S. This component reacts in precipitation tests with virus, and does so in definite proportions. The inhibitor progressively decreases in amount in infected embryos, and is destroyed *in vitro* by live virus and by cholera filtrate. It does not inhibit virus multiplication in the egg. Svedmyr briefly describes a second type of hemagglutinin inhibitor in allantoic fluid, an inhibitor which is not susceptible to inactivation by virus and which is not sedimentable at 27,000 r.p.m. These two inhibitors may well correspond to the two described before as being present in normal serum. Hardy and Horsfall (19) have suggested that in infected allantoic fluid some of the virus is linked to an inhibitor which it does not inactivate. It may be that this substance is the same as Svedmyr's unsedimentable inhibitor and the same as the thermostable inhibitor in normal serum described by McCrea in 1946 (7).

Svedmyr believes the thermostable particulate inhibitor may be derived directly from the cell receptors lining the allantoic cavity. This concept of the identity of the 'soluble' thermostable inhibitor, the receptors on red cells, and the receptors on susceptible tissue cells, constantly appears. On the basis of the latter part of this hypothesis, Dr Stone (23) in 1947 carried out a series of interesting experiments. She reasoned that if the hypothesis were correct, the cholera filtrate which readily destroyed red cell receptors should also destroy tissue cell receptors. With their receptors destroyed, the cells would not be susceptible to virus attack. Her experiments confirmed this. Prior treatment with cholera filtrate in the allantoic cavity of chick embryos prevented subsequent infection in all the eggs inoculated with up to 30 infective doses of CAM influenza virus, and in about 90 per cent of eggs inoculated with 50 ID₅₀ of NDV. The protective effect lasted for about two days following the cholera treatment. After about 54 hours the receptors were apparently regenerating and the cells again became susceptible.

You will remember that the salivary mucus of ferret, mouse and man had a particularly high titer of thermostable inhibitor, in these species clinical signs of influenza are readily produced. There is a suggestion, though no more, that the sheep, which is the only other animal known to have a high titer of inhibitor, may also be clini-

cally infected. These correlations seem more than coincidental, but their significance remains open to question. It may be that the presence of inhibitor in the respiratory secretions merely reflects the presence of virus receptors on respiratory cells, or the relation may be more direct in that virus more readily persists, and infects in the presence of a mucin to which it can be adsorbed and with which it can react enzymically. It is cogent to ask whether in other species the respiratory mucin, which is not so susceptible to virus action, prevents the rapid spread of virus over the respiratory cells.

Bacterial polysaccharides were reported by Levine and Frisch in 1932 (24) to inhibit the lysis of the corresponding bacteria by bacteriophage. Burnet and Gough (25, 26) confirmed the specificity of this inhibition and believed that it was due to the blocking of the bacteriophage by the polysaccharide. More recently, Maurer and Woolley (27) described the non-specific action of pectin in preventing lysis of bacteria after they have been infected with bacteriophage. Apple pectin has also been found by Green and Woolley (28, 29) to prevent hemagglutination due to Lee virus, and in doing so to adhere both to virus and to red cells. It was also partially or completely effective in inhibiting Lee virus multiplication in the fertile hen egg, even when given 1-2 hours after inoculation of the virus. The authors were stimulated to investigate apple pectin because it was thought to be possibly an analogue of the hypothetical cellular substrate of the influenza virus. Subsequently Woolley (30) has reviewed the subject of competition between analogues and has suggested (31) that such competition may be the basis of the inhibitory action of apple pectin.

It is not known whether the effect of these pectins is destroyed by virus or by cholera filtrates. Woolley (29) states that of the polysaccharides tested the most active were polygalacturonides but that those containing glucuronic acid and mannuronic acid had some potency.

So far I have referred to competitive adsorption and the prevention of receptor-virus union as the mechanism of inhibition. The point of view of Horsfall and Ginsberg (32) strikes a new note.

They believe that bacterial polysaccharides inhibit virus multiplication by an intracellular action. They believe that the virus can enter the cell but cannot multiply there because of the presence of the intracellular polysaccharide. The background of their thesis is briefly this:

Horsfall and McCarty (33) reported that substances, probably polysaccharides, found in several species of bacteria would inhibit multiplication of pneumonia virus of mice in the mouse lung. This effect was seen even if the polysaccharide was given up to 4 days after the initial infection. Ginsberg and Horsfall (32) later found that 1 mg of Friedlander bacillus polysaccharide would prevent the reproduction of mumps virus in the allantoic cavity of chick embryos so long as the inoculum was less than 10^5 ID₅₀. These authors placed polysaccharide, together with a large amount of virus in the allantoic cavity, and found some virus was absorbed. They assumed that if a smaller inoculum were used (such as 10^5 ID₅₀), a portion of that, too, would be absorbed. They believed therefore that polysaccharide inhibits mumps inside, not outside the cell.

To summarize, I have described two types of inhibitors of hemagglutination in serum. One is destroyed at 65° C and is possibly protein, the other is thermostable and is probably mucoid. Both act by combining reversibly with extracellular virus. Two inhibitors described in allantoic fluid, and the inhibitor found in mucous secretions, may be of the same type as those in serum. Certain bacterial polysaccharides have been postulated to inhibit intracellular virus multiplication.

Work in this field is a step toward unravelling the relation between virus and host cell. If it is true that the mucoid or vegetable polysaccharide inhibitors are analogues of the cell receptor, we have one phase of that relation reproduced in a simple system and in solution. On the other hand, as Horsfall, McCarty and Ginsberg have said, their work may open the way to a study of *intracellular* metabolic processes. Because of this I believe it is very important to have confirmation of their hypothesis that bacterial polysaccharide affects intracellular virus metabolism rather than adsorption of virus on cell.

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authors as precursor or immature form of the virus

A similar suggestion could serve as an explanation of the following data. If one injects a large dose of optimally prepared virus into the allantoic sac and studies thereafter, at hourly intervals, the allantoic fluids and membranes from representative numbers of eggs, for their infectivity, hemagglutinating and complement-fixing activities, it can be seen, allowing one hour for adsorption of the seed, that the infectivity in the fluid and tissue remains constant for 6 and 5 hours, respectively. On the other hand, development of hemagglutinins becomes measurable after the third hour, and they increase 30- to 100-fold before any rise in infectivity is noted. The complement-fixing antigen develops in the membranes even before the hemagglutinins, a fact recently reported also by Hoyle. This series of events is the more striking if one considers that the order of increasing sensitivity of these three tests is just the reverse, the complement fixation test being the least sensitive.

Further experiments revealed that the rise in hemagglutinins, prior to that of infectivity, is accompanied by the development of interfering activity as measured after inactivation of the various preparations by ultraviolet light. Although the origin of this non-infectious, but hemagglutinating, interfering and complement-fixing material is not definitely established, whether it is a breakdown product of the seed virus or an intermediary stage of virus formation, all indications point to the latter possibility at present.

As far as the interactions between host and interfering agent are concerned, the interference phenomenon can be induced only by direct contact of the host cells with the irradiated virus. The effect can not be counteracted either by flushing of the allantoic cavity with saline solution, or by the injection of potent immune serum immediately after the administration of the interfering agent. It is apparent, therefore, that interference is induced very rapidly, and possibly within less than one minute. The cells thus altered remain so for at least 6 days. It does not seem to matter whether a few or 10 million ID_{50} of active virus are used for the challenge, the results of interference appear identical. However, recent observations seem to indicate that the interfering activity may be overcome to some extent by the use of excessive amounts of active virus, a point which is at present under investigation.

The quantitative aspects of the interference phenomenon in this system have not been clearly worked out as yet. Rough estimates of the number of cells lining the allantoic cavity of the 11- to 12-day-old chick embryo by two different approaches led to the figure of about 10^8 cells. The equivalent of approximately 10^8 ID_{50} of virus in the inactivated state is required to produce interference to such an extent that no measurable amounts of hemagglutinin are formed. Thus it appears that the equivalent of about one ID_{50} per cell will cause interference. However, it is not known whether every cell lining the allantoic sac will support viral propagation, and the number of virus particles constituting one ID_{50} , likewise, has not been definitely determined although it appears to be of the order of 10 and possibly less. These considerations would imply that the quantitative aspects are very similar to those encountered in the bacteriophage system by Luria and Delbrück.

The interference reaction is non-specific in the sense that inactivated influenza virus interferes with the active agents, not only of the homologous but also of the heterologous types, as well as with some strains of mumps virus, equine encephalomyelitis, epidemic keratoconjunctivitis, Newcastle disease, and possibly other viruses. There is no obvious difference in the degree of cross-interference between influenza A and B viruses, if the irradiated virus is injected prior to the administration of the active agents. However, if the irradiated virus is injected after infection, during the first hour of the first infectious cycle, then the homologous irradiated virus may markedly affect viral propagation in the tissues, whereas heterologous irradiated virus inhibits only to a lesser extent. This would seem to indicate that interference may be obtained at two different levels of the infectious cycle, at the first, a relatively non-specific effect is obtained, whereas at the second it appears rather specific for the type.

Various hypotheses have been proposed to furnish an explanation for the interference reaction. Many of these can be disregarded since they obviously do not apply to the system under study. However, two of these, suggested by Ziegler and Horsfall, and by Delbrück and Luria, respectively, require discussion. They are 1) blockade or destruction by the primarily injected virus of cell receptors which mediate also infection of the host tissue by the second virus, and 2) blockade of a key enzyme in the host cell by the interfering

agent which is necessary also for the propagation of the excluded virus

The cell receptor theory, of course, is based on the studies of the hemagglutination phenomenon. As is well known, influenza virus is adsorbed onto and subsequently eluted from chicken red cells, and the erythrocytes thus treated become non-agglutinable, as measured by the addition of fresh homologous virus and, to a varying extent, by other strains of virus of the homologous and heterologous types. Similar phenomena of adsorption and elution have been observed by Hirst and by Stone, to occur in the lungs of ferrets and mice, and in the allantoic cavity of the chick embryo, under conditions which in all probability, would have precluded viral multiplication. Elution of active or inactivated virus has not been demonstrated as yet in the intact host. Furthermore, as has been pointed out previously, ultraviolet irradiation of influenza viruses destroyed the interfering property more rapidly than the hemagglutinating property. In other words, on prolonged irradiation a preparation may be obtained which does not cause interference but still is adsorbed and eluted from red cells to an apparently unaltered degree.

Finally, cells which have been treated with interfering preparations of irradiated virus still may adsorb additional virus of the homologous or heterologous types.

Although receptor blockade thus can be ruled out as the major cause of interference, it has not been entirely excluded as a contributing factor. Adsorption of virus onto the allantoic tissue amounts to only about 70 per cent in the average, the remainder of the seed virus being left free in the allantoic fluid. If this equilibrium is markedly

disturbed by secondary injection of large amounts of virus, the additional adsorption apparently is more pronounced than in the case of small amounts of challenge virus, where less additional adsorption seems to take place. These relationships will bear further clarification since the experimental conditions are such as to approach closely the limits of accuracy of the available techniques used for the assays.

As to the key enzyme hypothesis of Delbruck and Luria it seems to fit best to most of the available data. It is possible that the enzyme or enzymes affected are necessary both for viral and host cell multiplication. It was found that bacteria, treated with irradiated phage, discontinued multiplication, and, similarly, the allantoic sac of the 8-day-old chick embryo, injected with irradiated influenza virus, failed to grow thereafter at the normal rate. If the key enzyme hypothesis is correct, one would have to postulate now that one enzyme system must be involved in propagation of a number of viruses, and, consequently, its blockade would be responsible for the interference phenomenon between immunologically distinct viruses. The specific interference obtained by injection of irradiated virus of the homologous type within the first hour after infection, i.e., during the first infectious cycle, would seem to have to be caused by blockade of another enzyme system involved in production of components needed only for formation of virus of the homologous type.

It is obvious from these remarks that we are still far from having a satisfactory explanation for the interference phenomenon and, indeed, it may very well be that not a single explanation will ultimately fit all the observed facts.

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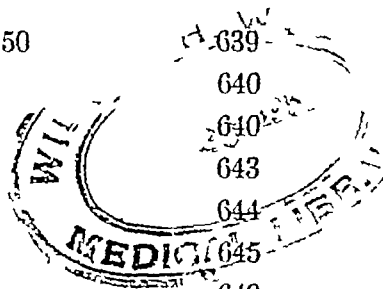
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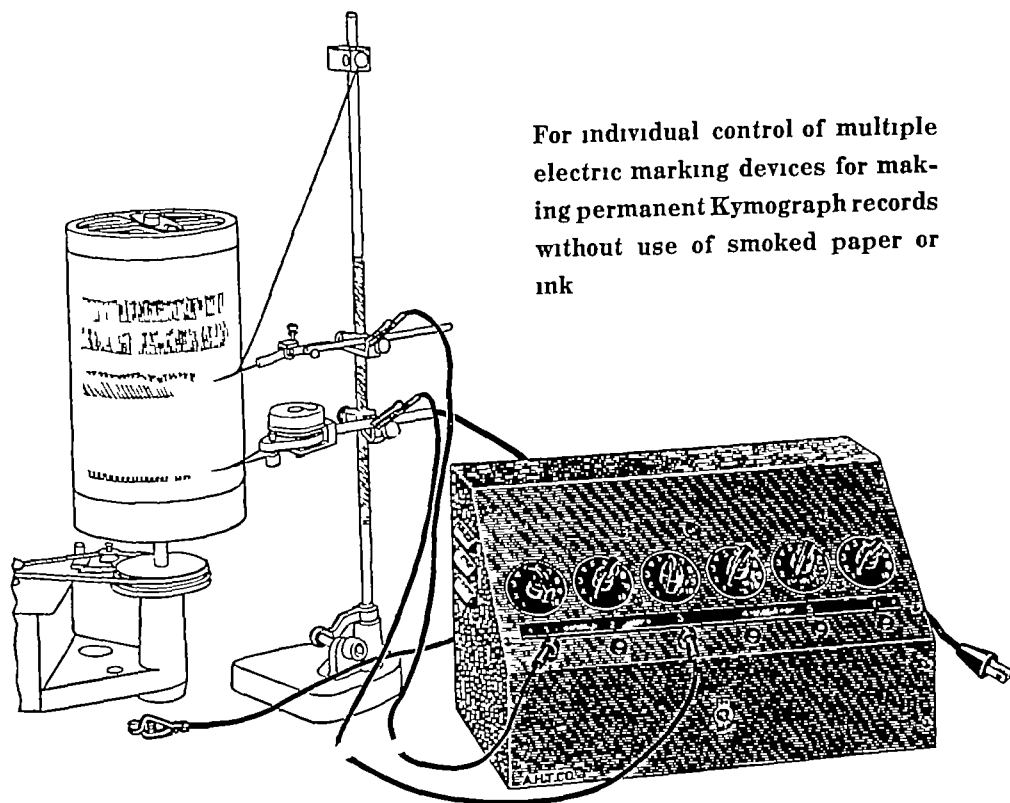
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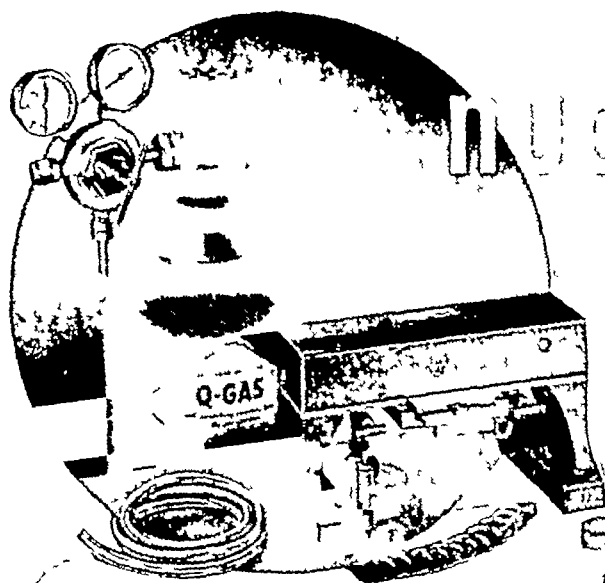
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FEDERATION PROCEEDINGS is published quarterly by the Federation of American Societies for Experimental Biology. The *March* issue consists of the program (Part II) of the Annual Meeting of the Federation, and the Abstracts (Part I) of the papers presented at the scientific sessions. The program includes an author index. The abstracts are arranged alphabetically according to the first author and segregated as to Societies. The *June* and *September* issues contain symposia and other special papers presented at Federation meetings as selected by the Editorial Board. The *December* issue contains the membership list and other matters pertinent to the Constituent Societies of the Federation.

Price Schedule

The subscription price is \$4.50 (\$5.25 foreign) payable in advance. Single issues may be purchased, if ordered in advance, at the following prices: No. 1 (Part I, March, Abstracts), \$3.00, No. 1 (Part II, March, Program), 50¢, Nos. 2 and 3 (June and September), \$1.50 an issue, No. 4 (December), \$2.00 an issue. Subscriptions and orders should be sent to the Federation of American Societies for Experimental Biology, 2101 Constitution Ave., Washington 25, D. C.

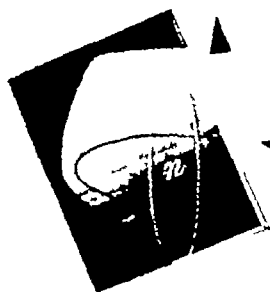
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To facilitate the publication of an accurate and up-to-date Membership list in the December issue of *FEDERATION PROCEEDINGS*, it is requested that *any member whose entry in the December 1949 issue is incorrect* furnish the Secretary of his Society with the following data, in duplicate, on 3" x 5" cards: Name, Degree (highest), Mailing Address (street address, city, zone, state), Present Position (title and department or branch), Society membership and year of election to membership in that society. If you are retired, give only degree(s), home address, and society membership(s) with year(s). If you are officially carried on the rolls of your society as a retired or honorary member, please so indicate.

Names and addresses of the Society Secretaries are as follows:

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American Society of Biological Chemists—Dr. Richard W. Jackson, Northern Regional Research Laboratory, Peoria 5, Ill.

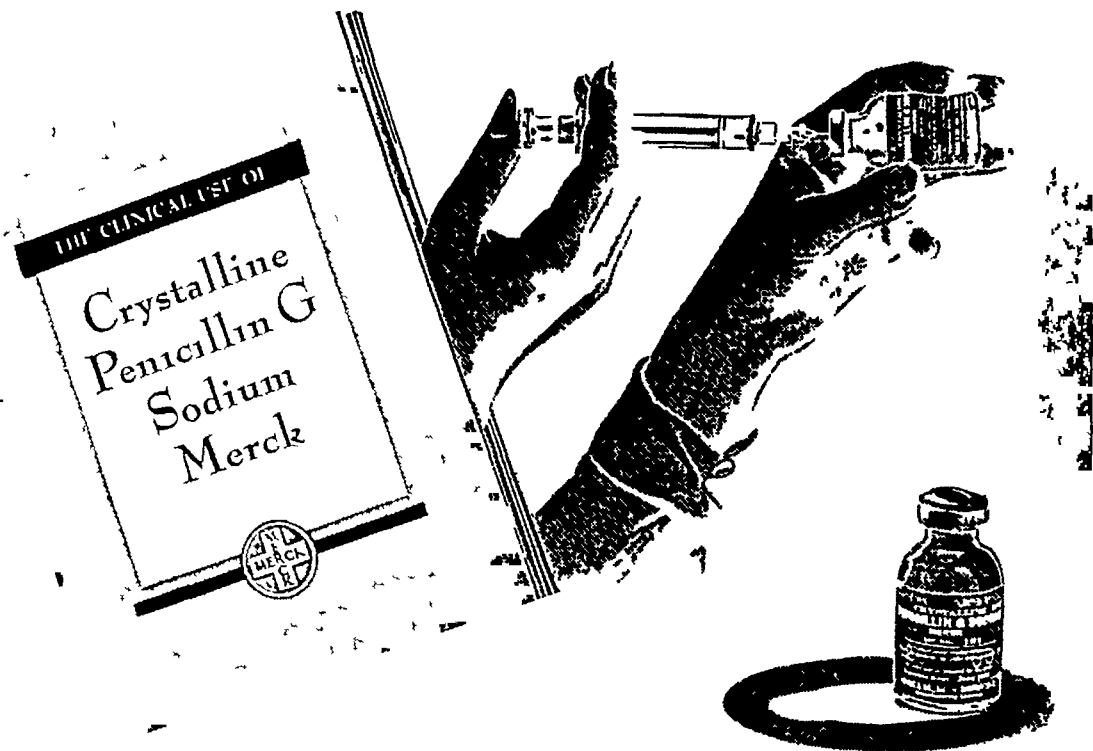
American Society for Pharmacology and Experimental Therapeutics—Dr. Harvey B. Haag, Medical College of Virginia, Dept. of Pharmacology, Richmond 19, Va.

The American Society for Experimental Pathology—Dr. Sidney C. Madden, Brookhaven National Laboratory, Upton, N. Y.

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The American Association of Immunologists—Dr. Jules Freund, Public Health Research Institute of the City of New York, Inc., Foot of East 15th St., New York City.

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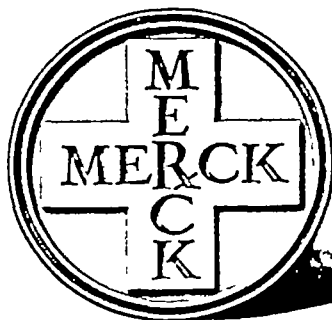
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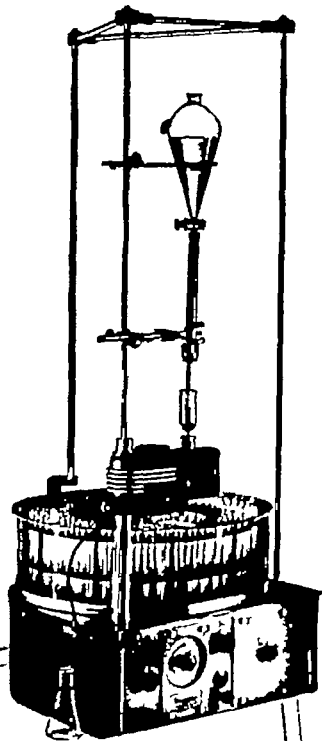
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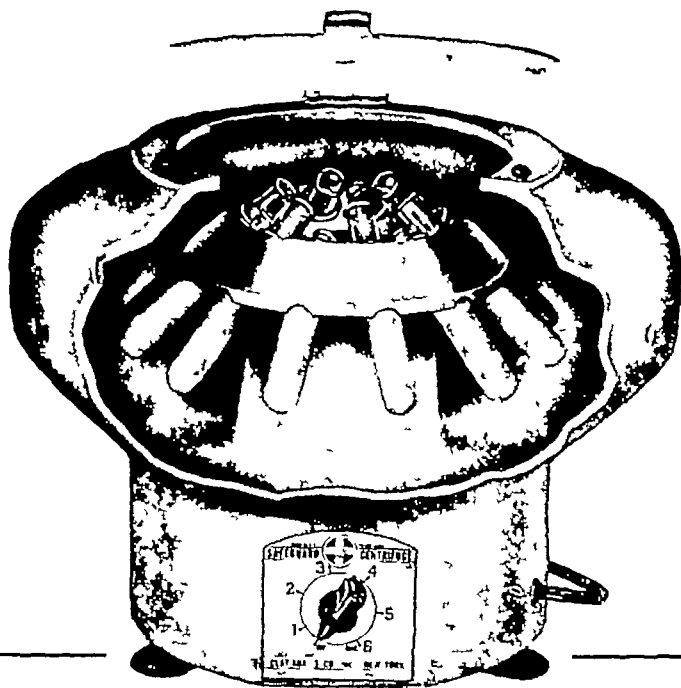
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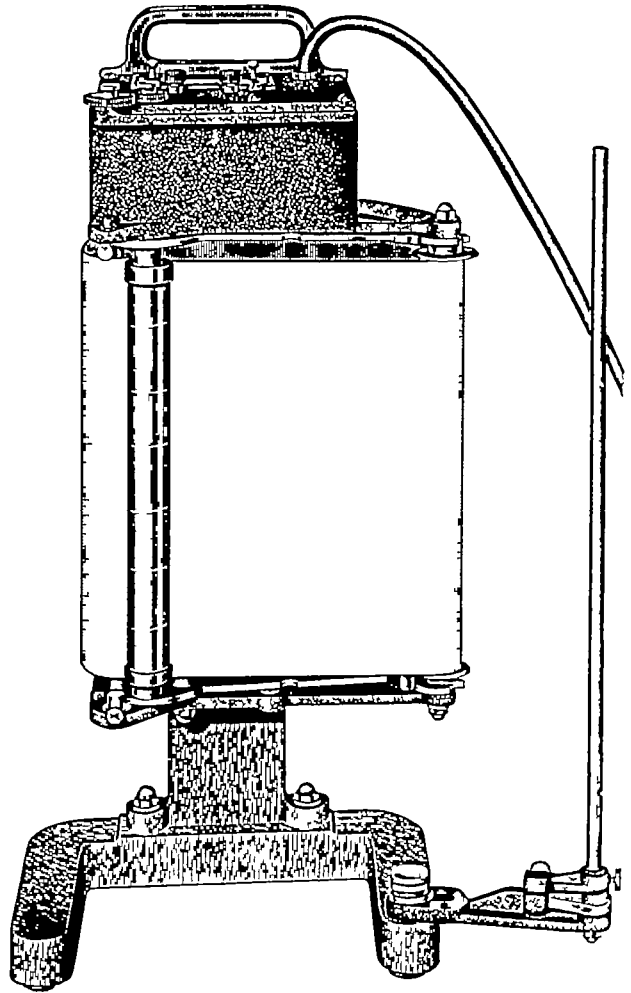
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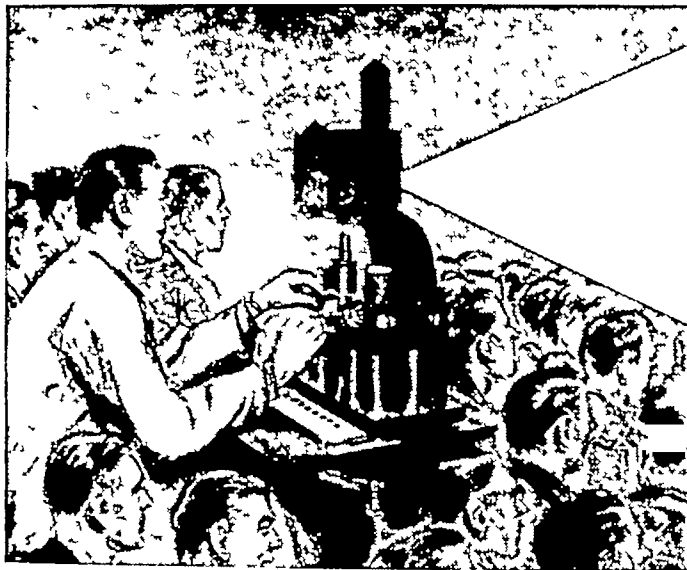
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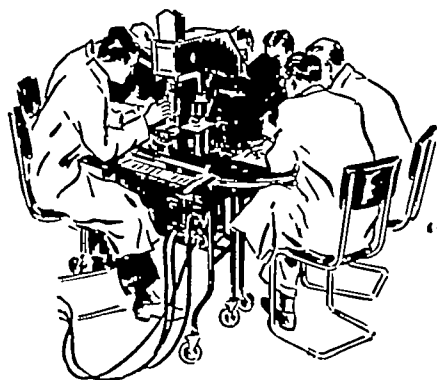
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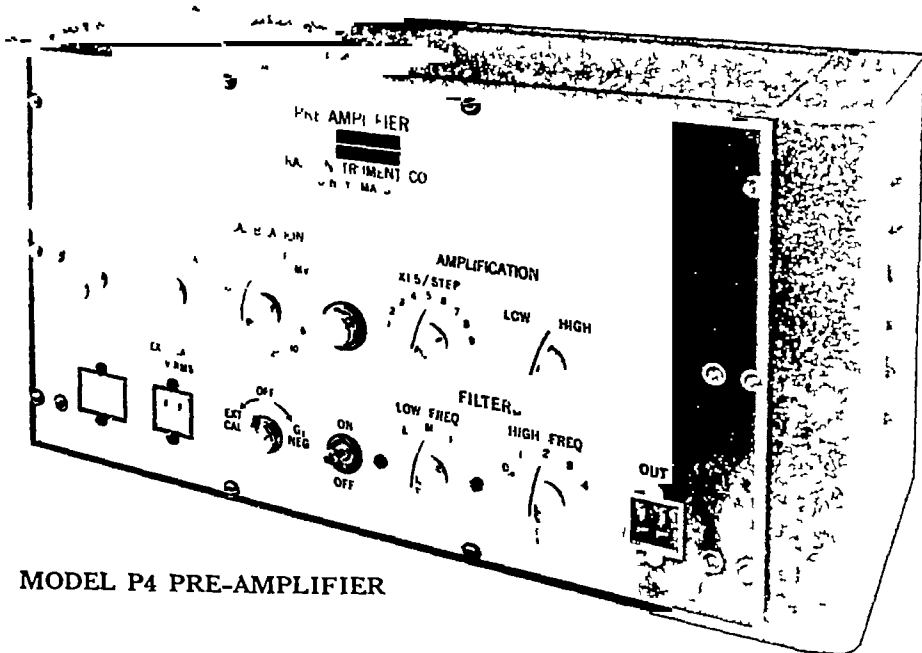
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Volume 8, 1949

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Federation Proceedings

VOLUME 8

December 1949

NUMBER 4

THIRTY-FOURTH ANNUAL MEETING

ATLANTIC CITY, NEW JERSEY

April 17-21, 1950

THE 1950 Convention of the Federation will be held in Atlantic City, New Jersey, April 17-21. The scientific sessions of the six constituent Societies will begin at 9 00 A M, Tuesday, April 18, in the Municipal Auditorium, and will continue through Friday afternoon, April 21. Sunday and Monday, April 16 and 17, will be devoted to meetings of Society Councils, the Federation Executive Committee and other Committees. The Federation Joint Session will be Tuesday evening, April 18, in the Ballroom of the Auditorium.

Registration will open at 9 00 A M, Monday, April 17, at the Municipal Auditorium. The registration desks will be open until 10 00 P M Monday evening and from 8 00 A M to 5 00 P M on Tuesday, Wednesday, Thursday and Friday. Since the scientific sessions begin at 9 00 A M on Tuesday, members are urged to complete registration on Monday, if possible. Members of any of the constituent Societies, guests, and other biologists and physicians who wish to attend the meetings may register. The official badge, issued at registration, must be worn to secure admission to the scientific sessions and other activities of the Convention. There will be a separate registration desk where ladies who are present as guests may register without charge. Programs, abstracts and tickets for various special functions will be on sale near the registration desk.

Headquarters Hotel will be the Hotel Traymore. Information on hotels and rates, and forms for making reservations will be distributed to members by the Society Secretaries. Hotel housing facilities are adequate in Atlantic City.

EXHIBITS

Commercial exhibits will be shown from Tuesday to Friday noon in the Municipal Auditorium. Exhibitors will be publishers and manufacturers and distributors of scientific equipment, apparatus, supplies, chemicals and pharmaceuticals.

In addition, there will be provision for small scientific exhibits by Federation members and by University laboratories, Foundations and Institutes. These will consist of exhibits (not demonstrations) of new apparatus, graphs, charts and pictures. These exhibits must be set up before Tuesday morning and must remain in place until

Friday noon. Gas, water and electric facilities will not be available. There will be no charge for this exhibit space.

MOTION PICTURES

Motion pictures will be shown at one session, to be scheduled in the program. The title and abstract of the film must be submitted to the Society Secretaries. Only 16 mm safety film can be shown, equipment for sound projection will be available.

DINNERS AND LUNCHEONS

There will be ample facilities for holding group breakfasts, luncheons and dinners. It is advantageous to schedule all of these through the Federation Secretary's office. Members and groups desiring to schedule these functions should make their plans early and inform the Federation Secretary of their requirements before March 1, 1950. Specifications should include attendance expected, desirable time and alternatives, conflicts to be avoided, and whether or not a discussion session is to follow the meal.

PLACEMENT SERVICE

An office of the Placement Service of the Federation will be located in the Auditorium during the meetings, for arranging interviews between applicants and employers. Applicants for positions should write to the Federation Placement Service, 2101 Constitution Avenue, Washington 25, D C, for application forms to be returned not later than April 1, 1950. Applicants who have previously filed forms with the Service are requested to notify the office and to send additional data not later than April 1, 1950. The application forms, comprising a résumé of education, training, experience, publications and type of position desired, will be on file at the Placement Service office.

Agencies seeking personnel should write to the Federation Placement Service for employers' registration cards on which to describe the positions and to record the Atlantic City addresses. The cards should be returned to the Federation office not later than April 1, 1950. Employers who have previously filed cards should notify the office of their Atlantic City address not later than April 1

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Society W B CANNON and A J CARLSON, The
Physiological Society

Boston, Dec 26-29, 1915

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Society W B CANNON and C W GREENE, The

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SHAFFER, The Biochemical Society

New York, Dec 27-30, 1916

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NON and C W GREENE, The Physiological Society
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Biochemical Society REID HUNT and L G ROWN-
TREE, The Pharmacological Society LUDVIG
HEKTOEN and HOWARD T KARSNER, The Patho-
logical Society

Baltimore, April 24-26, 1918

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REID HUNT and E D BROWN, The Pharmacologi-
cal Society H GIDEON WELLS and HOWARD T
KARSNER, The Pathological Society FREDERIC S
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Society

Cincinnati, Dec 29-31, 1919

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ological Society WARREN P LOMBARD and
CHARLES W GREENE, The Physiological Society
STANLEY R BENEDICT and VICTOR C MYERS, The
Biochemical Society

Chicago, Dec 28-30, 1920

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Rochester, N Y, April 14-16, 1927

E C KENDALL, *Chairman*, and F C KOCH, *Secretary*, The Biochemical Society JOHN AUER and E D BROWN, The Pharmacological Society W H BROWN and E B KRUMBHAAR, The Patho-

logical Society J ERLANGER and W J MEEK, The Physiological Society

Ann Arbor, April 12-14, 1928

CARL VOEGTLIN, *Chairman*, and E D BROWN, *Secretary*, The Pharmacological Society DAVID MARINE and CARL V WELLER, The Pathological Society JOSEPH ERLANGER and WALTER J MEEK, The Physiological Society E V MCCOLLUM and D WRIGHT WILSON, The Biochemical Society

Boston, Aug 19-24, 1929*(The XIIIth International Physiological Congress)*

EDWARD B KRUMBHAAR, *Chairman*, and CARL V WELLER, *Secretary*, The Pathological Society JOSEPH ERLANGER and WALTER J MEEK, The Physiological Society E V MCCOLLUM and D WRIGHT WILSON, The Biochemical Society CARL VOEGTLIN and E D BROWN, The Pharmacological Society

Chicago, March 26-29, 1930

WALTER J MEEK, *Chairman*, and ALFRED C REDFIELD, *Secretary*, The Physiological Society W R BLOOR and HOWARD B LEWIS, The Biochemical Society CARL VOEGTLIN and E D BROWN, The Pharmacological Society WILLIAM F PETERSEN and CARL V WELLER, The Pathological Society

Montreal, April 8-11, 1931

W R BLOOR, *Chairman*, and H B LEWIS, *Secretary*, The Biochemical Society GEORGE B WALLACE and E D BROWN, The Pharmacological Society FREDERICK L GATES and C PHILLIP MILLER, The Pathological Society WALTER J MEEK and ARNO B LUCKHARDT, The Physiological Society

Philadelphia, April 27-30, 1932

GEORGE B WALLACE, *Chairman*, and V E HENDERSON, *Secretary*, The Pharmacological Society SAMUEL R HAYTHORN and C PHILLIP MILLER, The Pathological Society WALTER J MEEK and ARNO B LUCKHARDT, The Physiological Society H C BRADLEY and HOWARD B LEWIS, The Biochemical Society

Cincinnati, April 10-12, 1933

PEYTON ROUS, *Chairman*, and C PHILLIP MILLER, *Secretary*, The Pathological Society ARNO B LUCKHARDT and FRANK C MANN, The Physiological Society H C BRADLEY and HOWARD B LEWIS, The Biochemical Society WM DEB MACNIDER and V E HENDERSON, The Pharmacological Society

New York, March 28-31, 1934

ARNO B LUCKHARDT, *Chairman*, FRANK C MANN, *Secretary*, and ALEXANDER FORBES, *Treasurer*, The Physiological Society W M CLARK and H A MATTILL, The Biochemical Society W DEB MACNIDER and V E HENDERSON, The Pharmacological Society CARL V WELLER and C PHILLIP MILLER, The Pathological Society

Detroit, April 10-13, 1935

W M CLARK, *Chairman* H A MATTILL, *Secretary*, and C H FISKE, *Treasurer*, The Biochemical Society CHARLES W GREENE and FRANK C MANN, The Physiological Society R A HATCHER and E M K GEILING, The Pharmacological Society S BURT WOLBACH and SHIELDS WARREN, The Pathological Society

Washington, March 25-28, 1936

V E HENDERSON, *Chairman*, E M K GEILING, *Secretary*, and C M GRUBER, *Treasurer*, The Pharmacological Society FRANK C MANN and ANDREW C IVY, The Physiological Society H B LEWIS and H A MATTILL, The Biochemical Society OSKAR KLOTZ and SHIELDS WARREN, The Pathological Society

Memphis, April 21-24, 1937

ALPHONSE R DOCHEZ, *Chairman*, and SHIELDS WARREN The Pathological Society FRANK C MANN and ANDREW C IVY, The Physiological Society HOWARD B LEWIS and H A MATTILL, The Biochemical Society V E HENDERSON and E M K GEILING, The Pharmacological Society D R HOOKER *Secretary*

Baltimore, March 30-April 2, 1938

WILLIAM T PORTER, *Honorary President*, WALTER E GARREY, *Chairman*, and ANDREW C IVY, The Physiological Society GLENN E CULLEN and H A MATTILL, The Biochemical Society ARTHUR L TATUM and G PHILIP GRABFIELD, The Pharmacological Society C PHILLIP MILLER and PAUL R CANNON, The Pathological Society D R HOOKER, *Secretary*

Toronto, April 26-29, 1939

GLENN E CULLEN, *Chairman*, and CHARLES G KING, The Biochemical Society ARTHUR L TATUM and G PHILIP GRABFIELD, The Pharmacological Society C PHILLIP MILLER and PAUL R CANNON, The Pathological Society WALTER E GARREY and ANDREW C IVY, The Physiological Society D R HOOKER, *Secretary*

New Orleans, March 13-16, 1940

E M K GEILING, *Chairman*, and G PHILIP GRABFIELD, The Pharmacological Society ERNEST W GOODPASTURE and PAUL R CANNON, The Pathological Society ANDREW C IVY and PHILIP BARD, The Physiological Society WILLIAM C ROSE and CHARLES G KING, The Biochemical Society D R HOOKER, *Secretary*

Chicago, April 15-19, 1941

SHIELDS WARREN, *Chairman*, and H P SMITH, The Pathological Society THORNE M CARPENTER and L A MAYNARD, The Institute of Nutrition ANDREW C IVY and PHILIP BARD, The Physiological Society WILLIAM C ROSE and CHARLES G KING, The Biochemical Society E M K GEILING and G PHILIP GRABFIELD, The Pharmacological Society D R HOOKER, *Secretary*

Boston, March 31-April 4, 1942

ALBERT G HOGAN, *Chairman*, and ARTHUR H SMITH, The Institute of Nutrition PHILIP BARD and CARL J WIGGERS, The Physiological Society RUDOLPH J ANDERSON and ARNOLD K BALLS, The Biochemical Society E M K GEILING and R N BIETER, The Pharmacological Society JESSE L BOLLMAN and H P SMITH, The Pathological Society SHIELDS WARREN, *Ex-Chairman* D R HOOKER, *Secretary*

1943, 1944, 1945 The meetings scheduled for Cleveland were cancelled because of war conditions

PHILIP BARD, *Chairman*, and WALLACE O FENN, The Physiological Society E A DOISY and ARNOLD K BALLS, The Biochemical Society E K MARSHALL, JR and RAYMOND N BIETER, The Pharmacological Society BALDUIN LUCKÉ and H P SMITH, The Pathological Society LEONARD A MAYNARD and ARTHUR H SMITH, The Institute of Nutrition JACQUES J BRONFENBRENNER and ARTHUR F COCA, The Association of Immunologists D R HOOKER, *Secretary*

Atlantic City, March 11-15, 1946

PHILIP BARD, *Chairman*, WALLACE O FENN, The Physiological Society A BAIRD HASTINGS and ARNOLD K BALLS, The Biochemical Society ERWIN E NELSON and RAYMOND N BIETER, The Pharmacological Society BALDUIN LUCKÉ and H P SMITH, The Pathological Society WILLIAM C ROSE and H E CARTER, The Institute of Nutrition JACQUES J BRONFENBRENNER and ARTHUR F COCA, The Association of Immunologists D R HOOKER, *Secretary*

Chicago, May 18-22, 1947

A BAIRD HASTINGS, *Chairman*, and OTTO A BESSEY, The Biochemical Society MAURICE H SEEVERS and HARVEY B HAAG, The Pharmacological Society PAUL R CANNON and FRIEDA S ROBSCHT-ROBBINS, The Pathological Society ARTHUR H SMITH and H E CARTER, The Institute of Nutrition MICHAEL HEIDELBERGER and ARTHUR F COCA, The Association of Immunologists WALLACE O FENN and MAURICE B VISSCHER, The Physiological Society WILLIAM H CHAMBERS, *Secretary*

Atlantic City, March 15-19, 1948

MAURICE H SEEVERS, *Chairman*, and HARVEY B HAAG, The Pharmacological Society DOUGLAS H SPRUNT and FRIEDA S ROBSCHT-ROBBINS, The Pathological Society R M BETHKE and H

E CARTER, The Institute of Nutrition LLOYD D FELTON and ARTHUR F COCA, The Association of Immunologists WALLACE O FENN and MAURICE B VISSCHER, The Physiological Society HANS T CLARKE and OTTO A BESSEY, The Biochemical Society WILLIAM H CHAMBERS, *Secretary*

Detroit, April 18-22, 1949

H P SMITH, *Chairman*, and FRIEDA S ROBSCHT-ROBBINS, The Pathological Society E M NELSON and J H ROE, The Institute of Nutrition MICHAEL HEIDELBERGER and JULES FREUND, The Association of Immunologists MAURICE B VISSCHER and D B DILL, The Physiological Society HANS T CLARKE and OTTO A BESSEY, The Biochemical Society CARL A DRAGSTEDT and HARVEY B HAAG, The Pharmacological Society M O LEE, *Federation Secretary*

FEDERATION BY-LAWS**BY-LAWS**

Adopted at the Washington Meeting, 1936, and amended at the Boston Meeting, 1942

1 The Presidents and Secretaries of the Constituent Societies, the Chairman of the Executive Committee of the preceding year and the Federation Secretary shall form the Executive Committee of the Federation

2 The Chairmanship of the Executive Committee shall be held in turn by the Presidents of the Constituent Societies, who shall succeed one another annually in the order of seniority of the Societies

3 The Executive Committee shall appoint annually from the membership of the Federation a Secretary-Treasurer, to be known as the Federation Secretary

4 The Federation Secretary shall (a) Keep the minutes of the Executive Committee and distribute copies to the Secretaries of the Constituent Societies (b) Make arrangements for the Annual Meeting with the Local Committee, with the approval of the Executive Committee (c) Print in convenient combined form and distribute to the membership of the Federation the programs of the Constituent Societies as received from their respective Secretaries (d) Undertake such other duties to be decided upon from time to time by the Executive Committee, as do not conflict with the complete autonomy of the Constituent Societies

5 The Executive Committee shall control all monies in the hands of the Federation Secretary, who shall make an annual report to the Executive Committee for audit and approval The expenses

of the Federation Secretary, as authorized by the Executive Committee, shall be the first charge on such monies and if insufficient for the purpose the Executive Committee shall prorate such expenses to the Constituent Societies of the Federation in proportion to their respective memberships

The Executive Committee may appropriate Federation monies annually for the uses of Local Committees and for the uses of other authorized Committees but in the latter cases an audit of expenditures shall be made and approved before such committees are discharged

6 The Executive Committee shall determine the place of the Annual Meeting, and the time shall be determined by the Local Committee, preferably within the period of March fifteenth to May first

7 The Local Committee at the place of meeting of the Federation shall charge such fee for registration as may be approved by the Executive Committee The monies thus collected shall be used to defray the expenses of the Local Committee and the remainder, after such expenses have been met, shall be turned over to the Federation Secretary

8 The Executive Committee shall consider measures of advantage to the Federation as a whole Any Constituent Society may refer similar measures to the Executive Committee No action, however, shall be taken by the Executive Committee unless specifically authorized by all the Constituent Societies

9 The Chairman of the Executive Committee may appoint committees when the purposes of such committees have been approved by all the

Constituent Societies of the Federation Such committees shall be appointed for a term of one year, but may be continued and their members reappointed Such committees shall report in writing to the Executive Committee, which shall in turn report thereon to the Constituent Societies either for information or recommendation The Secretaries of the Constituent Societies shall report the recommendations of their respective Societies to the Executive Committee for final action

10 All individuals whose names appear on the program by invitation or introduction and those registering from any recognized biological laboratory may be enrolled as Associate Members of the Federation for that Annual Meeting Such Associate Members may enjoy all the privileges of the Annual Meeting except that of voting

11 No person may present orally more than one paper during all of the scientific sessions of the Constituent Societies at the time of the Annual Meeting except upon invitation of the Executive Committee or a Council Papers must be submitted to the Secretary of the Society of which the proposer is a member The proposer may request transfer to another program, but this may only be done with the consent of the Secretary of the Society concerned Any Secretary who regards any paper submitted to him as better suited to the program of another Society may arrange this transfer with the Secretary of the Society concerned, if it be possible Such transfer shall be indicated on the program

12 Abstracts not to exceed two hundred and fifty words in length, of papers approved for presentation at all of the scientific sessions of all the Constituent Societies at the Annual Meeting,

shall receive publication in the *Federation Proceedings*

13 A Control Committee, consisting of at least one representative of each Constituent Society as designated by the several Councils, shall have editorial control over the *Federation Proceedings* which shall be financed as required by an annual assessment of all the members of each Constituent Society

14 The Control Committee shall have power to choose certain additional papers presented at the Annual Meetings and from other sources, including material heretofore published in the *Federation Yearbook*, for publication in the *Federation Proceedings*

PLACEMENT SERVICE

The Federation maintains a service to act as a medium of communication between persons seeking positions for teaching or research and institutions that wish to fill vacancies in these sciences

The service does not undertake to recommend or to pass judgment upon applicants It aims merely to serve as a clearing-house for such information as above stated and to bring into touch with one another candidates for positions and employers

Individuals, whether members of the Federation or not, universities, other institutions and organizations desiring to avail themselves of the Service may receive such information as is available By action of the Executive Committee in 1947, a registration fee of one dollar is required of each applicant for a position

All communications should be addressed to Federation Placement Service, 2101 Constitution Ave , Washington 25, D C

THE AMERICAN PHYSIOLOGICAL SOCIETY

Founded December 30, 1887, Incorporated June 2, 1923

OFFICERS, 1949-1950

President—CARL J WIGGERS, Western Reserve University, Cleveland, Ohio

President-Elect—H C BAZETT, University of Pennsylvania, School of Medicine, Philadelphia

Past-President—MAURICE B VISSCHER, University of Minnesota, Minneapolis

Council—CARL J WIGGERS, H C BAZETT, MAURICE B VISSCHER, EDWARD F ADOLPH (1950), EUGENE M LANDIS (1951), D B DILL (1952), R W GERARD (1953)

Executive Secretary—M O LEE, 2101 Constitution Ave, Washington, D C

Board of Publication Trustees—W O FENN, *Chairman* (1952), R F PITTS (1950), FRANK C MANN (1951)

Representative on the Division of Biology and Agriculture, National Research Council—MAURICE B VISSCHER (1952)

Representative on the Division of Medical Sciences, National Research Council—HALLOWELL DAVIS (1950)

Representative on the Council of the American Association for the Advancement of Science—J H BODINE (1952), LEIGH CHADWICK (1952)

Historian—WALTER J MEEK

Contributing Editor to Scientific Monthly—JOHN FIELD II

PAST OFFICERS

Organization Meeting, December 30, 1887

S WEIR MITCHELL, *President*

H N MARTIN, *Secretary*

1888 H P BOWDITCH, *President*, H N MARTIN, *Secretary-Treasurer*, J G CURTIS, H C WOOD, H SEWALL *Councilors* 1889 S WEIR MITCHELL, *President*, H N MARTIN, *Secretary-Treasurer*, H P BOWDITCH, J G CURTIS, H C WOODS *Councilors* 1890 S WEIR MITCHELL, *President*, H N MARTIN, *Secretary-Treasurer*, H P BOWDITCH, J G CURTIS, H H DONALDSON, *Councilors* 1891 H P BOWDITCH, *President*, H N MARTIN, *Secretary-Treasurer*, R H CHITTENDEN, J G CURTIS, H N DONALDSON, *Councilors* 1892 H P BOWDITCH, *President*, H N MARTIN, *Secretary-Treasurer*, R H CHITTENDEN, J G CURTIS, W H HOWELL, *Councilors* 1893 H P BOWDITCH, *President*, W P LOMBARD, *Secretary-Treasurer*, R H CHITTENDEN, J G CURTIS,

W H HOWELL, *Councilors* 1894 H P BOWDITCH, *President*, W P LOMBARD, *Secretary-Treasurer*, R H CHITTENDEN, W H HOWELL, J W WARREN, *Councilors* 1895 H P BOWDITCH, *President*, F S LEE, *Secretary-Treasurer*, R H CHITTENDEN, W H HOWELL, W P LOMBARD, *Councilors* 1896 R H CHITTENDEN, *President*, F S LEE, *Secretary-Treasurer*, H P BOWDITCH, W H HOWELL, J W WARREN, *Councilors* 1897 R H CHITTENDEN, *President*, F S LEE, *Secretary-Treasurer*, H P BOWDITCH, W H HOWELL, W P LOMBARD, *Councilors* 1898 R H CHITTENDEN, *President*, F S LEE, *Secretary-Treasurer*, H P BOWDITCH, W H HOWELL, W P LOMBARD, *Councilors* 1899 R H CHITTENDEN, *President*, F S LEE, *Secretary-Treasurer*, W H HOWELL, W P LOMBARD, W T PORTER, *Councilors* 1900 R H CHITTENDEN, *President*, F S LEE, *Secretary-Treasurer*, W H HOWELL, W P LOMBARD, W T PORTER, *Councilors* 1901 R H CHITTENDEN, *President*, F S LEE, *Secretary-Treasurer*, W H HOWELL, W P LOMBARD, W T PORTER, *Councilors* 1902 R H CHITTENDEN, *President*, F S LEE, *Secretary-Treasurer*, W H HOWELL, W P LOMBARD, W T PORTER, *Councilors* 1903 R H CHITTENDEN, *President*, F S LEE, *Secretary-Treasurer*, W H HOWELL, W P LOMBARD, W T PORTER, *Councilors* 1904 R H CHITTENDEN, *President*, W T PORTER, *Secretary-Treasurer*, F S LEE, W P LOMBARD, W H HOWELL, *Councilors* 1905 W H HOWELL, *President*, L B MENDEL, *Secretary*, W B CANNON, *Treasurer*, R H CHITTENDEN, S J MELTZER, *Councilors* 1906 W H HOWELL, *President*, L B MENDEL, *Secretary*, W B CANNON, *Treasurer*, A B MACALLUM, S J MELTZER, *Councilors* 1907 W H HOWELL, *President*, L B MENDEL, *Secretary*, W B CANNON, *Treasurer*, J J ABEL, G LUSK, *Councilors* 1908 W H HOWELL, *President*, R HUNT, *Secretary*, W B CANNON, *Treasurer*, J J ABEL, G LUSK, *Councilors* 1909 W H HOWELL, *President*, R HUNT, *Secretary*, W B CANNON, *Treasurer*, A J CARLSON, W P LOMBARD, *Councilors* 1910 W H HOWELL, *President*, A J CARLSON, *Secretary*, W B CANNON, *Treasurer*, J ERLANGER, F S LEE, *Councilors* 1911 S J MELTZER, *President*, A J CARLSON, *Secretary*, W B CANNON, *Treasurer*, J ERLANGER, F S LEE, *Councilors* 1912 S J MELTZER, *President*, A J CARLSON, *Secretary*, W B CANNON, *Treasurer*,

J ERLANGER, F S LEE, Councilors 1913 S J MELTZER, President, A J CARLSON, Secretary, J ERLANGER, Treasurer, W B CANNON, F S LEE, Councilors 1914 W B CANNON, President, A J CARLSON, Secretary, J ERLANGER, Treasurer, F S LEE, S J MELTZER, Councilors 1915 W B CANNON, President, C W GREENE, Secretary, J ERLANGER, Treasurer, W E GARREY, W H HOWELL, J J R MACLEOD, W J MEEK, Councilors 1916 W B CANNON, President, C W GREENE, Secretary, J ERLANGER, Treasurer, W E GARREY, W H HOWELL, J J R MACLEOD, W J MEEK, Councilors 1917 F S LEE, President, C W GREENE, Secretary, J ERLANGER, Treasurer, W B CANNON, W H HOWELL, J J R MACLEOD, W J MEEK, Councilors 1918 F S LEE, President, C W GREENE, Secretary, J ERLANGER, Treasurer, W B CANNON, W H HOWELL, J J R MACLEOD, W J MEEK, Councilors 1919 W P LOMBARD, President, C W GREENE, Secretary, J ERLANGER, Treasurer, W B CANNON, Y HENDERSON, J J R MACLEOD, W J MEEK, Councilors 1920 W P LOMBARD, President, C W GREENE, Secretary, J ERLANGER, Treasurer, W B CANNON, J J R MACLEOD, Y HENDERSON, C J WIGGERS, Councilors 1921 J J R MACLEOD, President, C W GREENE, Secretary, J ERLANGER, Treasurer, J A E EYSTER, Y HENDERSON, C J WIGGERS, A J CARLSON, Councilors 1922 J J R MACLEOD, President, C W GREENE, Secretary, J ERLANGER, Treasurer, Y HENDERSON, C J WIGGERS, A J CARLSON, J A E EYSTER, Councilors 1923 A J CARLSON, President, C W GREENE, Secretary, J ERLANGER, Treasurer, C J WIGGERS, A B LUCKHARDT, J A E EYSTER, J R MURLIN, Councilors 1924 A J CARLSON, President, W J MEEK, Secretary, C K DRINKER, Treasurer, A B LUCKHARDT, J A E EYSTER, J R MURLIN, W E GARREY, Councilors 1925 A J CARLSON, President, W J MEEK, Secretary, C K DRINKER, Treasurer, J A E EYSTER, J R MURLIN, W E GARREY, JOSEPH ERLANGER, Councilors 1926 J ERLANGER, President, W J MEEK, Secretary, A FORBES, Treasurer, J R MURLIN, W E GARREY, A B LUCKHARDT, C J WIGGERS, Councilors 1927 J ERLANGER, President, W J MEEK, Secretary, A FORBES, Treasurer, W E GARREY, A B LUCKHARDT, C J WIGGERS, R GESELL, Councilors 1928 J ERLANGER, President, W J MEEK, Secretary, A FORBES, Treasurer, A B LUCKHARDT, C J WIGGERS, R GESELL, A J CARLSON, Councilors 1929 W J MEEK, President, ALFRED C REDFIELD, Secretary, A FORBES, Treasurer, C J WIGGERS, R GESELL, A J CARLSON, J R MURLIN, Councilors 1930 W J MEEK, President, ARNO B LUCKHARDT, Secretary, A FORBES, Treasurer,

R GESELL, A J CARLSON, J R MURLIN, E G MARTIN, Councilors 1931 W J MEEK, President, ARNO B LUCKHARDT, Secretary, ALEXANDER FORBES, Treasurer, A J CARLSON, J R MURLIN, E G MARTIN, JOHN TAIT, Councilors 1932 ARNO B LUCKHARDT, President, FRANK C MANN, Secretary, ALEXANDER FORBES, Treasurer, E G MARTIN, W J MEEK, J R MURLIN, JOHN TAIT, Councilors 1933 ARNO B LUCKHARDT, President, FRANK C MANN, Secretary, ALEXANDER FORBES, Treasurer, HERBERT S GASSER, ERNEST G MARTIN, W J MEEK, JOHN TAIT, Councilors 1934 CHARLES W GREENE, President, FRANK C MANN, Secretary, ALEXANDER FORBES, Treasurer, HERBERT S GASSER, ARNO B LUCKHARDT, W J MEEK, JOHN TAIT, Councilors 1935 FRANK C MANN, President, ANDREW C IVY, Secretary, ALEXANDER FORBES, Treasurer, CHARLES H BEST, HERBERT S GASSER, ARNO B LUCKHARDT, W J MEEK, Councilors 1936 FRANK C MANN, President, ANDREW C IVY, Secretary, WALLACE O FENN, Treasurer, CHARLES H BEST, PHILIP BARD, HERBERT S GASSER, ARNO B LUCKHARDT, Councilors 1937 WALTER E GARREY, President, ANDREW C IVY, Secretary, WALLACE O FENN, Treasurer, CHARLES H BEST, PHILIP BARD, HERBERT S GASSER, ARNO B LUCKHARDT, Councilors 1938 WILLIAM T PORTER, Honorary President, WALTER E GARREY, President, ANDREW C IVY, Secretary, WALLACE O FENN, Treasurer, ARNO B LUCKHARDT, CHARLES H BEST, PHILIP BARD, HERBERT S GASSER, Councilors 1939 ANDREW C IVY, President, PHILIP BARD, Secretary, WALLACE O FENN, Treasurer, CHARLES H BEST, HERBERT S GASSER, ARNO B LUCKHARDT, MAURICE B VISSCHER, Councilors 1940 ANDREW C IVY, President, PHILIP BARD, Secretary, CARL J WIGGERS, Treasurer, CHARLES H BEST, HERBERT S GASSER, ARNO B LUCKHARDT, MAURICE B VISSCHER, Councilors 1941 PHILIP BARD, President, CARL J WIGGERS, Secretary, HALLOWELL DAVIS, Treasurer, CHARLES H BEST, ARNO B LUCKHARDT, MAURICE B VISSCHER, HIRAM E ESSEX, Councilors 1942, 1943, 1944, 1945 PHILIP BARD, President, WALLACE O FENN, Secretary, HALLOWELL DAVIS, Treasurer, CHARLES H BEST, MAURICE B VISSCHER, HIRAM E ESSEX, W F HAMILTON, Councilors 1946 WALLACE O FENN, President, MAURICE B VISSCHER, Secretary, D B DILL, Treasurer, CHARLES H BEST, HIRAM E ESSEX, W F HAMILTON, H C BAZETT, Councilors 1947 WALLACE O FENN, President, MAURICE B VISSCHER, Secretary, D B DILL, Treasurer, EUGENE M LANDIS, HIRAM E ESSEX, W F HAMILTON, H C BAZETT, Councilors 1948 MAURICE B VISSCHER, President, CARL J WIGGERS, President-Elect, WALLACE O FENN, Past-President, W F HAMILTON, H C BAZETT, EUGENE M LANDIS, D B DILL, Councilors

CONSTITUTION

I

1 This Society shall be named "THE AMERICAN PHYSIOLOGICAL SOCIETY, INCORPORATED "

2 The Society is instituted to promote the advance of Physiology and to facilitate personal intercourse between American Physiologists

II

1 The Society shall consist of members and honorary members

2 Any person who has conducted and published meritorious original researches in Physiology and who is a resident of North America shall be eligible for membership in the Society

3 Members who have been relieved by the Council of the payment of the annual assessment shall retain all the rights of members

4 Distinguished men of science who have contributed to the advance of Physiology shall be eligible for election as honorary members of the Society. Honorary members shall pay no membership fee. They shall have the right of attending the meetings of the Society, and of taking part in its scientific discussions, but they shall have no vote

III

1 The management of the Society shall be vested in a Council consisting of a President-Elect, President, Past-President for the previous year, and four other members. The President-Elect and one member of the Council shall be chosen by ballot at each annual meeting. The President-Elect shall automatically assume the duties of President at the adjournment of the annual meeting following his or her election. The four additional members shall be elected for terms of four years and shall not be eligible to succeed themselves. A person who has once been President shall not be eligible for re-election as President-Elect. The Council shall have the power to elect its Secretary-Treasurer from among its own members. It shall also have the power to appoint and to compensate an Executive Secretary of the Society, who shall not be a voting member of the Council but shall assist it in carrying on the functions of the Society, including the receipt and disbursement of funds under the direction of the Council. If the annual meeting is not held all the members of the Council shall continue in office until their successors are chosen in the prescribed manner and succession

2 The Council shall have the power to fill all interim vacancies that may occur in its membership or in any Committee or board of the Society except those for which other provisions have been made

IV

1 At least a fortnight before the annual meeting the Secretary shall send to each member a notice of the place and time of each meeting, and shall make such other announcements as the Council shall direct

2 The annual assessment shall be determined by the Council, and shall be due in advance at the time of the annual meeting. No allocation or disbursement of funds of the Society shall be made except upon prior approval of the Council. Appropriations shall be made by the Council for the conduct of the necessary and appropriate business of the Society

3 Any member whose assessment is two years in arrears shall cease to be a member of the Society, unless at the next annual meeting he shall be reinstated by special vote of the Society, and it shall be the duty of the Treasurer to inform the Secretary that he may notify the said delinquent of his right to appeal to said meeting

4 Any member who has retired because of illness or age may, upon application to the Council, be relieved from payment of the annual assessment

V

1 Meetings of the Society for the conduct of business and the presentation of papers and demonstrations shall be held annually except for national emergencies or other exceptional circumstances when the Council may cancel the proposed meeting. The time and place of such meetings shall be determined by the Council in consultation with the Executive Committee of the Federation of American Societies for Experimental Biology

2 Special meetings may be held at such times and places as the Council may determine

VI

1 Proposed amendments to the Constitution must be brought up at one meeting for preliminary discussion and approval by a majority vote and cannot be adopted except by a two-thirds vote at a business session at the next annual meeting. Notice of such changes shall be sent to all members at least two weeks prior to the meeting at which they are scheduled for adoption

2 At all business meetings of the Society twenty-five members shall form a quorum

3 By-laws for the conduct of the Society may be adopted, altered, or repealed at any business meeting by two-thirds vote of the ballots cast

VII

1 The Council may, from the names of the candidates proposed in writing by at least two members of the Society, nominate candidates for election to membership. The names of the candidates so nominated and a statement of their quali-

fications for membership signed by their proposers shall be available for inspection during the business sessions of the Society at which their election is considered. The candidates may be balloted for at any session of the same meeting and a majority vote shall elect. If an annual meeting is not held, the Council shall elect the candidates to membership subject to Society approval at the next annual meeting.

2 Honorary members shall be proposed by the Council, and shall be elected by a majority ballot of the members present at an annual business session of the Society.

VIII

1 If a majority of the Council shall decide that the interests of the Society require the expulsion of a member, the Secretary shall send a notice of this decision to each member at least two weeks before the next annual meeting. At this meeting the Secretary shall on behalf of the Council, propose the expulsion, and if two-thirds of the members present vote for it, the member shall be expelled, and his assessment for the current year shall be returned to him, and he shall cease to be a member of the Society.

IX

1 The official organs of the Society shall be the *American Journal of Physiology*, the *Physiological Reviews* and such other publications as the Society shall establish. These the Society shall own and they shall be managed according to the provisions of Article X.

X

1 The President of the Society shall appoint, in consultation with the Council and subject to the approval of the Society, three members of the Society to serve as members of a Board of Publication Trustees.

2 The initial appointments shall be for one, two and three years. Thereafter, each member shall be appointed for three years, and shall be eligible for one immediate reappointment. He may be subse-

quently reappointed, but only after the lapse of at least one year between reappointments.

3 The Board of Publication Trustees shall be vested with full power of the Society to control and manage, both editorially and financially, all of the publications owned in whole or in part by the Society, to appoint editorial boards, to appoint and compensate a Managing Editor, and to control all publication funds, none of which, however, may be diverted from support of publications of the Society except by consent of the Council.

4 The Board of Publication Trustees shall make a full report to the Council at each annual meeting of the financial condition and publication policy of the Journals or other publications.

BY-LAWS

1 All papers read before the Society shall be limited to a length of ten minutes. No person may orally present more than one paper. In case of joint authorship the name of the individual who will orally present the paper shall stand first.

2 Abstracts in duplicate, not to exceed two hundred and fifty words in length, of all papers to be presented at the annual meeting of the Society shall be required by the Secretary for publication in the *Federation Proceedings*, in accordance with rules approved by the Council.

3 The Council shall upon the request of twenty-five members call a regional meeting of the Society at any time and place, for the reading of papers and the promotion of personal intercourse. Such a request shall be made in writing at least six weeks before the proposed date of meeting. Such meeting shall be held in accordance with the Constitution and By-laws of the Society, and if the regular officers of the Society cannot be present the President shall appoint a committee from among the petitioners to conduct the meeting. The Committee through a Secretary chosen by them shall forward an account of the scientific proceedings of the meeting to the official Secretary of the Society for insertion in the minutes.

4 No general business of the Society shall be transacted at such regional meetings.

AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, INCORPORATED

Founded December 6, 1906, Incorporated September 12, 1919

OFFICERS, COMMITTEES AND REPRESENTATIVES FOR 1949-1950

President—CARL F CORI, Washington University School of Medicine, St Louis, Mo

Vice-President—HUBERT B VICKERY, Connecticut Agricultural Experiment Station, New Haven, Conn

Secretary—RICHARD W JACKSON, Northern Regional Research Laboratory, Peoria, Ill

Treasurer—E A EVANS, JR, University of Chicago, Chicago, Ill

Council—HANS T CLARKE, CARL F CORI, E A EVANS, JR, A BAIRD HASTINGS, RICHARD W JACKSON, J MURRAY LUCK, HUBERT B VICKERY

Editorial Committee—HENRY A MATTILL, *Chairman*, W R BLOOR, R KEITH CANNAN, JOHN T EDSALL, C G KING, J MURRAY LUCK, ALFRED N RICHARDS, PHILIP A SHAFFER, D WRIGHT WILSON

Editorial Board—RUDOLPH J ANDERSON, *Managing Editor*, R M ARCHIBALD, W MANSFIELD CLARK, HANS T CLARKE, CARL F CORI, E A DOISY, VINCENT DU VIGNEAUD, JOSEPH S FRUTON, WENDELL H GRIFFITH, A BAIRD HASTINGS, HOWARD B LEWIS, E V MCCOLLUM, E E SNELL, WARREN M SPERRY, WILLIAM G STADIE, EDWARD L TATUM, HUBERT B VICKERY, HARLAND G WOOD

Nominating Committee—C G KING, *Chairman and Secretary*, ERIC G BALL, H E CARTER, C A ELVEHJEM, JOSEPH S FRUTON, D M GREENBERG, HOWARD B LEWIS, SEVERO OCHOA, HARLAND G WOOD

Committee on Armed Forces—WENDELL H GRIFFITH, *Chairman*, OTTO A BESSEY, C G KING

Committee on Biochemical Nomenclature (jointly with the American Institute of Nutrition)—HUBERT B VICKERY, *Chairman*, E M NELSON

Committee on Clinical Chemistry—WARREN M SPERRY, *Chairman*, HANS T CLARKE, R N FARGER, R M HILL, A E OSTERBERG

Committee on Professional Training of Biochemists—C A ELVEHJEM, *Chairman*, W MANSFIELD CLARK, E A DOISY, VINCENT DU VIGNEAUD, HOWARD B LEWIS

Representatives to the Council of the American Association for the Advancement of Science—W D ARMSTRONG, DEAN BURK

Representative to the American Documentation Institute—ATHERTON SEIDELL

Representatives to the National Research Council—Division of Biology and Agriculture, W M CLARK, Division of Medical Sciences, W C STADIE

Historian—PHILIP A SHAFFER

Representative on the International Committee for Biochemistry—R W JACKSON

PAST OFFICERS

1907 RUSSELL H CHITTENDEN, President, J J ABEL, Vice-President, W J GIES, Secretary, L B MENDEL, Treasurer, W JONES, W KOCH, J MARSHALL, T B OSBORNE, Councilors 1908 JOHN J ABEL, President, OTTO FOLIN, Vice-President, Wm J GIES, Secretary, L B MENDEL, Treasurer, A B MACALLUM, A P MATHEWS, F G NOVY, Councilors 1909 OTTO FOLIN, President, T B OSBORNE, Vice-President, Wm J GIES, Secretary, L B MENDEL, Treasurer, J J ABEL, P A LEVENE, G LUSK, Councilors 1910 THOMAS B OSBORNE, President, L B MENDEL, Vice-President, A N RICHARDS, Secretary, WALTER JONES, Treasurer, A B MACALLUM, A P MATHEWS, V C VAUGHAN, Councilors 1911 LAFAYETTE B MENDEL, President, A B MACALLUM, Vice-President, A N RICHARDS, Secretary, WALTER JONES, Treasurer, Wm J GIES, A S LOEVENHART, P A SHAFFER, Councilors 1912 ARCHIBALD B MACALLUM, President, G LUSK, Vice-President, A N RICHARDS, Secretary, WALTER JONES, Treasurer, H P ARMSBY, L B MENDEL, H G WELLS, Councilors 1913 ARCHIBALD B MACALLUM, President, G LUSK, Vice-President, P A SHAFFER, Secretary, D D VAN SLYKE, Treasurer, H P ARMSBY, L B MENDEL, H G WELLS, Councilors 1914 GRAHAM LUSK, President, C L ALSBERG, Vice-President, P A SHAFFER, Secretary, D D VAN SLYKE, Treasurer, J J ABEL, A B MACALLUM, T B OSBORNE, Councilors 1915 WALTER JONES, President, C L ALSBERG, Vice-President, P A SHAFFER, Secretary, D D VAN SLYKE, Treasurer, OTTO FOLIN, G LUSK, L B MENDEL, Councilors 1916 WALTER JONES, President, F P UNDERHILL, Vice-President, S R BENEDICT, Secretary, D D VAN SLYKE, Treasurer, OTTO FOLIN, A B MACALLUM, P A SHAFFER, Councilors 1917 CARL L ALSBERG, President, A P MATHEWS, Vice-President, S R BENEDICT, Secretary, H C BRADLEY, Treasurer, L J HENDERSON, P A

SHAFER, F P UNDERHILL, Councilors 1918 CARL L ALSBERG, President, A P MATHEWS, Vice-President, S R BENEDICT, Secretary, H C BRADLEY, Treasurer, W J GIES, ANDREW HUNTER, E V McCOLLUM, Councilors 1919 STANLEY R BENEDICT, President, D D VAN SLYKE, Vice-President, V C MYERS, Secretary, H C BRADLEY, Treasurer, ANDREW HUNTER, E V McCOLLUM, L B MENDEL, Councilors 1920 STANLEY R BENEDICT, President, D D VAN SLYKE, Vice-President, V C MYERS, Secretary, H C BRADLEY, Treasurer, OTTO FOLIN, WALTER JONES, L B MENDEL, Councilors 1921 DONALD D VAN SLYKE, President, P A SHAFER, Vice-President, V C MYERS, Secretary, H C BRADLEY, Treasurer, S R BENEDICT, OTTO FOLIN, WALTER JONES, Councilors 1922 DONALD D VAN SLYKE, President, P A SHAFER, Vice-President, V C MYERS, Secretary, W R BLOOR, Treasurer, S R BENEDICT, H C BRADLEY, A P MATHEWS Councilors 1923 PHILIP A SHAFER, President, H C SHERMAN, Vice-President, V C MYERS, Secretary, W R BLOOR, Treasurer, H C BRADLEY, ANDREW HUNTER, A P MATHEWS, Councilors 1924 PHILIP A SHAFER, President, HENRY C SHERMAN, Vice-President, D WRIGHT WILSON, Secretary, WALTER R BLOOR, Treasurer, OTTO FOLIN, ANDREW HUNTER, VICTOR C MYERS, Councilors 1925 HENRY C SHERMAN, President, EDWARD C KENDALL, Vice-President, D WRIGHT WILSON, Secretary, WALTER R BLOOR, Treasurer, OTTO FOLIN, LAFAYETTE B MENDEL, PHILIP A SHAFER, Councilors 1926 EDWARD C KENDALL, President, ELMER V McCOLLUM, Vice-President, FRED C KOCH, Secretary, GLENN E CULLEN, Treasurer, J B COLLIP, EDWARD A DOISY, ALBERT P MATHEWS, Councilors 1927 E V McCOLLUM, President, W R BLOOR, Vice-President, D WRIGHT WILSON, Secretary, G E CULLEN, Treasurer, E A DOISY, F C KOCH, D D VAN SLYKE, Councilors 1928 E V McCOLLUM, President, W R BLOOR, Vice-President, D WRIGHT WILSON, Secretary, G E CULLEN, Treasurer, W M CLARK, F C KOCH, D D VAN SLYKE, Councilors 1929 W R BLOOR, President, H C BRADLEY, Vice-President, H B LEWIS, Secretary, G E CULLEN, Treasurer, W M CLARK, C L A SCHMIDT, P A SHAFER, Councilors 1930 W R BLOOR, President, H C BRADLEY, Vice-President, H B LEWIS, Secretary, G E CULLEN, Treasurer, W M CLARK, P A SHAFER, D W WILSON, Councilors 1931 H C BRADLEY, President, W M CLARK, Vice-President, H B LEWIS, Secretary, C H FISKE, Treasurer, W C ROSE, P A SHAFER, D W WILSON, Councilors 1932 H C BRADLEY, President, W M CLARK, Vice-President, H B LEWIS, Secretary, C H FISKE, Treasurer, P E HOWE,

W C ROSE, D W WILSON, Councilors 1933 W M CLARK, President, H B LEWIS, Vice-President, H A MATTILL, Secretary, C H FISKE, Treasurer, H C BRADLEY, P E HOWE, W C ROSE, Councilors 1934 W M CLARK, President, H B LEWIS, Vice-President, H A MATTILL, Secretary, C H FISKE, Treasurer, H C BRADLEY, E A DOISY, P E HOWE, Councilors 1935 H B LEWIS, President, G E CULLEN, Vice-President, H A MATTILL, Secretary, C H FISKE, Treasurer, H C BRADLEY, J B COLLIP, E A DOISY, Councilors 1936 H B LEWIS, President, G E CULLEN, Vice-President, H A MATTILL, Secretary, A B HASTINGS, Treasurer, J B COLLIP, E A DOISY, W C ROSE, Councilors 1937 G E CULLEN, President, W C ROSE, Vice-President, H A MATTILL, Secretary, A B HASTINGS, Treasurer, E A DOISY, H B LEWIS, H B VICKERY, Councilors 1938 G E CULLEN, President, W C ROSE, Vice-President, CHARLES G KING, Secretary, A B HASTINGS, Treasurer, H B LEWIS, H A MATTILL, H B VICKERY, Councilors 1939 W C ROSE, President, R J ANDERSON, Vice-President, CHARLES G KING, Secretary, A B HASTINGS, Treasurer, H B LEWIS, H A MATTILL, G E CULLEN, Councilors 1940 WILLIAM C ROSE, President, RUDOLPH J ANDERSON, Vice-President, CHARLES G KING, Secretary, A B HASTINGS, Treasurer, H A MATTILL, GLENN E CULLEN, E A DOISY, Councilors 1941 R J ANDERSON, President, E A DOISY, Vice-President, A K BALLS, Secretary, W C STADIE, Treasurer, H B LEWIS, W C ROSE, Councilors 1942 R J ANDERSON, President, E A DOISY, Vice-President, A K BALLS, Secretary, W C STADIE, Treasurer, W C ROSE, C A KING, H T CLARKE, Councilors 1943 E A DOISY, President, A B HASTINGS, Vice-President, A K BALLS, Secretary, W C STADIE, Treasurer, W C ROSE, H T CLARKE, R J ANDERSON Councilors 1944 E A DOISY, President, A B HASTINGS, Vice-President, A K BALLS, Secretary, W C STADIE, Treasurer, R J ANDERSON, H T CLARKE, V DU VIGNEAUD, Councilors 1945 A B HASTINGS, President, H T CLARKE, Vice-President, A K BALLS, Secretary, W C STADIE, Treasurer, R J ANDERSON, C F CORI, V DU VIGNEAUD, Councilors 1946 A B HASTINGS, President, H T CLARKE, Vice-President, OTTO A BESSEY, Secretary, E A EVANS, Jr, Treasurer, V DU VIGNEAUD, C F CORI, A K BALLS, Councilors 1947 HANS T CLARKE, President, CARL F CORI Vice-President, OTTO A BESSEY, Secretary, E A EVANS, Jr, Treasurer, A K BALLS, A BAIRD HASTINGS, J MURRAY LUCK, Councilors 1948 HANS T CLARKE, President, CARL F CORI, Vice-President, OTTO A BESSEY, Secretary, E A EVANS, Jr, Treasurer, A K BALLS, A BAIRD HASTINGS, J MURRAY LUCK, Councilors

CONSTITUTION

FROM THE ARTICLES OF INCORPORATION

1 The name of the proposed corporation is "AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, INCORPORATED"

2 The purposes for which this corporation is formed are to further the extension of biochemical knowledge and to facilitate personal intercourse between American investigators in biological chemistry

BY-LAWS

ARTICLE I—*Membership*

SECTION 1 *Eligibility for Membership*—Qualified investigators who have conducted and published meritorious original investigations in biological chemistry shall be eligible for membership in the Society

SEC 2 *Nomination*—Nominations for membership shall be made and seconded by members of the Society on blanks furnished by the Secretary. Nominations shall be submitted to the Council who shall determine eligibility and make recommendation to the Society at a regular meeting

SEC 3 *Election to Membership*—A A nominee for membership may be voted for by ballot at any meeting of the Society after the Council has reported its findings on his eligibility. The eligible candidate shall be reported by the Council as "eligible" or as "eligible and indorsed" B A majority of the ballots cast shall elect

SEC 4 *Forfeiture*—A Any member who may grant the use of his name for (a) the advertisement of a patent medicine, a proprietary food preparation, or any other commercial article of doubtful value to the public or possibly harmful to the public health, or (b) who may concede its use for the purpose of encouraging the sale of individual samples (of any such product) that he has not examined, shall forfeit his membership

B The Council shall have authority to announce forfeiture of membership, provided that the copy of the charges, together with a written notice of a hearing thereon by the Council at a place and time specified in such notice, shall have been delivered to the member charged with violating the preceding section either personally or mailed to him at his last known address at least thirty days before the date of such hearing

SEC 5 *Expulsion*—Upon the recommendation of the Council any member may be expelled by a majority vote of the total membership at a meeting of the Society, provided that a copy of the charges against him, together with a written notice of a hearing thereon by the Council at a place and time specified in such notice shall have been delivered to him personally or mailed to him at his last known address at least thirty days before the date of such hearing

ARTICLE II—*Meetings and Quorum*

SECTION 1 *Annual*—The annual meeting of the Society shall be held on the date fixed by the Certificate of Incorporation

SEC 2 *Special*—A special meeting may be called at any time by the President, or in case of his absence or disability, by the Vice-President, and must be called at the request of a majority of the Council or fifteen members of the Society. A notice specifying the purpose of such meeting shall be mailed to each member at least ten days previous thereto. The Council shall select the places at which meetings shall be held

SEC 3 *Quorum*—Fifteen members shall constitute a quorum at all meetings of the Society, but in absence of a quorum any number shall be sufficient to adjourn to a fixed date

ARTICLE III—*Officials*

SECTION 1 *Officers*—The officers shall be a President, a Vice-President, a Secretary, and a Treasurer, who shall be elected annually by the members of the Society

SEC 2 *Council*—A The officers so elected and three additional members, one of whom shall be elected at each annual meeting of the Society to serve a three year term, shall constitute the Board of Directors of the corporation and shall be known as "The Council" (When this provision is first put into effect three members will need to be elected for a one, a two and a three year period)

B No two members of the Council may be from the same institution, and none of the officers so elected shall be eligible for re-election for more than two years except the Secretary and Treasurer, who shall be eligible for re-election for five years. The three additional members of the Council shall be ineligible for re-election (until after the lapse of one year)

SEC 3 *Duties of Officers*—The powers and duties of the officers elected by the Society shall be such as usually devolve upon their respective positions

SEC 4 *Assistant Treasurer*—A The Council may from time to time appoint a trust company, or some member of the Society, to serve during the pleasure of the Council as Assistant Treasurer, and to act as depository of the investments and income of the "Christian A. Herter Memorial Fund" and of such other funds as the Society may from time to time commit to its or his charge

B The Assistant Treasurer shall have and exercise the following powers and duties, viz, the custody and safe-keeping of securities and cash belonging to the "Christian A. Herter Fund" and the collection of income and other moneys due to the Fund, with power to receipt for the same and to endorse for deposit all checks payable to the Society or the Treasurer, or to the Journal of Biological Chemistry for income or other moneys

due to the Fund, the investment or reinvestment of the capital of the Fund subject to the approval of the Council, the disbursement of principal under the direction of the Council and the disbursement of income under the direction of the Editorial Board of the Journal of Biological Chemistry, such disbursement to be made under a resolution of the Council or Board, or with the approval of two members of either the Council or Board, as the case may be. The Assistant Treasurer shall keep books of account and render statements, annually or oftener upon the request of the Council or Board setting forth the condition of the Fund and the receipts and disbursements since the date of the preceding statement.

ARTICLE IV — *The Council*

SECTION 1 *Powers* —The general management of the Society during the intervals between meetings shall be vested in the Council, which shall regularly perform the ordinary duties of an executive committee and possess all the powers conferred upon the Board of Directors of a membership corporation by the Membership Corporation Law of the State of New York.

SEC 2 *Reports* —The Council shall report to the Society as promptly as possible its findings on the eligibility of candidates for membership, and on all charges of a violation of these By-Laws.

SEC 3 *Journal of Biological Chemistry* —The Council shall have power to appoint the persons to act as proxies for the society at all meetings of the stockholders of the "Journal of Biological Chemistry" (a corporation) of which all the stock is owned by the Society, and also to designate the persons to be elected as Directors of such corporation.

SEC 4 *Herter Fund* —It shall be the duty of the Council to see that the "Christian A. Herter Memorial Fund" is administered in accordance with the terms of the Trust Agreement, Dated May 16, 1911, executed by the Journal of Biological Chemistry and the donors of said Fund.

ARTICLE V — *Nominating Committee*

SECTION 1 *Membership* —A The Nominating Committee shall consist of nine members from nine different institutions elected at each annual meeting to serve for the ensuing year. Members who have served on the Nominating Committee for two consecutive years shall be ineligible for re-election until after the lapse of one year.

B The member of the Nominating Committee who is elected to the Committee by the largest number of votes shall become Chairman and Secretary of the Committee.

SEC 2 *Nomination of Officials* —A The Nomi-

nating Committee shall make at least one nomination for each of the four offices and for each of the three additional positions in the Council to be filled by vote of the members.

B The nominations by the Nominating Committee must be transmitted to the Secretary at least one month before the annual meeting at which they are to be considered.

C The Secretary shall send to every member, at least two weeks before the annual meeting, two copies of the list of nominees presented to him by the Nominating Committee and at the same time shall notify all the members that they may vote by proxy.

D At the opening of the first executive session of the ensuing annual meeting the Secretary shall formally present the regular nominations for the Nominating Committee.

E Additional nominations for the offices and for membership in the Council may be made by any member at the opening of the first executive session of any annual meeting.

F Nominations for membership on the Nominating Committee shall be made by or for individual members, either in person or by proxy, and not otherwise, at the opening of the first executive session of any annual meeting.

SEC 3 *Election of Officials* —A The Secretary shall receive and present to the tellers, appointed by the President to take charge of the election, all signed ballots forwarded by absent members. When such ballots are presented to the tellers the Secretary shall announce the names of the members voting by proxy, and he shall record the same names in the minutes of the meeting.

B All elective officials shall be selected by ballot at the close of the first executive session of each annual meeting.

C A majority of the votes cast shall be necessary to elect an official.

D Elective officials shall take office on July 1st following the annual meeting.

SEC 4 *Filling of Vacancies* —A The Nominating Committee shall fill all vacancies in elective positions except such as may occur at a meeting of the Society.

B The President of the Society shall fill all vacancies in appointive positions.

ARTICLE VI — *Financial*

SECTION 1 *Dues* —Annual assessments shall be determined by majority vote at the annual meetings, upon the recommendation of the Council, and shall be due January 15th in each year. Members who have reached the age of 65 years, or who have become incapacitated, may, by vote of the Council, be exempted from the payment of dues.

SEC 2 *Expenditures* —No expenditures from the general funds of the Society except those required

in the performance of the ordinary official duties shall be made except by vote of the Society or the Council, but this section shall not apply to expenditures from the "Christian A. Herter Memorial Fund."

SEC 3 *Privileges of Membership Begin with Payment of Dues*—Candidates for membership, if elected, shall not be entitled to any of the privileges of membership, before they pay the dues of the fiscal year succeeding their election.

SEC 4 *Penalty for Non-Payment of Dues*—A Member in arrears for dues for a period of three consecutive years shall thereupon forfeit their membership.

B Delinquent members may be reinstated by the Council provided all indebtedness to the Society is liquidated.

SEC 5 *Herter Fund*—The "Christian A. Herter Memorial Fund" shall be held and invested separately from the general funds of the Society and the income thereof shall be expended under the direction of the Editorial Board exclusively for the maintenance and support of the Journal of Biological Chemistry, subject to the supervision and control of the Editorial Committee in accordance with the terms of the Trust Agreement mentioned in ARTICLE IV, SECTION 4, and the provisions of Article VII of the By-Laws.

ARTICLE VII—*Journal of Biological Chemistry*

SECTION 1 *Editorial Committee*—There shall be an Editorial Committee consisting of nine members of the Society who shall be nominated by the Nominating Committee and elected by the Society in the same manner as officers. The nine members first elected shall divide themselves by lot into three classes of three in each class, to serve for two, four, and six years respectively, and thereafter three members shall be elected at each alternate annual meeting of the Society to succeed the members of the outgoing class and to serve for a term of six years. Members of the Committee shall be eligible to re-election.

SEC 2 *Powers of Committee*—The Committee shall have power to elect an Editorial Board and shall have final authority in matters pertaining to the general policy of the Journal.

SEC 3 *Editorial Board*—The members of the Board shall hold office until their successors are elected and shall appoint a Managing Editor from among their own number who shall have direct responsibility and authority for the active editorial conduct of the Journal, and who shall have discretionary power in arranging the details as to the conduct of the Journal. The expenditures of the income of the "Christian A. Herter Memorial Fund" shall be under the direction of the Board,

and the approval of any two members of the Board shall be a sufficient warrant to authorize payments from such income.

ARTICLE VIII—*Papers on Scientific Subjects*

SECTION 1 *Presentation of Papers*—The Secretary shall request each member who signifies his intention of reading a paper at any session to specify the length of time which its presentation will require. The time thus specified shall be printed on the official program, and the presiding officer shall have no authority to extend it unless a majority of the members present signify their wish to the contrary. In the absence of any specification of time required not more than ten minutes shall be allotted for the reading of any one paper.

SEC 2 *Number of Papers*—No member shall be permitted to present more than one paper, either alone or in collaboration, until every member shall have had the opportunity of presenting one paper.

ARTICLE IX—*Corporate Seal*

SECTION 1 The corporate seal of the corporation shall be a circle surrounded by the words, "AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS," and including the word, "INCORPORATED."

ARTICLE X—*Amendments*

SECTION 1 *Amendments*—These By-Laws, after having been approved by the Council, and adopted by the Society at its first annual meeting, shall not be amended except as hereinafter provided.

SEC 2 *Manner of Presentation*—Proposed amendments to the By-Laws must be sent to the Secretary at least one month before the date of the meeting at which they are to be considered and must be indorsed in writing by at least three members.

SEC 3 *Notice of Intended Amendments*—The Secretary shall give every member notice of proposed amendments at least two weeks before the meeting at which they are to be considered and shall notify all members that they may vote by proxy.

SEC 4 *Adoption of Amendments*—A The Secretary shall receive and present to the tellers appointed by the President all signed ballots forwarded by absent members. When such ballots are presented to the tellers, the Secretary shall announce the names of members voting by proxy, and he shall record the same names in the minutes of the meeting.

B Votes upon amendments shall be cast at the opening of the second executive session of the meeting at which they are considered.

C Affirmative votes from three-fifths of the members voting shall be required for the adoption of an amendment.

AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, INCORPORATED

Founded December 28, 1908, Incorporated June 19, 1933

OFFICERS, 1949-1950

President—CARL F SCHMIDT, University of Pennsylvania School of Medicine, Philadelphia

Vice-President—J C KRANTZ, JR, University of Maryland Medical School, Baltimore

Secretary—HARVEY B HAAG, Medical College of Virginia, Richmond

Treasurer—K K CHEN, Lilly Research Laboratories, Indianapolis, Ind

Council—THOMAS C BUTLER, ARTHUR C DEGRAFF, ROBERT A WOODBURY, CARL F SCHMIDT, J C KRANTZ, JR, HARVEY B HAAG, K K CHEN

Membership Committee—ROBERT P WALTON (1950), JOHN C KRANTZ, JR (1951), J H COMROE, JR (1952)

Nominating Committee—OTTO KRAYER, *Chairman*, W T SALTER, WALTON VAN WINKLE, JR, ALFRED GILMAN, M H SEEVERS

Historian—WILLIAM DEB MACNIDER

Committee on International Pharmacological Congress—M L TAINTER, *Chairman*, OTTO KRAYER, H B VAN DYKE

PAST OFFICERS

1909 J J ABEL, President, REID HUNT, Secretary, A S LOEVENHART, Treasurer, S J MELTZER, T SOLLMANN, C W EDMUNDS, A C CRAWFORD, Councilors 1910 J J ABEL, President, REID HUNT, Secretary, A S LOEVENHART, Treasurer, A C CRAWFORD, G B WALLACE, Councilors 1911 J J ABEL, President, REID HUNT, Secretary, A S LOEVENHART, Treasurer, G B WALLACE, W DEB MACNIDER, Councilors 1912 J J ABEL, President, J AUER, Secretary, A S LOEVENHART, Treasurer, G B WALLACE, REID HUNT, Councilors 1913 T SOLLMANN, President, J AUER, Secretary, A S LOEVENHART, Treasurer, J J ABEL, W DEB MACNIDER, Councilors 1914 T SOLLMANN, President, J AUER, Secretary, W DEB MACNIDER, Treasurer, J J ABEL, A S LOEVENHART, Councilors 1915 T SOLLMANN, President, J AUER, Secretary, W DEB MACNIDER, Treasurer, WORTH HALE, D E JACKSON, Councilors 1916 REID HUNT, President, J AUER, Secretary, W DEB MACNIDER, Treasurer, A D HIRSCHFELDER, G B ROTH, Councilors 1917 REID HUNT, President, L G ROWNTREE, Secretary, W DEB MACNIDER, Treasurer, J AUER, CARL VOEGTLIN, Councilors 1918 REID HUNT, President, E D BROWN, Secretary, W DEB MACNIDER, Treasurer, HUGH

McGUIGAN, CARL VOEGTLIN, Councilors 1919 A S LOEVENHART, President, E D BROWN, Secretary, W DEB MACNIDER, Treasurer, REID HUNT, E K MARSHALL, JR, Councilors 1920 A S LOEVENHART, President, E D BROWN, Secretary, W DEB MACNIDER, Treasurer, D E JACKSON, E K MARSHALL, JR, Councilors 1921 C W EDMUNDS, President, E D BROWN, Secretary, HUGH McGUIGAN, Treasurer, JOHN AUER, J P HANZLIK, Councilors 1922 C W EDMUNDS, President, E D BROWN, Secretary, HUGH McGUIGAN, Treasurer, J P HANZLIK, H G BARBOUR, Councilors 1923 C W EDMUNDS, President, E D BROWN, Secretary, HUGH McGUIGAN, Treasurer, J P HANZLIK, H G BARBOUR, Councilors 1924 JOHN AUER, President, E D BROWN, Secretary, A L TATUM, Treasurer, J P HANZLIK, H G BARBOUR, Councilors 1925 JOHN AUER, President, E D BROWN, Secretary, A L TATUM, Treasurer, H G BARBOUR, W DEB MACNIDER, Councilors 1926 JOHN AUER, President, E D BROWN, Secretary, A L TATUM, Treasurer, H G BARBOUR, W DEB MACNIDER, Councilors 1927 CARL VOEGTLIN, President, E D BROWN, Secretary, A L TATUM, Treasurer, V E HENDERSON, C W EDMUNDS, Councilors 1928 CARL VOEGTLIN, President, E D BROWN, Secretary, A L TATUM, Treasurer, V E HENDERSON, C W EDMUNDS, Councilors 1929 CARL VOEGTLIN, President, E D BROWN, Secretary, O H PLANT, Treasurer, V E HENDERSON, C W EDMUNDS, Councilors 1930 GEORGE B WALLACE, President, E D BROWN, Secretary, O H PLANT, Treasurer, H G BARBOUR, C M GRUBER, Councilors 1931 GEORGE B WALLACE, President, VELYIEN E HENDERSON, Secretary, O H PLANT, Treasurer, PAUL D LAMSON, WILLIAM DEB MACNIDER, Councilors 1932 WM DEB MACNIDER, President, A N RICHARDS, Vice-President, V E HENDERSON, Secretary, O H PLANT, Treasurer, G B ROTH, A L TATUM, Councilors 1933 WM DEB MACNIDER, President, A L TATUM, Vice-President, V E HENDERSON, Secretary, O H PLANT, Treasurer, C M GRUBER, G B ROTH, Councilors 1934 R A HATCHER, President, A L TATUM, Vice-President, E M K GEILING, Secretary, O H PLANT, Treasurer, WM DEB MACNIDER, R L STEHLE, Councilors 1935 V E HENDERSON, President, O H PLANT, Vice-President, E M K GEILING, Secretary, C M GRUBER, Treasurer, FLOYD DE EDS, M S DOOLEY, Councilors 1936 V E HENDERSON, President,

O H PLANT, Vice-President, E M K GEILING, Secretary, C M GRUBER, Treasurer, C W EDMUNDS, G B WALLACE, Councilors 1937 A L TATUM, President, E M K GEILING, Vice-President, G P GRABFIELD, Secretary, C M GRUBER, Treasurer, V E HENDERSON, M H SEEVERS, Councilors 1938 A L TATUM, President, E M K GEILING, Vice-President, G P GRABFIELD, Secretary, C M GRUBER, Treasurer, E K MARSHALL, JR, C F SCHMIDT, Councilors 1939 O H PLANT, President, E M K GEILING, Vice-President, G P GRABFIELD, Secretary, E E NELSON, Treasurer, A L TATUM, C A DRAGSTEDT, Councilors 1940 E M K GEILING, President, C F SCHMIDT, Vice-President, G PHILIP GRABFIELD, Secretary, E E NELSON, Treasurer, B H ROBBINS, C H THIENES, Councilors 1941 E M K GEILING, President, C F SCHMIDT, Vice-President, RAYMOND N BIETER, Secretary, E E NELSON, Treasurer, E G GROSS, R G SMITH, Councilors 1942 E K MARSHALL, JR, President, CARL A DRAGSTEDT, Vice-President, RAYMOND N BIETER, Secretary, E E NELSON, Treasurer, MCK CATTELL, R G SMITH, Councilors 1943 E K MARSHALL, JR, President, CARL A DRAGSTEDT, Vice-President, RAYMOND N BIETER, Secretary, E E NELSON, Treasurer, MCK CATTELL, R G SMITH, Councilors 1944, 1945 E E NELSON, President, C M GRUBER, Vice-President, R N BIETER, Secretary, MCKEEN CATTELL, Treasurer, HARRY BECKMAN, NATHAN B EDDY, Councilors 1946 MAURICE H SEEVERS, President, H B VAN DYKE, Vice-President, HARVEY B HAAG, Secretary, MCKEEN CATTELL, Treasurer, HAMILTON H ANDERSON, JOHN C KRANTZ, JR, Councilors 1947 MAURICE H SEEVERS, President, CARL A DRAGSTEDT, Vice-President, HARVEY B HAAG, Secretary, K K CHEN, Treasurer, HAMILTON H ANDERSON, JOHN C KRANTZ, JR, Councilors 1948 CARL A DRAGSTEDT, President, H B VAN DYKE, Vice-President, HARVEY B HAAG, Secretary, K K CHEN, Treasurer, ARTHUR C DEGRAFF, ROBERT A WOODBURY, GORDEN A ALLES, Councilors

CONSTITUTION

ARTICLE I—Name

The name of this organization shall be the "AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, INCORPORATED"

ARTICLE II—Objects

The purpose of this Society shall be to promote these branches of science and to facilitate personal intercourse between investigators who are actively engaged in research in these fields

ARTICLE III—Membership

SECTION 1 Any person who has conducted and published a meritorious investigation in pharmacology or experimental therapeutics, and who is an active investigator in one of these fields, shall be eligible to membership, subject to the conditions of the other sections of Article III

SEC 2 A Candidates for membership to this Society shall be proposed by two members who are not members of the Council The names so proposed shall be sent to the Secretary at least three months prior to the Annual Meeting

B The Membership Committee shall investigate the qualifications of the candidates and report to the Council

C Candidates reported upon by the Membership Committee to the Council may be recommended for admission by the Council only provided they have been approved by four-fifths of the combined membership of the Membership Committee and the Council

D The names of the candidates recommended for admission by the Council shall be posted by the Secretary not later than the day preceding the election for members

E The election of members shall be by individual ballot, one opposing vote in every eight cast shall be sufficient to exclude a candidate from membership

SEC 3 Forfeiture of Membership

A Any member whose assessment is three years in arrears shall cease to be a member of the Society, unless he shall be reinstated by a special vote of the Council, and it shall be the duty of the Treasurer to inform the Secretary that he may notify the said delinquent of his right to appeal to the Council

B If the Council shall decide that it is for the best interests of the Society that a member be expelled, the member shall be notified and given an opportunity of a hearing before the Council Upon the recommendation of the Council the member then may be expelled by a three-fourths vote of those present at a regular meeting of the Society

SEC 4 Honorary Members

A Distinguished men of science who have contributed to the advance of pharmacology or experimental therapeutics shall be eligible for election as honorary members of the Society

B Nominations for honorary members shall take the same course as nominations for ordinary members (Art III, Sec 2), but their election shall require the unanimous vote of the members present at the election

C Honorary members shall pay no membership fee They shall have the right to attend all meet-

ings of the Society, and to take part in its discussions, but they shall have no vote

D The conditions for continuation of membership shall be the same for honorary as for ordinary members (Art III, Sec 3), except that forfeiture for arrears of fees does not apply to honorary members

ARTICLE IV—*Officers and Elections*

SECTION 1 *Officers and Committees*

A The management of the Society shall be vested in a Council of seven officers, consisting of the President, Vice-President, Secretary, and Treasurer of the Society, and three Councilors-at-Large

B The four ex officio members shall serve for one year but shall be eligible for re-election

C The three Councilors-at-Large shall serve for a period of three years, and shall not be eligible for immediate re-election

D At the first meeting of the Society under this amended Constitution one Councilor-at-Large shall be elected to serve for a period of three years, one for two years, and one for one year, and subsequently one Councilor-at-Large shall be elected annually to serve for a period of three years

E There shall be a Membership Committee, consisting of three members. No two members shall be from the same institution. The election of the Membership Committee shall be held annually at the time when the election of officers occurs. At the first meeting of the Society under this constitution, one member shall be elected to serve on the Committee for three years, one for two years, and one for one year, and subsequently one member shall be elected each year to serve for a period of three years

F There shall be a Nominating Committee of five members. No two members shall be from the same institution. Members of the Nominating Committee shall serve for one year. They are eligible for re election, but shall not hold membership in the Committee for more than two consecutive years

SEC 2—*Nomination of Officials and Committeemen*

A The Nominating Committee shall make at least one nomination for each office and for the position on the Membership Committee to be filled by vote of the members. The nominations so made shall be transmitted to the Secretary and by him in turn to the members, at least one month before the annual meeting

B Nominations for membership on the Nominating Committee shall be made by individual members at the time of the annual election. The five nominees who receive the highest numbers of votes shall be declared elected. The Nominating

Committee shall select its own Chairman who shall serve as Secretary to the Committee

SEC 3 *Election of Officials and Committeemen*

A At the opening of the first executive session of the annual meeting the Secretary shall give to each member present a printed ballot showing the nominations of the Nominating Committee. After accepting additional nominations from individual members present a complete list of nominees shall be posted. A preliminary vote shall then be taken and the tellers, appointed by the President to conduct the election, shall post immediately a final list showing the two nominees for each office receiving the highest number of votes. At the close of the first session, a final vote shall be taken. A majority of votes cast shall be necessary to a choice

B Such vacancies as may occur in the offices and in the various committees in the interval between annual meetings shall be filled by a majority vote of the Council

ARTICLE V—*Meetings*

SECTION 1 The annual meeting of the Society shall be held at a time and place determined by the Council in consultation with the Executive Committee of the Federation of American Societies for Experimental Biology

SEC 2 Special meetings may be held at such times and places as the Council may determine

SEC 3 At least four weeks before the annual meeting the Secretary shall send to each member a notice of the time and place of such meeting and shall make such announcements as the Council may direct

ARTICLE VI—*Financial*

SECTION 1 The annual assessment shall be determined by majority vote at the annual meetings, upon the recommendation of the Council, and shall be due in advance at the time of the meeting

SEC 2 Beyond the ordinary expenditures required by the routine business of the Society no money shall be disbursed save by the authority of the Council or Society

SEC 3 The treasurer shall make an annual report to the Society

SEC 4 All publication funds shall be kept in a separate account, subject to the control of the Board of Publications Trustees except that none of these funds may be diverted from the support of publications of the Society except by consent of the Council or the Society. A financial report shall be made by the Board at each annual meeting

ARTICLE VII—*Quorum*

Ten members shall constitute a quorum for the transaction of business

ARTICLE VIII — *By-Laws*

By-Laws shall be adopted, altered or repealed at any meeting by two-thirds vote of the ballots cast

ARTICLE IX — *Amendments*

SECTION 1 Intended amendments to the Constitution shall be sent to the Secretary at least one month before the date of the meeting at which they are to be considered, and must be indorsed in writing by at least three members

SEC 2 The Secretary shall give all members due notice of proposed amendments

SEC 3 A four-fifths vote of the members present shall be required for the adoption of an amendment

ARTICLE X — *Official Publications*

SECTION 1 The President of the Society shall appoint, in consultation with the Council, and subject to the approval of the Society, three members of the Society to serve as members of a Board of Publications Trustees, these members shall elect a Managing Editor for each of the official journals of the Society, and each Managing Editor, during the term of his service, shall act as an additional voting member of the Board of Publications Trustees and shall participate in all the activities of the Board except that of election of managing editors of the journals of the Society

SEC 2 The members of the Board of Publications Trustees, hereinafter termed "The Board," shall each serve for a term of three years, shall be subject to reappointment, and may hold office concurrently in the Society. At the first appointment, however, one member shall be appointed for three years, one member for two years, and one member for one year, in order that in the future, appointments may be made annually in rotation. The Board shall meet at least once annually (a quorum shall consist of three members) and shall report directly to the Society

SEC 3 The special functions of The Board shall be to consider and to investigate thoroughly all matters pertaining to the fiscal and editorial policies of the journals which may come to the Society or to its Council, to the Managing Editors, and to

the members of The Board. The Board shall (1) administer the finances of the journals, (2) establish the publication policies of the journals, and (3) elect a Managing Editor for each of the journals of the Society (as described in Sec 1)

SEC 4 The Managing Editor of each journal shall nominate to The Board, members of the Society acceptable to him as Associate Editors. From those nominated, The Board shall elect, for each journal, Associate Editors in such number as they shall consider adequate to fulfill the duties of that Editorial Board. The Managing Editor and each Associate Editor shall serve for three years, subject to reappointment, and may hold office concurrently in the Society. In the choice of The Boards of Editors, The Board is charged with the responsibility of obtaining editors with expert knowledge in the several fields of pharmacological research and with evidenced ability for critical and grammatical expression

SEC 5 The Boards of Editors of the journals shall meet on call of the respective Managing Editor, if possible just prior to or during the regular meetings of the Society, and may make recommendations to The Board concerning the improvement of the publication policies of the journals

BY-LAWS

1 Papers to be read shall be submitted by the members of the Society to the Secretary, who, with the President, shall be empowered to arrange the program. No person may orally present more than one paper. In case of joint authorship, the name of the individual who will orally present the paper shall stand first. Papers not read shall appear on the program as read by title

2 An abstract of a paper to be read before the Society shall be sent to the Secretary with the title. As early as possible after each meeting, the Secretary shall edit and publish the Proceedings of the Society together with abstracts in a publication authorized by the Society

3 All applications for membership shall be accompanied by a copy of as many reprints as possible of the published work of the applicant

4 Any member who has been an active member for thirty years, or who has retired for disability or age, may upon notification to the Treasurer be relieved from payment of dues

THE AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY

Founded December 29, 1913

OFFICERS, 1949-1950

President—JOHN G KIDD, Cornell University Medical College, New York City

Vice-President—JAMES F RINEHART, University of California Medical School, San Francisco

Secretary - Treasurer—SIDNEY C MADDEN, Brookhaven National Laboratory, Upton, Long Island, N Y

Councilors—F S ROBSCHT-ROBBINS, D MURRAY ANGEVINE, JOHN G KIDD, JAMES F RINEHART, SIDNEY C MADDEN

Representative on the Division of Medical Sciences of the National Research Council—JOHN G KIDD (1949-1952)

Representatives on the Council of the American Association for the Advancement of Science—MALCOLM H SOULE, E B KRUMBHAAR (1949-1952)

Representatives on the Eli Lilly Award Committee (jointly with the Society of American Bacteriologists)—*For nominations* PAUL R CANNON (1951), S BURT WOLBACH (balance of 1949) *For award* PEYTON ROUS (1951), JOHN G KIDD (balance of 1949)

Representative on the Committee for the Placement Service—DOUGLAS H SPRUNT

PAST OFFICERS

1914 R M PEARCE, President, JOHN F ANDERSON, Vice-President, G H WHIPPLE, Secretary-Treasurer, HARVEY CUSHING, DAVID MARINE, Councilors 1915 THEOBALD SMITH, President, G H WHIPPLE, Vice-President, PEYTON ROUS, Secretary-Treasurer, DAVID MARINE, R M PEARCE, Councilors 1916 SIMON FLEXNER, President, LEO LOEB, Vice-President, PEYTON ROUS, Secretary-Treasurer, DAVID MARINE, R M PEARCE, Councilors 1917 LUDVIG HEKTOEN, President, LEO LOEB, Vice-President, HOWARD T KARSNER, Secretary-Treasurer, PAUL A LEWIS, L G ROWNTREE, Councilors 1918 H GIDEON WELLS, President, W G MACCALLUM, Vice-President, HOWARD T KARSNER, Secretary-Treasurer, L G ROWNTREE, LUDVIG HEKTOEN, Councilors 1919 W G MACCALLUM, President, WILLIAM H PARK, Vice-President, HOWARD T KARSNER, Secretary-Treasurer, LUDVIG HEKTOEN, E L OPIE, Councilors 1920 WILLIAM H PARK, President, F G NOVY, Vice-President, HOWARD T KARSNER, Secretary-Treasurer, E L OPIE, WADE H BROWN, Councilors 1921 F G NOVY, President, HOWARD T KARSNER, Vice-President, WADE H BROWN, Secretary-Treasurer, PAUL A

LEWIS, A R DOCHEZ, Councilors 1922 HOWARD T KARSNER, President, EUGENE L OPIE, Vice-President, WADE H BROWN, Secretary-Treasurer, A R DOCHEZ, GEORGE H WHIPPLE, Councilors 1923 EUGENE L OPIE, President, ALDRED S WARTHIN, Vice-President, WADE H BROWN, Secretary-Treasurer, GEORGE H WHIPPLE, H GIDEON WELLS, Councilors 1924 ALDRED S WARTHIN, President, GEORGE H WHIPPLE, Vice-President, EDWARD B KRUMBHAAR, Secretary-Treasurer, H GIDEON WELLS, FREDERICK L GATES, Councilors 1925 GEORGE H WHIPPLE, President, WADE H BROWN, Vice-President, EDWARD B KRUMBHAAR, Secretary-Treasurer, FREDERICK L GATES, DAVID MARINE, Councilors 1926 WADE H BROWN, President, DAVID MARINE, Vice-President, EDWARD B KRUMBHAAR, Secretary-Treasurer, FREDERICK L GATES, WILLIAM F PETERSEN, Councilors 1927 DAVID MARINE, President, EDWARD B KRUMBHAAR, Vice-President, CARL V WELLER, Secretary-Treasurer, WILLIAM F PETERSEN, FREDERICK L GATES, Councilors 1928 EDWARD B KRUMBHAAR, President, WILLIAM F PETERSEN, Vice-President, CARL V WELLER, Secretary-Treasurer, FREDERICK L GATES, SAMUEL R HAYTHORN, Councilors 1929 WILLIAM F PETERSEN, President, FREDERICK L GATES, Vice-President, CARL V WELLER, Secretary-Treasurer, SAMUEL R HAYTHORN, PEYTON ROUS, Councilors 1930 FREDERICK L GATES, President, SAMUEL R HAYTHORN, Vice-President, C PHILLIP MILLER, Secretary-Treasurer, PEYTON ROUS, CARL V WELLER, Councilors 1931 SAMUEL R HAYTHORN, President, PEYTON ROUS, Vice-President, C PHILLIP MILLER, Secretary-Treasurer, CARL V WELLER, S BURT WOLBACH, Councilors 1932 PEYTON ROUS, President, CARL V WELLER, Vice-President, C PHILLIP MILLER, Secretary-Treasurer, S BURT WOLBACH, OSKAR KLOTZ, Councilors 1933 CARL V WELLER, President, S BURT WOLBACH, Vice-President, C PHILLIP MILLER, Secretary-Treasurer, OSKAR KLOTZ, ALPHONSE R DOCHEZ, Councilors 1934 S BURT WOLBACH, President, OSKAR KLOTZ, Vice-President, SHIELDS WARREN, Secretary-Treasurer, C PHILLIP MILLER, ALPHONSE R DOCHEZ, Councilors 1935 OSKAR KLOTZ, President, ALPHONSE R DOCHEZ, Vice-President, SHIELDS WARREN, Secretary-Treasurer, MORTON MCCUTCHEON, C PHILLIP MILLER, Councilors 1936 ALPHONSE R DOCHEZ, President, C PHILLIP MILLER, Vice-President, SHIELDS WARREN, Secretary-Treasurer, MORTON MCCUTCHEON, ERNEST

W GOODPASTURE, Councilors 1937 C PHILIP MILLER, President, MORTON McCUTCHEON, Vice-President, PAUL R CANNON, Secretary-Treasurer, ERNEST W GOODPASTURE, SHIELDS WARREN, Councilors 1938 MORTON McCUTCHEON, President, ERNEST W GOODPASTURE, Vice-President, PAUL R CANNON, Secretary-Treasurer, SHIELDS WARREN, JESSE L BOLLMAN, Councilors 1939 ERNEST W GOODPASTURE, President, SHIELDS WARREN, Vice-President, PAUL R CANNON, Secretary-Treasurer, JESSE L BOLLMAN, BALDUIN LUCKÉ, Councilors 1940 SHIELDS WARREN, President, JESSE L BOLLMAN, Vice-President, H P SMITH, Secretary-Treasurer, BALDUIN LUCKÉ, PAUL R CANNON, Councilors 1941 JESSE L BOLLMAN, President, BALDUIN LUCKÉ, Vice-President, H P SMITH, Secretary-Treasurer, PAUL R CANNON, DOUGLAS H SPRUNT, Councilors 1942, 1943, 1944, 1945 BALDUIN LUCKÉ, President, PAUL R CANNON, Vice-President, H P SMITH, Secretary-Treasurer, DOUGLAS H SPRUNT, FRIEDA S ROBSCHUIT-ROBBINS, Councilors 1946 PAUL R CANNON, President, DOUGLAS H SPRUNT, Vice-President, FRIEDA S ROBSCHUIT-ROBBINS, Secretary-Treasurer, H P SMITH, JOHN G KIDD, Councilors 1947 DOUGLAS H SPRUNT, President, H P SMITH, Vice-President, FRIEDA S ROBSCHUIT-ROBBINS, Secretary-Treasurer, JAMES F RINEHART, JOHN G KIDD, Councilors 1948 H P SMITH, President, JOHN G KIDD, Vice-President, FRIEDA S ROBSCHUIT-ROBBINS, Secretary-Treasurer, JAMES F RINEHART, SIDNEY C MADDEN, Councilors

CONSTITUTION

ARTICLE I—*Name*

The Society shall be named "THE AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY"

ARTICLE II—*Object*

The object of this Society is to bring the productive investigators in pathology, working essentially by experimental methods, in closer affiliation with the workers in the other fields of experimental medicine

ARTICLE III—*Time and Place of Meeting*

The Society shall meet at the same time and place as the Federation of American Societies for Experimental Biology, which comprises at present the American Physiological Society, the American Society of Biological Chemists, the American Society for Pharmacology and Experimental Therapeutics, the American Society for Experimental Pathology, the American Institute of Nutrition and the American Association of Immunologists

ARTICLE IV—*Membership*

SECTION 1 Any American investigator who, through the use of experimental methods, has, within three years prior to his candidacy, contributed meritorious work in pathology, is eligible to membership

SEC 2 It shall be the policy of the Society to restrict its membership to as small numbers as is compatible with the maintenance of an active existence

SEC 3 There shall be two classes of members active and honorary members

Active members Candidates for active membership shall be nominated at or before an annual meeting by two members of the Society. The nominators shall present to the Secretary in writing evidence of the candidate's qualifications for membership. Nominations approved by the Council shall be presented to the Society for election at the next annual meeting following nomination. For election a favorable ballot by a majority of the members present is necessary.

Honorary members These may be elected from the active list or from the group of distinguished investigators at home or abroad who have contributed to the knowledge of pathology by experimental study. They shall be elected only by the unanimous vote of the members present at time of nomination.

SEC 4 Active members shall pay such annual dues as are determined upon, from year to year, by the Council. Honorary members shall pay no dues, are not eligible to office, and have no vote in the business affairs of the Society, but they shall have all the privileges of the active members in the scientific proceedings.

SEC 5 Upon failure of an active member to pay dues for two years, notice shall be given to the member by the Secretary. At the end of the third year, if dues are still unpaid, such failure constitutes forfeiture of membership.

SEC 6 A motion for expulsion of a member must be thoroughly investigated by the Council, at this investigation the accused shall be afforded a hearing or may be represented by a member. Expulsion can be accomplished only after a unanimous vote by the Council in favor of expulsion, sustained by a four-fifths vote of the members present at the meeting.

ARTICLE V—*Officers*

The management of the Society shall be vested in a Council of five members, consisting of a President, a Vice-President, a Secretary-Treasurer, and two other members who shall be nominated by the Council and elected by the Society. Officers are elected by a majority vote and remain in office until July 1 following the Federation Meeting.

Vacancies shall be filled by the Council for the unexpired term

The President and Vice-President shall hold office for one year and are ineligible for re-election during the following year The Secretary-Treasurer is eligible for re election Councilors shall hold office for two years and are elected on alternate years At the first election one Councilor shall be elected for a short term of one year

ARTICLE VI —*Quorum*

SECTION 1 —Three constitute a quorum of the Council The Council decides by a majority vote

SEC 2 A Quorum of the Society for transaction of business shall be one-fourth of the total membership In all questions brought before the Society a majority vote of those present shall decide, except as elsewhere provided for

ARTICLE VII —*Annual Meeting*

SECTION 1 Papers shall be limited to ten minutes However, on motion and with unanimous consent, the time may be prolonged by a period not exceeding five minutes The Council may make provision for longer papers on suitable occasions

SEC 2 The subjects of papers must be confined to experimental work in pathology In doubtful cases a liberal interpretation by the President and Secretary may prevail The Council may invite, however, presentations dealing with any subject which it considers of considerable interest to the Society

ARTICLE VIII —*Change of Constitution*

A motion concerning a change of the Constitution must be presented to the Council in writing by three members, and must be communicated to the members by the Secretary at least four weeks before the annual meeting At this meeting such a change may be established when accepted by a four-fifths vote of the members present

BY-LAWS

1 There must be in each year at least one meeting of the Council, which shall take place not later than the evening before the annual meeting

2 At the end of the first session of the annual meeting the Secretary shall read the report of the Council This report shall include (1) names of persons recommended for membership, (2) nominations for offices, (3) matters of general interest The Secretary shall exhibit in a conspicuous place the names of candidates for membership recommended by the Council, together with the evidence of the qualifications of the candidates

3 The election of officers and of new members, changes in the Constitution, etc, shall be voted upon at the end of the first session

4 Changes in the By-Laws may be determined by a majority vote of those present

5 In the year that a new Secretary-Treasurer is elected the incoming Council Member elected that year, or another member of the Council, shall become Assistant Secretary-Treasurer for the duration of the term of the Secretary-Treasurer

AMERICAN INSTITUTE OF NUTRITION

Founded April 11, 1933, Incorporated November 16, 1934

Member of Federation 1940

OFFICERS, 1949-1950

President—C G KING, Nutrition Foundation, Inc., New York City

Vice-President—W H GRIFFITH, University of Texas Medical School, Galveston

Secretary—J H ROE, George Washington University School of Medicine, Washington, D C

Treasurer—N R ELLIS, Bureau of Animal Industry, U S Department of Agriculture, Beltsville, Md

Councilors—A D HOLMES, E N TODHUNTER, A H SMITH, C G KING, W H GRIFFITH, J H ROE, N R ELLIS

Nominating Committee—ICIE MACY HOOBLER, *Chairman*, E W CRAMPTON, H J DEUEL, JR., L A MAYNARD, F J STARE

Committee on Rules for Awards—W C RUSSELL, *Chairman*, H E CARTER, R R SEALOCK, C D TOLLE, J WADDELL

Committee on Program Policy—W H GRIFFITH, *Chairman*, G R COWGILL, F S DAFT, R M LEVERTON, E W MCHENRY

Committee on Registry of Nutritional Pathology—R E JOHNSON *Chairman*, O A BESSEY, P L PHILLIPS, W H SEBRELL

Representatives on Committee on Biochemical Nomenclature (joint with American Society of Biological Chemists)—H J ALMQUIST, C A ELVEHJEM

PAST-OFFICERS

1933 L B MENDEL, President, H C SHERMAN, Vice-President, J R MURLIN, Secretary-Treasurer, E F DuBois, M S ROSE, Councilors 1934 J R MURLIN, President, E F DuBois, Vice-President, ICIE G MACY, Secretary, W M BOOTHBY, Treasurer, A H SMITH, AGNES FAY MORGAN, R M BETHKE, Councilors 1935 J R MURLIN, President, E F DuBois, Vice-President, ICIE G MACY, Secretary, G R COWGILL, Treasurer, A H SMITH, R M BETHKE, L A MAYNARD Councilors 1936 E F DuBois, President, MARY SWARTZ ROSE, Vice-President, G R COWGILL, Treasurer, ICIE G MACY, Secretary, R M BETHKE, L A MAYNARD, C A ELVEHJEM, Councilors 1937 MARY S ROSE, President, E V MCCOLLUM, Vice-President, G R COWGILL, Treasurer, ICIE G MACY, Secretary, L A MAYNARD, C A ELVEHJEM, P E HOWE, Councilors 1938 E V MCCOLLUM, President, T M CARPENTER, Vice-President, G R COWGILL, Treasurer,

L A MAYNARD, Secretary, C A ELVEHJEM, P E HOWE, HELEN S MITCHELL, Councilors 1939 H C SHERMAN, President, T M CARPENTER, Vice-President, G R COWGILL, Treasurer, L A MAYNARD, Secretary, P E HOWE, HELEN S MITCHELL, A H SMITH, Councilors 1940 THORNE M CARPENTER, President, A G HOGAN, Vice-President, L A MAYNARD, Secretary, W H SEBRELL, JR., Treasurer, HELEN S MITCHELL, ARTHUR H SMITH, LYDIA J ROBERTS, Councilors 1941 A G HOGAN, President, L A MAYNARD, Vice-President, ARTHUR H SMITH, Secretary, W H SEBRELL, JR., Treasurer, T H JUKES, LYDIA J ROBERTS, H B LEWIS, Councilors 1942 L A MAYNARD, President, H B LEWIS, Vice-President, ARTHUR H SMITH, Secretary, W H SEBRELL, JR., Treasurer, LYDIA J ROBERTS, GENEVIEVE STEARNS, T H JUKES, Councilors 1943 H B LEWIS, President, ICIE G MACY-HOGBLER, Vice-President, ARTHUR H SMITH, Secretary, LYDIA J ROBERTS, GENEVIEVE STEARNS, T H JUKES, Councilors 1944 ICIE G MACY-HOGBLER, President, WM C ROSE, Vice-President, ARTHUR H SMITH, Secretary, E M NELSON, Treasurer, GENEVIEVE STEARNS, T H JUKES and C A ELVEHJEM, Councilors 1945 WM C ROSE, President, ARTHUR H SMITH, Vice-President, H E CARTER, Secretary, E M NELSON, Treasurer, T H JUKES, C A ELVEHJEM, D W WOOLLEY, Councilors 1946 ARTHUR H SMITH, President, R M BETHKE, Vice-President, H E CARTER, Secretary, E M NELSON, Treasurer, C A ELVEHJEM, D W WOOLLEY, H J ALMQUIST, Councilors 1947 R M BETHKE, President, E M NELSON, Vice-President, H E CARTER, Secretary, N R ELLIS, Treasurer, D W WOOLLEY, H J ALMQUIST, A D HOLMES, Councilors 1948 E M NELSON, President, C G KING, Vice-President, J H ROE, Secretary, N R ELLIS, Treasurer, H J ALMQUIST, A D HOLMES, E N TODHUNTER, Councilors

CONSTITUTION

1 The name of the proposed society is the "AMERICAN INSTITUTE OF NUTRITION"

2 The purposes of the society are to further the extension of the knowledge of nutrition and to facilitate personal contact between investigators in nutrition and closely related fields of interest

3 The management of the American Institute of Nutrition shall be vested in a council consisting

of the President Vice-President, Secretary, Treasurer and three additional members

BY-LAWS

ARTICLE I—*Membership*

SECTION 1 *Eligibility for membership* Members Qualified investigators who have independently conducted and published meritorious original investigations in some phase of the chemistry or physiology of nutrition and who have shown a professional interest in nutrition for at least 5 years shall be eligible for membership in the Society

SEC 2 *Nomination* Nominations for membership shall be made and seconded by members of the Society on blanks furnished by the Secretary. Nominations shall be submitted to the Council who shall determine eligibility and make recommendation to the Society at a regular meeting

SEC 3 *Election to membership* A A nominee for membership may be voted for by ballot at any meeting of the Society after the Council has reported its findings on his eligibility B A majority of the ballots cast shall elect

SEC 4 *Forfeiture* If a majority of the Council after due notice to the member in question and opportunity for a hearing, shall decide that the interests of the Society require the expulsion of a member, the Secretary shall send a notice of this decision to each member at least two weeks before the next annual meeting At this meeting the Secretary shall, on behalf of the Council, propose the expulsion, and if two-thirds of the members present vote for it, the member shall be expelled, his assessment for the current year shall be returned to him, and he shall cease to be a member of the Society

ARTICLE II—*Meetings and Quorum*

SECTION 1 *Annual* The annual meeting of the Society shall be held on the date fixed by the Certificate of Incorporation

SEC 2 *Special* A special meeting may be called at any time by the President, or in case of his absence or disability, by the Vice-President, and must be called at the request in writing of a majority of the Council or fifty members of the Society Notice specifying the purpose of such meeting shall be mailed to each member at least ten days previous thereto The Council shall select the places at which meetings shall be held

SEC 3 *Quorum* Thirty members shall constitute a quorum at all meetings of the Society, but in the absence of a quorum any number shall be sufficient to adjourn to a fixed date

ARTICLE III—*Officials*

SECTION 1 *Officers* The officers shall be a President, and a Vice-President, who shall be elected

annually, and a Secretary and Treasurer, each of whom shall be elected to serve for a term of three years These officers shall be elected by the members of the Society Their terms of office shall commence on July 1 of the year in which they are elected

SEC 2 *Council* The officers so selected and three additional members, one of whom shall be elected at each annual meeting to serve a term of three years, shall constitute a Board of Trustees and shall be known as "The Council" (When this provision is first put into effect one member shall be elected for 1 year, one for 2 years and the third for 3 years)

SEC 3 *Duties of Officers* The powers and duties of the officers elected by the Society shall be such as usually devolve upon their respective positions

ARTICLE IV—*The Council*

SECTION 1 *Powers* The general management of the Society during the intervals between meetings shall be vested in the Council, which shall regularly perform the ordinary duties of an executive committee and possess all the powers conferred upon the Board of Trustees of an educational institution chartered by the Education Department of the University of the State of New York A permanent charter was issued to the American Institute of Nutrition under date of November 16, 1934

SEC 2 *Reports* The Council shall report to the Society its findings on the eligibility of candidates for membership, and on all charges of a violation of these By-Laws

ARTICLE V—*Nominating Committee*

SECTION 1 *Membership* A The Nominating Committee shall consist of five members appointed for the coming year by the retiring President Members who have served on the Nominating Committee for two consecutive years shall be ineligible for reappointment until after a lapse of one year B The President shall designate one member to be Chairman of the Nominating Committee

SEC 2 *Nomination of Officials* A The Nominating Committee shall make at least one nomination for each of the four offices, for each of the additional positions on the Council to be filled by vote of the members and for each of the positions on the Editorial Board to be vacated at the time of the annual meeting Any member of the Institute may submit nominations to the Nominating Committee for its consideration along with those nominations made by the members of the Nominating Committee B The nominations by the Nominating Committee shall be transmitted to the Secretary at least six weeks before the annual meeting at which they are to be considered C The Secretary shall send to every member, at least two weeks before

the annual meeting, a printed ballot containing the list of nominees and space for such additional names as the member wishes to propose, and at the same time shall notify the members that they may vote by mail, returning to the Secretary the marked ballot in the envelope provided, at such a time and place as the Secretary may designate, or the ballot may be delivered to the Secretary at the beginning of the business session at which the elections are to take place

SEC 3 *Election of Officials* A At the beginning of the business session the Secretary shall present to the tellers, appointed by the President, the ballots submitted by the members and the ballots shall be counted forthwith B A majority of votes cast shall be necessary to elect an official

SEC 4 *Filling of Vacancies* A The Nominating Committee shall fill all vacancies in elective positions except such as may occur at a meeting of the Society B The President of the Society shall fill all vacancies in appointive positions

ARTICLE VI—*Financial*

SECTION 1 *Dues* The dues shall be the annual cost of subscription to *The Journal of Nutrition* for members plus an annual assessment which shall be determined by majority vote at the annual meetings, upon recommendation of the Council, and shall be due within a month after the annual meeting A member on attaining the age of 65 may elect to be relieved from all financial obligations to the Institute including subscription to *The Journal of Nutrition*

SEC 2 *Expenditures* No expenditures from the general funds of the Society except those required in the performance of the ordinary official duties shall be made except by vote of the Society or the Council

SEC 3 *Penalty for non-payment of dues* A Members in arrears for dues for two consecutive years shall forfeit their membership B Delinquent members may be reinstated by the Council provided all indebtedness to the Society is liquidated

ARTICLE VII—*The Journal of Nutrition*

SECTION 1 The American Institute of Nutrition designates *The Journal of Nutrition* as its official organ of publication

SEC 2 In accordance with the expressed wish of the Wistar Institute of Anatomy and Biology, owner and publisher of *The Journal of Nutrition*, the American Institute of Nutrition shall nominate members of the Editorial Board for its official organ A The editorial management of *The Journal of Nutrition* shall be vested in an Editorial Board consisting of an Editor and twelve Board Members B The Editor shall be chosen by the Editorial Board to serve a term of five years beginning July 1 of the year in which he is chosen, and shall be eligible for reelection The Editor shall have the power to designate one of the Board Members to serve as his assistant, and such an appointee shall be called Associate Editor C Three members of the Institute shall be nominated by the Nominating Committee for membership on the Editorial Board each year to serve a term of four years, replacing three retiring members and taking office May 1 of the year in which they are elected In the event of a vacancy in the membership of the Editorial Board occurring through death or other reason, the Nominating Committee, for each such vacancy to be filled shall make an additional nomination In this event the nominees elected who receive the greatest number of votes shall serve the longest term of vacancies to be filled D Retiring members of the Editorial Board shall not be eligible for renomination until one year after their retirement

ARTICLE VIII—*Papers on Scientific Subjects*

SECTION 1 The Secretary shall be authorized to arrange programs for the scientific sessions at the annual meetings

ARTICLE IX—*Changes in Constitution and By-Laws*

SECTION 1 Proposed changes in the Constitution and By-Laws must be sent in writing to the Secretary at least one month before the date of the meeting at which they are to be considered, and must be signed by at least three members The Secretary shall send a printed copy of any proposed change to each member at least two weeks before the next meeting and shall notify all members that they may vote by proxy

SEC 2 If at this meeting two-thirds of the votes cast shall favor the proposed change, it shall be made

THE AMERICAN ASSOCIATION OF IMMUNOLOGISTS

Founded June 19, 1913, Member of Federation 1942

OFFICERS, 1949-1950

President—THOMAS FRANCIS, JR., School of Public Health, Ann Arbor, Mich

Honorary President—ARTHUR F COCA, Oradell, N J

Vice President—MICHAEL HEIDELBERGER, College of Physicians and Surgeons, New York City

Secretary Treasurer—JULIUS FREUND, Public Health Research Institute, New York City

Council—JACQUES J BRONFENBRENNER, GEORGE EDSELL, COLIN MACLEOD, KARL F MEYER, SANFORD B HOOKER, *ex officio*, THOMAS FRANCIS, JR., MICHAEL HEIDELBERGER, JULIUS FREUND

PAST OFFICERS

Presidents—1913 GERALD B WEBB 1915 JAMES W LOBING 1916 RICHARD WILL 1917 JOHN A KOELMER 1918 WILLIAM H PARK 1919 HANS ZINSSER 1920 RUFUS I COLE 1921 FREDERICK P GAY 1922 GEORGE W MCCOY 1923 HIRSH GIDION WEISS 1924 FREDERICK G NOVY 1925 WILFRIED H MANWARING 1926 LUDVIG HERTZEN 1927 KARL LANDSTAMMER 1928 EUGENE L OPIE 1929 OSWALD T AVERY 1930 STANHOPE BAYNE JONES 1931 ALPHONSE R DOCHET 1932 AUGUSTUS B WADSWORTH 1933 THOMAS M RIVERS 1934 FRANCIS G BLAKE 1935 WARFIELD T LONGCOPE 1936 SANFORD B HOOKER 1937 CARL TINBROECK 1938 DONALD T FRASER 1939 GEORGE P BLISS 1940 PAUL R CANNON 1941 KARL F MEYER 1942-1945 JACQUES J BRONFENBRENNER 1945-1947 MICHAEL HEIDELBERGER 1947 LLOYD D FELTON 1948 MICHAEL HEIDELBERGER

Vice-Presidents—1913-1915 GEORGE W ROSS 1915 GEORGE P SANBORN 1916 JOHN A KOELMER 1947 MICHAEL HEIDELBERGER 1948 LLOYD D FELTON

Secretary—1913-1918 MARTIN J SYNOT

Treasurer—1913-1918 WILLARD I STONE

Secretary - Treasurer—1918-1947 ARTHUR F COCA 1948 JULIUS FREUND

CONSTITUTION

(As revised, 1949)

ARTICLE I—Name

This association shall be called THE AMERICAN ASSOCIATION OF IMMUNOLOGISTS

ARTICLE II—Object

The purpose of the Association shall be to advance knowledge of immunology and related disci-

plines, and to facilitate interchange of ideas and information among investigators in the various fields

ARTICLE III—Members

SECTION 1 The Association shall consist of active members, members emeriti and honorary members

SECTION 2 Any qualified person engaged in the study of problems related to the purpose of the Association may apply for active membership. Candidates for active membership shall be nominated by two members of the Association on blanks furnished by the Secretary. Applications must be accompanied by letters of recommendation of the sponsors, a curriculum vitae, and a list of reprints of publications. The Council shall determine eligibility and post a list of candidates at the annual meeting. The membership shall elect new members by majority vote.

SECTION 3 Failure to pay dues for three successive years shall annul membership. The Council may reinstate a member if an acceptable explanation is submitted and all indebtedness to the Association is liquidated. Payment of such indebtedness may be waived by unanimous vote of the Council if circumstances justify such action.

SECTION 4 If a two thirds majority of the Council decides that the best interests of the Association require the expulsion of a member, the Secretary shall notify the affected member in writing of the charges. The Council shall allow a reasonable time for the presentation of his defense before acting. Upon recommendation of a two thirds majority of the Council, the Secretary shall send a notice of the decision to each active member at least six weeks before the next annual meeting. At this meeting the Secretary shall, on behalf of the Council, propose the expulsion, and on a two thirds vote of the members present, the member shall be expelled, his assessment for the current year shall be returned, and he shall cease to be a member of the Association.

SECTION 5 An active member of the Association for twenty years upon retirement because of age or disability, may elect to accept the status of member emeritus. As such he shall retain voting privileges but shall be relieved of all financial obligations to the Association and shall not receive the Journal without payment.

SECTION 6 The Council may nominate for honorary membership persons of extraordinary achievement in the field of endeavor of this Association. Election to honorary membership shall follow the same procedure as that for election for

office in the Association, and the Secretary, on order of the Council, shall place nominations for honorary membership on the annual ballot

ARTICLE IV—*Officers*

SECTION 1 The Association shall be governed by a Council, which shall consist of the Officers of the Association and five Councilors, one of whom shall be the Editor-in-Chief of the Journal of Immunology

SECTION 2 The Officers of the Association shall be a President, a Vice President, a Secretary, and a Treasurer. The duties of the two latter may be performed by one or two individuals as determined by vote of the Association (See By-Law # 7)

SECTION 3 The President, the Vice President, the Secretary, and the Treasurer shall be elected at the regular annual meeting of the Association to serve for one year. Only the Secretary and the Treasurer are eligible to serve for successive terms in their respective offices. The term of office shall begin the day after the end of the annual meeting.

SECTION 4 One Councilor shall be elected each year to serve for a term of four years. He may however serve in any other elective office immediately after expiration of his term as Councilor.

SECTION 5 The President shall appoint a Nominating Committee of three (or more) members not currently serving on the Council of the Association and shall designate the Chairman. The Committee shall submit nominations for the offices of President, Vice President, Secretary, Treasurer, and Councilor. They may nominate the member currently serving as Vice President for the office of President, and the Councilor who is serving his fourth year for the office of Vice President. They shall submit the names of three members of the Association as candidates for the office of Councilor for a four-year term. The Nominating Committee shall confer at least three months before the annual meeting and shall transmit its nominations, over the signatures of the majority of the Committee, to the Secretary at least two months before the annual meeting. Other names may be added to the ballot upon petition of at least twenty-five members of the Association to the Secretary at least two months before the annual meeting. The Secretary shall send to each member of the Association, at least six weeks before the annual meeting, a ballot containing the list of all such nominees.

SECTION 6 The members of the Association shall vote on the nominations by secret ballot by mail. All ballots must be in the hands of the Secretary one week before the annual meeting. At the time of the annual meeting the Secretary shall present all the valid sealed ballots received by him to the tellers appointed by the President. A plurality of votes shall be sufficient for election.

SECTION 7 The Vice President shall substitute

for the President when necessary. If both the President and Vice President are unable to serve, the senior Councilor shall temporarily assume the duties of President. Should a vacancy occur in the office of Secretary or Treasurer, or Councilor, the Council by a majority vote shall appoint a member to fill the unexpired term until the following election. In the event of a vacancy in the Council, the members shall in the following election choose two Councilors from among six nominees whose names shall be selected by the Nominating Committee and included on the ballot as above. Moreover, in this event the terms of the four Councilors shall be adjusted so as to provide an orderly progression within the general sense of the Constitution.

ARTICLE V—*Meetings*

SECTION 1 A regular meeting of the Association shall be held annually at such time and place as the Council shall determine. Regular meetings shall be open to all members of the Association. The members present at the annual meeting of the Association shall constitute a quorum.

SECTION 2 A meeting of the Council shall be held prior to the annual business meeting of the Association. A quorum of the Council for this meeting shall be five.

SECTION 3 In case of equal division of votes, the President shall cast the decisive ballot.

SECTION 4 Minutes of the annual meeting of the Council and of the annual business meeting of the Association shall be published in the form of a news-letter and be distributed only to members of the Association. The annual report of the Treasurer shall be included in this news-letter.

ARTICLE VI—*Business*

SECTION 1 The fiscal year of the Association shall begin March first.

SECTION 2 Annual dues, upon recommendation of the Council, shall be determined by a majority vote at the annual meeting of the Association.

ARTICLE VII—*Publication*

SECTION 1 The Journal of Immunology, which is the property and official organ of this Association, shall be administered for the Association by an Editorial Board.

SECTION 2 An Editor-in-Chief shall be elected from the membership by a majority vote of the Council to serve for four years. His term of office shall be subject to renewal. He shall appoint with the consent of the Council an Editorial Board of four to six members to serve for four years. The Editor-in-Chief and the members of the Editorial Board shall be responsible for the editorial conduct of the Journal.

SECTION 3 The Editor-in-Chief shall, with the consent of the Council, appoint from the active

members Associate Editors as needed These Associate Editors are to be chosen because of their ability in specialized fields

SECTION 4 The Editor-in-Chief shall make a report of his stewardship of the Journal at the annual meeting and in this report shall summarize the editorial situation and all matters dealing with finances He shall submit to the Council statements relating to the number of manuscripts received, rejected, accepted, and published during the year, changes in editorial personnel of editors, a complete summary of circulation and of finances and of any other information which the Editorial Board may feel to be pertinent or which may be required by the Council

ARTICLE VIII—*Amendments*

SECTION 1 Proposed changes in the Constitution shall be submitted by at least five members in writing through the Secretary to the President The President shall then appoint a committee of at least three members which shall communicate its recommendations to the President for consideration by the Council The Council shall then advise the Secretary to submit the recommendations of the committee to the membership of the Association for approval with the annual ballot A change in the Constitution shall require a two-thirds majority of the votes cast by the members at the annual meeting and shall require confirmation by a majority in a mail vote in which at least 50% shall have participated

SECTION 2 As an alternative procedure a proposed change in the Constitution may be submitted directly to the Council by petition of one-tenth of the total active membership Under this circumstance the Council is required to arrange for a vote by the members as heretofore described

SECTION 3 Proposed changes in By-Laws may be adopted by two-thirds vote of members present at the annual meeting

BY-LAWS

1 The Past Presidents shall have the right of attending, without vote, the meetings of the Council

2 The President may appoint a Past President

or a Past Councilor as pro tempore Councilor at any stated meeting of the Council at which a quorum is not present

3 The Council may transact and vote by mail on such business as cannot be conveniently transacted at meetings

4 The Secretary shall arrange the program for the scientific meetings, with the advice of the other officers of the Association Any member in good standing shall have the right to submit a paper The privilege of presentation may be extended to non-members upon recommendation of active members Papers intended for presentation at the meetings shall conform to the standards of the Journal of Immunology In case of doubt, the Secretary shall have the right to submit papers to the scrutiny of three or more members of the Editorial Board of the Journal of Immunology whose decision shall be final

5 Each member who signifies his intention of reading a paper at any session may specify the length of time which its presentation shall require In the absence of any specification of time required not more than ten minutes shall be allotted for the reading of any one paper The time allotted shall be printed on the official program, and the presiding officer shall have no authority to extend it unless a majority of the members present signify their wish to the contrary

6 If by force of circumstances it should be impossible to hold the annual meeting, the election of Officers and Council may be carried out entirely by mail

7 The offices of Secretary and Treasurer are combined and shall be held by one person

8 Official or invited addresses presented at the annual meeting shall be given immediate priority in publication, unless the Editor-in-Chief shall be otherwise instructed by majority vote of the Council

9 Dues are payable on or before January 1st and subscriptions to the Journal will be lapsed if not paid by this date

10 If the Editor-in-Chief is unable to attend the stated meeting of the Council, he may designate an alternate from the membership of the Editorial Board

ALPHABETICAL LIST OF MEMBERS OF THE SIX SOCIETIES

- (1) The American Physiological Society
- (2) American Society of Biological Chemists
- (3) The American Society for Pharmacology and Experimental Therapeutics
- (4) The American Society for Experimental Pathology
- (5) The American Institute of Nutrition
- (6) The American Association of Immunologists

Number and year in parentheses following each name indicate Society affiliation and year of election

HONORARY MEMBERS

- Adrian, E D** Cambridge University, Dept of Physiology, Cambridge, England (1, 1946)
- Castaneda, M Ruiz, M D** Investigaciones Medicas, Hospital General, Mexico, D F *Director, Department of Medical Research* (6, 1942)
- Chopra, R N, M D, Sc D** School of Tropical Medicine, Calcutta, India *Director, Professor of Pharmacology* (3, 1938)
- Coca, Arthur F, A M, M D** Pearl River, N Y (6, 1916)
- Dale, H H** The Wellcome Trustees, Dilke House, Malet St, London, W C, England (3, 1926)
- Hektoen, Ludvig, M D** 629 S Wood St, Chicago, Ill *President, Chicago Tumor Institute* (6, 1919)
- Hitchens, Arthur P, M D** Public Building, Wilmington 33, Del *Health Commissioner, Wilmington* (6, 1913)
- Houssay, Bernardo A, M D** Viamonte 2790, Buenos Aires, Argentina *Director and Professor of Physiology* (1, 1942)
- Huntoon, F M, M D** Woodbridge, Conn (6, 1918)
- Lapicque, L** The Sorbonne, Laboratory of Physiology, Paris, France (1, 1946)
- Loewi, Otto, M D** New York University College of Medicine, 477 First Ave, New York City *Research Professor in Pharmacology* (3, 1941)
- McCoy, George Walter, M D** Louisiana State University Medical School, New Orleans *Director, Department of Public Health* (6, 1916)
- Novy, Frederick G, M D, Sc D** 721 Forest Ave, Ann Arbor, Mich *Dean Emeritus and Professor Emeritus of Bacteriology, University of Michigan Medical School* (6, 1920)
- Orbeli, L A** Academy of Sciences of the USSR, Moscow, USSR (1, 1946)
- Sherrington, Sir Charles S, Sc D, M D** "Broomside," Valley Road, Ipswich, England, *Former Waynefleete Professor of Physiology, Oxford University Former President of the Royal Society* (1, 1904)
- Sordelli, A** Department of Public Health, Institute of Bacteriology, Buenos Aires, Argentina *Director* (6, 1942)

RETIRED MEMBERS

- Addison, William H, M D** Univ of Pennsylvania, Philadelphia *Prof of Histology and Embryology* (1, 1928)
- Allen, William F, Ph D** Univ of Oregon Med School, Portland *Prof Emeritus of Anatomy* (1, 1929)
- Alvarez, Walter C, M D** Mayo Clinic, Rochester, Minn *Prof of Medicine, Mayo Foundation* (1, 1917, 3, 1921)
- Babkin, B P, M D, D Sc** McGill Univ, Montreal, Quebec, Canada *Prof of Physiology* (1, 1924)
- Bachmann, George, M D** Emory Univ School of Medicine, Emory University, Ga *Prof Emeritus of Physiology* (1, 1912)
- Benedict, Francis G, Ph D, M D** Machiasport, Maine (1, 1904, 2, 1906)
- Brown, Edgar D, Pharm D, M D** Paynesville, Minn *Assoc Prof Emeritus of Pharmacology* (1, 1907, 3, 1909)
- Burton-Opitz, Russell, Ph D** 218 Bridle Way, Palisades, N J *Attending Cardiologist, Lenox Hill Hospital, Attending Physician, Cumberland Hospital, Consulting Cardiologist, Englewood, North Hudson, Holy Name, and Hackensack Hospitals* (1, 1902, 3, 1919)
- Campbell, H Louise, Ph D** 900 Windsor Ave, Windsor, Conn (5, 1933)
- Chambers, Robert, Ph D** Marine Biological Lab, Woods Hole, Mass *Dir of Lab of Cellular Physiology, Research Prof Emeritus, N Y U* (1, 1932)
- Child, Charles M, Ph D** Stanford Univ, Stanford, Calif *Prof Emeritus, of Zoology Univ of Chicago* (1, 1923)
- Clark, Elliot R, M D** Univ of Pennsylvania, Philadelphia *Prof and Head of Dept of Anatomy* (1, 1919)
- Coca, Arthur F, M D** Pearl River, N Y *Med Dir* (4, 1924, 6, 1916)
- Cole, Rufus, M D, D Sc** Mount Kisco, N Y *Member Emeritus, Rockefeller Inst for Med Research* (6, 1917)
- Culler, Elmer A, Ph D** Univ of Rochester, Roch-

- ester, N Y *Prof of Psychology and Dir of Lab* (1, 1936)
- Dawson, Percy M, M D 665 E Maryland Ave, Claremont, Calif (1, 1900)
- Dooley, M S, M D Syracuse Univ College of Medicine Syracuse, N Y *Prof of Pharmacology* (3, 1923)
- Dochez, A Raymond, M D Presbyterian Hospital, 620 W 168th Street, N Y C *John E Borne Prof of Medicine and Surgical Research, Columbia Univ* (4, 1917, 6, 1922)
- Durrant, Edwin P, Ph D Ohio State Univ, Columbus *Assoc Prof Emeritus of Physiology* (1, 1928)
- Erlanger, Joseph, M D, Sc D Washington Univ School of Medicine, St Louis, Mo *Prof Emeritus of Physiology* (1, 1901)
- Famulener, Lemuel W, Ph D, M D 275 Engle St, Englewood, N J (6, 1920)
- Fitzgerald, Mabel P, 54 A George Sq, Edinburgh, Scotland (1, 1913)
- Forbes, Henry S M D Harvard Med School, Boston, Mass *Assoc in Neuropathology* (1, 1931)
- Githens, Thomas S, M D The Cambridge-Alden Park, Wissahickon and School Lane, Germantown, Philadelphia, Pa (1, 1915)
- Glaser, O C, Ph D Amherst College, Amherst, Mass *Prof Emeritus of Biology* (1, 1913)
- Hadley, Philip B, Ph D Western Pennsylvania Hospital, Inst of Pathology, Pittsburgh *Chief of Bacteriological Service and Research Bacteriologist* (4, 1927)
- Hale, Worth, M D Antrim, N H (1, 1908, 3, 1908)
- Halsey, John T, M D P O Box 264, Waveland, Miss *Prof Emeritus of Pharmacology Tulane Univ* (3, 1929)
- Herrick, C Judson, Ph D Univ of Chicago, Chicago, Ill *Prof Emeritus of Neurology* (1, 1907)
- Hober, Rudolf Univ of Pennsylvania Med School, Philadelphia *Visiting Prof of Physiology* (1, 1936)
- Hodgkins, R G, Ph D, M D 86 Varick Rd, Waban 68, Mass (1, 1911)
- Jackson, Dennis Emerson, Ph D, M D 114 Louis Ave, Cincinnati 20, Ohio (1, 1910, 3, 1912)
- Jobbing, James W, M D Columbia Univ, 630 W 168th St, New York City *Prof Emeritus of Pathology* (4, 1913)
- King, Jesse, Ph D Towson, Md (1, 1914)
- Knowlton, Frank P, M D Syracuse Univ College of Medicine, Syracuse, N Y *Prof Emeritus of Physiology* (1, 1911)
- Kyes, Preston, Sc D M D North Jan, Maine *Prof Emeritus* (6, 1918)
- Laurens, Henry D, Ph D, M D The Rockefeller Inst, New York City *Associate* (1, 1913)
- Lewis, Warren H, M D Wistar Inst of Anatomy and Biology, Philadelphia, Pa *Member* (1, 1919)
- Loebel, Robert O, M D 205 East 78th St, New York City (1, 1928)
- Mackenzie, George M, M D Mary Imogene Bassett Hospital, Cooperstown, N Y *Physician-in-Chief, Dir, Otsego County Lab* (6, 1912)
- MacPhillamy, Betty B, Ph D 145 Greene Ave, Madison, N J *Bacteriologist* (6, 1944)
- Moulton, C Robert, Ph D 5602 Dorchester Ave, Chicago, Ill (5, 1933)
- Parker, George H, Sc D 16 Berkeley St, Cambridge, Mass *Prof Emeritus of Zoology, Harvard Univ* (1, 1909)
- Pilcher, J Douglas, M D City Hospital, Scranton Rd, Cleveland, Ohio *Assoc Prof of Pediatrics, Western Reserve Univ School of Medicine* (3, 1911)
- Pohlman, Augustus G, M D Univ of Southern California, Los Angeles *Assoc Prof, Dept of Otolaryngology* (1, 1934)
- Pratt, Frederick H, M D Boston Univ School of Medicine, Boston, Mass *Prof Emeritus of Physiology* (1, 1919)
- Quinby, William C, M D Harvard Univ Med School, Boston, Mass *Clin Prof of Genito urinary Surgery* (1, 1916)
- Riddle, Oscar, Ph D Cold Spring Harbor, L I, N Y *Visiting Prof from the U S (in South America)* (1, 1919)
- Robertson, Oswald H, M D Univ of Chicago, Chicago, Ill *Prof of Medicine* (4, 1932)
- Rogers, Charles G, Ph D Oberlin College, Oberlin, Ohio *Prof of Comparative Physiology* (1, 1911)
- Roth, George B, M D 3814 T St N W, Washington D C *Prof Emeritus of Pharmacology, George Washington Univ* (1, 1914, 3, 1911)
- Sabin, Florence R, M D, Sc D 1333 E 10th Ave, Denver 3, Colo *Member Emeritus, Rockefeller Inst* (1, 1923)
- Sacks, Ernest, M D 97 Arundel Pl, St Louis, Mo *Prof Emeritus of Clin Neurological Surgery, Washington Univ Med School* (1, 1910)
- Sappington, Samuel W, M D, D Sc P O Box 528 Bryn Mawr, Pa *Prof of Pathology, Hahnemann Hospital* (6, 1913)
- Scamman, Richard E, Ph D 172 S E Bedford St, Minneapolis, Minn *Distinguished Service Prof in Grad School* (1, 1923)
- Schultz, W H, Ph D 3102 18th St, N W, Washington, D C *Prof Emeritus of Pharmacology, Univ of Maryland* (1, 1907, 3, 1909)
- Slonaker, James R, Ph D 334 Kingsley Ave, Palo Alto, Calif *Prof of Physiology, Leland Stanford Jr Univ* (1, 1917)
- Smith, Sybil L, A M 1421 44th St, N W, Washington, D C (5, 1940)

- Snyder, Charles D , Ph D 4709 Keswick Rd , Baltimore, Md *Prof Emeritus of Exper Physiology, Johns Hopkins Univ* (1, 1907)
- Sweet, J E , M D , Sc D Unadilla N Y *Prof Emeritus of Surgical Research, Cornell Univ Med College* (1, 1913)
- Walker, Ernest Linwood, Sc D 50 Winchester St , San Francisco, Calif (3, 1931)
- Weed, Lewis H , M D , Ph D Natl Research Council, 2101 Constitution Ave Washington, D C (1, 1919)
- Wood, Horatio C , Jr , M D , Ph M 319 S 41st St , Philadelphia 4, Pa *Prof of Pharmacology and Therapeutics, Univ of Pennsylvania, Prof of Materia Medica, Philadelphia College of Pharmacy and Science* (3, 1908)
- Wulzen, Rosalind, Ph D Oregon State College, Corvallis *Asst Prof of Zoology* (1, 1916)
- Yerkes, Robert M , Ph D Yale Labs of Primate Biology, New Haven Conn *Prof Emeritus of Psychobiology, Yale Univ* (1, 1904)

MEMBERS

- Abbot, Lynn D F , Ph D Med College of Virginia, Richmond 19 *Assoc Prof of Biochemistry* (2, 1948)
- Abramson, David I , M D Univ of Illinois, Dept of Medicine, Chicago 12 *Asst Clin Prof, Attending Physician, Hines Veterans Hospital* (1, 1937)
- Abramson, Harold A , M D 133 E 58th St , New York City *Asst Prof of Physiology, Columbia Univ College of Physicians and Surgeons* (1, 1930, 2, 1934)
- Abreu, Benedict E , Ph D , M D Pitman-Moore Co , Indianapolis, Ind *Pharmacologist, Research Dept* (3, 1941)
- Acheson, George H , M D Univ of Cincinnati College of Medicine, Cincinnati, Ohio *Prof of Pharmacology* (1, 1942, 3, 1945)
- Adams, Georgian, M A , D Sc U S Dept of Agriculture, Washington 25, D C *Sr Exper Station Admin* (5, 1946)
- Adams, John M , M D Univ of Minnesota, Dept of Pediatrics, W 205 Univ Hospital, Minneapolis *Assoc Prof of Pediatrics* (4, 1947)
- Adams, Mildred, Ph D Takamine Lab , Clifton, N J *Research Chemist* (2, 1934)
- Adams, R Charles, M S , M D Mayo Clinic, Rochester, Minn *Instr in Anesthesia, Mayo Foundation, Member of Mayo Clinic Staff, Section on Anesthesia* (3, 1942)
- Adams, W Lloyd, M D , Ph D U S Public Health Service Hosp , Lexington, Ky *Chief of E E N T Service and Personnel Physician* (3, 1942)
- Adams, Wright R , M D Univ of Chicago, Dept of Medicine, Chicago 37, Ill *Assoc Prof of Medicine* (1, 1946)
- Ades, Harlow Whiting, Ph D Box 731, Emory Univ , Ga (1, 1945)
- Adler, Harry F , Ph D , M D School of Aviation Medicine, Randolph AFB , Tex *Dir Med Sciences Div* (1, 1943)
- Adolph, Edward Frederick, Ph D Univ of Rochester School of Medicine and Dentistry, Rochester, N Y *Prof of Physiology* (1, 1921)
- Adolph, William H , Ph D Peiping Union Med College, Peiping, China *Prof of Biochemistry* (2, 1946, 5, 1934)
- Ahlquist, Raymond P , Ph D Univ of Georgia School of Medicine, Dept of Pharmacology, Augusta *Prof and Chairman of Dept of Pharmacology* (3, 1945)
- Albanese, Anthony A , Ph D St Luke's Convalescent Hospital, Nutrition Research Lab , King St , Greenwich, Conn *Chief of Nutritional Research* (2, 1944)
- Albaum, Harry G , Ph D Brooklyn College, Bedford Ave and Ave H, Brooklyn, N Y *Asst Prof of Biology* (2, 1947)
- Albert, A , Ph D , M D Mayo Foundation, Rochester, Minn *Research Assoc* (1, 1947)
- Albritton, Errett C , M D George Washington Univ Med School, 1339 H St , N W , Washington, D C *Prof and Head of Dept of Physiology* (1, 1933)
- Alden, Roland H , Ph D Univ of Tennessee, Div of Anatomy, Memphis 3 *Assoc Prof* 1, 1949)
- Alexander, Robert S , Ph D Western Reserve Univ School of Medicine, 2109 Adelbert Rd , Cleveland, Ohio *Instr in Physiology* (1, 1946)
- Algire, Glenn H , M D Natl Cancer Inst , Bethesda, Md *Sr Surgeon, USPHS* (4, 1945)
- Allan, Frank N , M D Lahey Clinic, 605 Commonwealth Ave , Boston, Mass *Exec Dir of the Med Dept* (4, 1930)
- Allen, Charles Robert, Ph D , M D Univ of Texas School of Medicine, Galveston *Assoc Prof of Anesthesiology* (1, 1943)
- Allen, Frank W , Ph D Univ of California, 1557 Life Science Building, Berkeley *Assoc Prof* (2, 1947)
- Allen, Frederick M , M D 1031 Fifth Ave , New York City *Prof of Medicine, Polyclinic Med School and Hospital* (1R, 1924, 4, prior to 1920)
- Allen, J Garrott, M D Univ of Chicago, Univ Clinics, Chicago, Ill *Instr in Surgery* (1, 1943)
- Allen, Lane, Ph D , M D Univ of Georgia School of Medicine, Univ Place, Augusta *Assoc Prof of Anatomy* (1, 1939)
- Allen, Shannon C , Ph D Cornell Univ Med School, Depts of Physiology and Pharmacology, 1300 York Ave , New York City *Research Assoc and Dir of Aviation Research Unit* (1, 1945)

- Allen, Thomas H , Ph D Columbia Univ College of Physicians and Surgeons, Dept of Physiology, 630 W 168th St, New York City 32 *Asst Prof of Physiology* (1, 1947)
- Allen, Willard M , M D Washington Univ School of Medicine, 630 S Kingshighway, St Louis, Mo *Prof of Obstetrics and Gynecology* (1, 1934)
- Alles, Gordon A , Ph D 770 S Arroyo Parkway, Pasadena, Calif *Lecturer in Pharmacology, Univ of California Med School, and Research Assoc in Biology, California Inst of Technology* (1, 1932, 3, 1941)
- Alling, Eric L , M D Univ of Rochester School of Medicine and Dentistry, Rochester 7, N Y *Assoc in Radiology* (4, 1947)
- Allison, James B , Ph D Rutgers Univ , New Brunswick, N J *Dir of Bureau of Biological Research, Prof of Physiology and Biochemistry* (2, 1946, 5, 1949)
- Almquist, Herman J , Ph D The Grange Co , Modesto, Calif *Dir of Research and Vice-Pres* (2, 1937, 5, 1937)
- Altschul, Aaron M , Ph D Southern Regional Research Lab , 2100 R E Lee Blvd , New Orleans, 19, La *Biochemist, U S Dept of Agriculture* (2, 1949)
- Altire-Werber, Erna Jewish Hospital of Brooklyn, Brooklyn, N Y *Dir of Research Lab* (6, 1948)
- Alving, Alf Sven, M D Billings Hospital, Univ of Chicago, 950 E 59th St , Chicago, Ill *Assoc Prof of Medicine* (1, 1939)
- Amberg, Samuel, M D Mayo Clinic, Rochester, Minn *Assoc in Pediatrics, Assoc Prof of Pediatrics, Mayo Foundation* (1R, 1903, 2, 1906, 3R, 1909)
- Amberson, William R , Ph D Univ of Maryland School of Medicine, Baltimore *Prof of Physiology* (1, 1924)
- Ambrose, Anthony M , Ph D Western Regional Research Lab , 800 Buchanan St , Albany, Calif *Pharmacologist, U S Dept of Agriculture, Bureau of Agricultural Chemistry and Engineering* (3, 1937)
- Ames, Stanley R , Ph D Distillation Products, Inc , 755 Ridge Road W , Rochester 13, N Y *Sr Research Chemist* (2, 1948)
- Amoss, Harold L , M D , D P H , Sc D 68 Deerfield Drive, Greenwich, Conn (4, 1922, 6, 1917)
- Andersch, Marie A , Ph D Univ Hospital, Baltimore, Md *Biochemist, Instr in Medicine, Univ of Maryland* (2, 1940)
- Andersen, Dorothy H , M D Babies Hospital, Broadway and 167th St , New York City *Asst Prof of Pathology, Columbia Univ* (4, 1935)
- Anderson, Evelyn, M D Ph D Natl Insts of Health, Bethesda 14, Md (1, 1934)
- Anderson, Hamilton H , M S , M D Pharmacology Lab , Univ of California Med School, San Francisco *Prof of Pharmacology* (3, 1931)
- Anderson, Oscar Daniel, Ph D Cornell Univ , Dept of Psychology, Ithaca, N Y (1, 1939)
- Anderson, Rubert S , Ph D Univ of South Dakota School of Medicine, Vermillion *Prof of Physiology* (1, 1948)
- Anderson, Rudolph J , Ph D Sterling Lab , Yale Univ , New Haven, Conn *Prof of Chemistry* (2, 1915)
- Anderson, W A D , M A , M D Marquette Univ School of Medicine, Milwaukee, Wis *Prof of Pathology and Bacteriology* (4, 1941)
- Anderson, William E , M A Eastern State Farmers' Exchange, Westbrook Farm, Rockville, Conn *Biochemist* (2, 1931, 5, 1933)
- Andervont, H B , Sc D Natl Cancer Inst , Bethesda, Md *Biologist, USPHS* (4, 1939)
- Andrews, James C , Ph D Univ of North Carolina, Chapel Hill *Prof of Biological Chemistry and Nutrition* (2, 1925)
- Andrus, E Cowles, M D 24 E Eager St , Baltimore 2, Md *Asst Visiting Physician, Assoc Prof of Medicine, Johns Hopkins Univ* (1, 1925)
- Anfinsen, Christian B , Jr , Ph D Harvard Univ Med School, 25 Shattuck St , Boston 15, Mass *Asst Prof , Div of Biophysics* (2, 1946)
- Angerer, Clifford, Ph D Ohio State Univ , Columbus *Assoc Prof of Physiology* (1, 1943)
- Angevine, D Murray, M D Univ of Wisconsin Med School, Madison *Prof of Pathology* (4, 1940)
- Anker, H S , M D , Ph D Univ of Chicago, Dept of Biochemistry, 947 E 58th St , Chicago 37, Ill *Research Assoc and Asst Prof* (2, 1949)
- Annegers, John H , Ph D , M D Northwestern Univ Med School, Dept of Physiology, 313 E Chicago Ave , Chicago 11, Ill *Asst Prof of Physiology* (1, 1949)
- Ansbacher, Stefan, D Sc 17 Loel Court, Rockville Center, N Y (2, 1939)
- Anslow, W Parker, Jr , Ph D New York Univ College of Medicine, 477 First Ave , New York City 16 *Asst Prof of Physiology* (2, 1948)
- Anson, Mortimer L , Ph D Continental Foods, Inc , Hoboken, N J *Dir of Chemical Research* (2, 1937)
- Apperly, Frank L , M A , D Sc , M D Med College of Virginia, Richmond *Prof of Pathology* (4, 1936)
- Archibald, Reginald M , Ph D , M D Rockefeller Inst for Med Research, 66th St and York Ave , New York City 21 *Member, Physician to Hospital* (2, 1947)
- Arkin, Aaron, M D , Ph D Suite 2006, 25 E Washington St , Chicago, Ill *Rush Prof of Medicine, Univ of Illinois, Prof and Chairman of Dept of Medicine, Cook County Grad School* (1, 1914, 3, 1919)
- Armstrong, Harry G , M D Headquarters US Air Force Washington, 25, DC *Surgeon General* (1, 1948)

- Armstrong, Philip B**, M D Syracuse Univ College of Medicine, Syracuse 10, N Y *Prof of Anatomy* (1, 1945)
- Armstrong, W D**, M D, Ph D Univ of Minnesota, 17 Med Sciences Bldg, Minneapolis *Prof and Head of Physiological Chemistry* (2, 1938)
- Arnold, Aaron**, Ph D Sterling-Winthrop Research Inst, Rensselaer, N Y *Head of Nutritional Research Lab* (5, 1947)
- Arnold, Lloyd, A M**, M D 1538 E 57th St, Chicago, Ill (4, 1930, 6, 1925)
- Arnow, L Earle**, Ph D, M D Sharp & Dohme, Med Research Div, Glenolden, Pa *Dir of Research* (2, 1940)
- Aronson, Joseph D**, M D Univ of Pennsylvania, Phipps Inst, Philadelphia 4 *Assoc Prof of Bacteriology* (4, 1927, 6, 1925)
- Artom, Camillo**, M D Wake Forest College, Bowman Gray School of Medicine, Winston-Salem, N C *Prof of Biochemistry* (2, 1944)
- Ascham, Leah**, Ph D Kansas State College, Manhattan *Prof in School of Home Economics* (5, 1935)
- Asenjo, Conrado F**, Ph D School of Tropical Medicine, San Juan, Puerto Rico *Assoc Prof of Chemistry and Head of Dept of Chemistry and Nutrition* (2, 1944)
- Ashburn, Llewellyn L**, M D U S Marine Hospital, Baltimore 11, Md *Sr Surgeon, U S Public Health Service* (4, 1947)
- Ashby, Winifred M**, Ph D 305 10th St, N E Washington, D C *Sr Scientist, Federal Security Agency* (6, 1923)
- Ashman, Richard**, Ph D Louisiana State Univ School of Medicine, New Orleans *Prof of Physiology* (1, 1925)
- Astwood, Edwin Bennet**, M D, Ph D Pratt Diagnostic Hospital, 30 Bennet St, Boston, Mass *Research Prof of Medicine, Tufts Med School* (1, 1939)
- Atkin, Lawrence**, Ph D The Fleischmann Labs, 810 Grand Concourse, New York City 51 *Asst to Dir of Research* (2, 1946, 5, 1946)
- Aub, Joseph C**, M D Massachusetts General Hospital, Fruit St, Boston 14 *Prof of Research Medicine, Harvard Med School* (1, 1919, 5, 1933)
- Austin, J Harold**, M D 711 Maloney Clinic, 36th and Spruce Sts, Philadelphia 4, Pa *Dir of Pepper Lab* (2, 1922)
- Avery, O T**, M D, Sc D Hoods Hill Rd, Nashville, Tenn *Member Emeritus, Rockefeller Inst for Med Research* (4, 1921, 6R, 1920)
- Axelrod, A E**, Ph D Univ of Pittsburgh, Dept of Chemistry, Pittsburgh, Pa *Research Biochemist, Inst of Pathology, Western Pennsylvania Hospital, Asst Research Prof, Dept of Chemistry* (5, 1949)
- Axelrod, Bernard**, Ph D Western Regional Research Lab, 800 Buchanan St, Albany, Calif *Assoc Chemist, Enzyme Research Div* (2, 1948)
- Axtmayer, Joseph H**, Ph D Univ of Puerto Rico, Rio Piedras *Prof of Chemistry* (5, 1935)
- Bach, L M N**, Ph D Tulane Univ School of Medicine, New Orleans, La *Prof of Physiology* (1, 1948)
- Bachem, Albert**, Ph D Univ of Illinois College of Medicine, Chicago 12 *Prof of Biophysics* (1, 1933)
- Bachman, Carl**, M D Univ of Pennsylvania School of Medicine, 3400 Spruce St Philadelphia 4 *Prof and Dir of Obstetrics and Gynecology* (2, 1941)
- Bachrach, William H**, M D, Ph D Univ of Southern California School of Medicine, Dept of Physiology, Los Angeles 7 *Research Assoc* (1, 1949)
- Baer, Erich**, Ph D Banting Inst, Banting and Best Dept of Med Research, 100 College St, Toronto, Ont, Canada *Assoc Prof* (2, 1942)
- Baernstein, Harry D**, Ph D Natl Insts of Health, Bethesda, Md *Sr Biochemist* (2, 1934)
- Baetjer, Anna M**, D Sc Johns Hopkins School of Hygiene and Public Health, 615 N Wolfe St, Baltimore 5, Md *Asst Prof of Physiological Hygiene* (1, 1929)
- Bahrs, Alice M**, Ph D 2735 Orchard St, Corvallis Ore (1, 1933)
- Bailey, Cameron Vernon**, C M, M D New York Univ and Hospital 303 E 20th St, New York City *Chn Prof of Medicine* (2, 1920, 5, 1933)
- Bailey, Orville T**, M D Harvard Univ Med School, 25 Shattuck St, Boston 15, Mass *Asst Prof of Pathology* (4, 1939)
- Bailey, Percival**, M D, Ph D Univ of Illinois College of Medicine, 1853 W Polk St, Chicago 12, Ill *Prof of Neurology and Neurosurgery* (1, 1941)
- Baitsell, George Alfred**, Ph D Yale Univ, Osborn Zoological Lab, 165 Prospect St, New Haven, Conn *Prof of Biology* (1, 1915)
- Baker, A B**, M D Univ of Minnesota Hospital, 19 Millard Hall, Minneapolis *Dir and Prof of Neurology and Neuropathology* (4, 1940)
- Baker, James A**, D V M, Ph D Cornell Univ New York State Veterinary College, Ithaca *Prof of Bacteriology* (4, 1947)
- Baker, Roger D**, M D Med College of Alabama, Birmingham 5 *Prof of Pathology* (4, 1939)
- Baldes, Edward J**, Ph D 427 Fifth Ave, S W, Rochester, Minn *Asst Prof of Physics, Mayo Foundation, Univ of Minnesota Grad School* (1, 1930)
- Baldwin, Francis Marsh**, Ph D Univ of Southern California, Los Angeles *Prof of Zoology and Dir of Exper Marine Biology* (1, 1919)
- Bale, William F**, Ph D Univ of Rochester School of Medicine and Dentistry, Rochester, N Y *Prof of Radiation Biology* (1, 1943)

- Ball, Eric G**, Ph D Harvard Med School, 25 Shattuck St, Boston 15, Mass *Prof of Biological Chemistry* (2, 1934)
- Ball, Howard A**, M D 233 A St, San Diego, Calif *Pathologist, Paradise Valley Hospital* (4, 1937)
- Balls, Arnold Kent**, Ph D Enzyme Research Lab, U S Bureau of Agricultural and Industrial Chemistry, Western Regional Research Lab, 800 Buchanan St, Albany 6, Calif *Head Chemist* (2, 1932)
- Bang, Frederick B**, M D Johns Hopkins Hospital, Baltimore, Md *Assoc Prof in Medicine* (4, 1947)
- Banus, Mario Garcia**, D Sc Bright Meadows, Chestertown, Md (1, 1927)
- Bard, Philip**, Ph D, Johns Hopkins Univ School of Medicine, 710 N Washington St, Baltimore, Md *Prof and Dir of Dept of Physiology* (1, 1929)
- Barker, H A**, Ph D Univ of California, 3048 Life Sciences Bldg, Berkeley 4, *Prof of Plant Nutrition (Agric)* (2, 1946)
- Barker, S B**, Ph D State Univ of Iowa College of Medicine, Iowa City *Assoc Prof of Physiology* (1, 1938)
- Barlow, O W**, M D, Ph D 3 Warwick Rd, Winchester, N H (3, 1944)
- Barnes, B O**, Ph D Box 967, Station Hospital, KAAF, Kingman, Ariz *Prof of Health Education, Univ of Denver* (1, 1932)
- Barnes, LaVerne A**, Ph D Naval Med Research Inst, Natl Naval Med Center, Bethesda 14, Md *Head, Bacteriology Facility* (6, 1931)
- Barnes, Richard Henry**, Ph D Sharp & Dohme, Glenolden, Pa *Dir of Biochemical Research, Med Research Div* (2, 1941, 5, 1944)
- Barnes, Thomas C**, D Sc Hahnemann Med College and Hosp of Philadelphia, Philadelphia, Pa *Assoc Prof of Pharmacology* (1, 1942, 3, 1948)
- Barnum, Cyrus P, Jr**, Ph D Univ of Minnesota, 210 Millard Hall, Minneapolis 14 *Assoc Prof of Physiological Chemistry* (2, 1946)
- Barott, Herbert G**, E E U S Dept of Agriculture, Natl Agricultural Research Center, Beltsville, Md *Biophysicist, Animal Nutrition Div, Bur of Animal Industry* (5, 1938)
- Barrera, S Eugene**, M D Albany Med College, New Scotland Ave, Albany, N Y (1, 1937)
- Barron, Donald H**, Ph D Yale Univ School of Medicine, New Haven, Conn *Assoc Prof of Physiology* (1, 1943)
- Barron, L S Guzman**, M D Univ of Chicago, Dept of Medicine, Chicago 37, Ill *Assoc Prof of Biochemistry* (2, 1931)
- Bartley, S Howard**, Ph D P O Box 763, East Lansing, Mich (1, 1935)
- Bass, Allan D**, M D Univ of Syracuse School of Medicine, Syracuse, N Y *Prof of Pharmacology* (3, 1944)
- Batchelder, Esther L**, Ph D 8433 Woodcliff Court, Silver Spring, Md *Head of Food and Nutrition Div, Bureau of Human Nutrition and Home Economics* (5, 1933)
- Bateman, John B**, Ph D Physical and Chemical Div, Camp Detrick, Frederick, Md (1, 1945)
- Bates, Robert W**, Ph D E R Squibb and Sons, Biological Labs, New Brunswick, N J *Head of Endocrine Development Dept* (2, 1936)
- Batson, Herbert C**, Ph D Army Med Center, AMDR & GS, Washington, D C *Scientific Dir, Dept of Biological Products* (6, 1949)
- Batterman, Robert C**, M D New York Univ College of Medicine, 477 First Ave, New York City *Instr in Therapeutics* (3, 1941)
- Baudisch, Oskar**, Ph D Saratoga Springs, N Y *Dir of Research, Saratoga Springs Authority of the State of New York* (2, 1931)
- Bauer, J H**, M D Rockefeller Foundation, 20 Rue de la Baume, Paris, (8^e) France (4, 1935)
- Bauer, Walter**, M D Massachusetts General Hospital, Boston *Assoc Prof and Tutor in Medicine, Harvard Med School* (1, 1929)
- Bauernfeind, J C**, Ph D Hoffmann-LaRoche, Inc, Nutley 10, N J *Chief of Applied Nutrition* (5, 1947)
- Bauman, Louis**, M D Columbia Presbyterian Med Center, 180 Fort Washington Ave New York City 32 *Asst Prof of Clin Medicine (retired), Columbia Univ* (2, 1912)
- Baumann, Carl A**, Ph D Univ of Wisconsin, Biochemistry Dept, Madison *Prof of Biochemistry* (2, 1938, 5, 1938)
- Baumann, Emil J**, Ph D 7 Church Lane, Scarsdale, N Y *Chemist, Montefiore Hospital* (2, 1922)
- Baumberger, J Percy**, Sc D Stanford Univ, Physiology Dept, Stanford Univ, Calif *Prof of Physiology* (1, 1921)
- Baxter, James H**, M D Johns Hopkins Hospital, Dept of Medicine, Baltimore, Md (3, 1948)
- Bayne-Jones, Stanhope**, M A, M D New York Hospital, Cornell Med Center, 525 E 68th St, New York City 21 *Pres, Joint Admin Board* (4, 1927, 6, 1917)
- Bazett, Henry C**, M A, M D Univ of Pennsylvania School of Medicine, Philadelphia *Prof of Physiology* (1, 1921)
- Beach, Elot F**, Ph D Metropolitan Life Insurance Co, 1 Madison Ave, New York City 10 *Research Biochemist* (2, 1941, 5, 1942)
- Beadle, Buell W**, M S, Ph D George W Gooch Lab, Ltd, 2580 E 8th St, Los Angeles 23, Calif *Director* (2, 1947)
- Bean, John W**, Ph D, M D Univ of Michigan, Ann Arbor *Prof of Physiology* (1, 1932)
- Beard, Howard H**, Ph D Terrell's Labs of

- Clinical Medicine, Med Arts Bldg, Rm 24, Fort Worth, Tex *Biochemist* (2, 1928, 5, 1933)
- Beard, Joseph W**, M D Duke Hospital, Durham, N C *Prof of Surgery, Assoc Prof of Virology* (4, 1938, 6, 1940)
- Beatty, Clarissa H**, Ph D Univ of Oregon Med School, Portland *Research Fellow* (1, 1949)
- Beazell, James Myler**, Ph D, M D 104 S Michigan Ave, Chicago, Ill *Instr in Physiology and Pharmacology, Northwestern Univ School of Medicine* (1, 1939)
- Beck, Claude S**, M D Lakeside Hospital, Cleveland, Ohio *Prof of Neurosurgery, Western Reserve Univ, Assoc Surgeon, Lakeside Hospital* (4, 1930)
- Beck, Lyle V**, Ph D 5609 Roosevelt St, Bethesda, Md (1, 1941)
- Becker, R Frederick**, M S, Ph D Daniel Baugh Inst of Anatomy, 307 S 11th St, Philadelphia, Pa (1, 1949)
- Beckman, Harry**, M D Marquette Univ School of Medicine, Milwaukee, Wis *Prof and Dir of Dept of Pharmacology* (3, 1937)
- Beecher, Henry K**, M D Massachusetts General Hospital, Boston *Anesthetist-in-Chief, Dorr Prof of Research in Anesthesia, Harvard Med School* (3, 1940)
- Behnke, Albert R**, M S, M D Naval Med Research Inst, Bethesda, Md *Exec Dir* (1, 1946)
- Behre, Jeanette Allen**, Ph D Columbia Univ College of Physicians and Surgeons, Dept of Biochemistry, 630 W 168th St, New York City 32 *Associate* (2, 1925)
- Behrens, Otto K**, Ph D Lilly Research Labs, Eli Lilly and Co, Indianapolis 6, Ind *Head, Bio-organic Chemical Research* (2, 1949)
- Behrmann, Vivian G**, Ph D Wayne Univ, Detroit, Mich *Instr, Grad School, Res Physiologist, Henry Ford Hospital* (1, 1948)
- Belding, David L**, M D Boston Univ School of Medicine, Boston, Mass *Prof Emeritus of Bacteriology and Exper Pathology* (4, 1927)
- Belding, Harwood S**, Ph D QMC Climatic Lab, Lawrence, Mass *Director* (1, 1945)
- Belkin, Morris**, Ph D Natl Cancer Inst, Bethesda 14, Md *Sr Pharmacologist* (3, 1949)
- Bell, E T**, M D 110 Anatomy Bldg, Univ of Minnesota, Minneapolis *Prof Emeritus of Pathology* (4, 1931)
- Bender, M B**, M D New York Univ College of Medicine, New York City *Assoc Prof of Neurology and Head of Lab of Exper Medicine* (1, 1947)
- Benditt, Earl P**, M D Univ of Chicago Clinics, Dept of Pathology, Chicago 37, Ill *Asst Prof* (4, 1947)
- Benham, Olive Ray**, B S Connecticut State Dept of Health, Bureau of Labs, Hartford *Chief Serologist* (6, 1944)
- Bennett, A Lawrence**, Ph D, M D Univ of Nebraska College of Medicine, Omaha *Prof of Physiology and Pharmacology* (1, 1941)
- Bennett, Granville A**, M D Univ of Illinois College of Medicine, 1853 W Polk St, Chicago *Prof of Pathology* (4, 1931)
- Bennett, Henry S**, M D Univ of Washington School of Medicine, Seattle *Dept of Anatomy* (1, 1946)
- Bennett, Leslie L**, M D Univ of California, Berkeley 4 *Asst Prof of Physiology* (1, 1945)
- Bennett, Mary Adelia**, Ph D Inst for Cancer Research, 7701 Burholme St Fox Chase, Philadelphia 11, Pa *Research Biochemist* (2, 1941)
- Benson, Clara C**, Ph D 160 Dorset St, West, Port Hope, Ontario, Canada *Prof Emeritus of Food Chemistry, Univ of Toronto* (2, 1906)
- Benton, Joseph G**, Ph D, M D Goldwater Memorial Hospital, 111 New York Univ Med Research Service, Welfare Island 17, N Y *Research Asst, Instr in Physical Medicine, New York Univ College of Medicine* (3, 1949)
- Berg, Benjamin N**, M D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 *Assoc in Pathology* (4, 1928)
- Berg, Clarence P**, Ph D State Univ of Iowa, Dept of Biochemistry, Chemistry Bldg, Iowa City *Prof of Biochemistry* (2, 1933, 5, 1936)
- Berg, William N**, Ph D 225 W 106th St, New York City *Biochemist* (2, 1906)
- Bergeim, Olaf**, Ph D Univ of Illinois College of Medicine, 1853 W Polk St, Chicago 12 *Prof of Biochemistry* (1, 1916, 2, 1914)
- Bergmann, Werner**, Ph D Yale Univ, Sterling Chemistry Lab, New Haven, Conn *Prof of Chemistry* (2, 1934)
- Berkson, Joseph**, M D, D Sc Mayo Clinic, Rochester, Minn (1, 1933)
- Bernard, Richard**, Ph D Laval Univ, Dept of Biology, Blvd de l'Entente, Quebec, Canada *Asst Prof of Physiology* (1, 1947)
- Bernheim, Frederick**, Ph D Box 3109, Duke Univ Med School, Durham, N C *Prof of Pharmacology* (2, 1933, 3, 1935)
- Bernthal, Theodore G**, M S, M D Med College, State of South Carolina, Dept of Physiology, Charleston 16 *Prof of Physiology* (1, 1932)
- Berry, George Packer**, M D Harvard Med School, Boston 15, Mass *Dean and Prof of bacteriology* (4, 1938, 6, 1934)
- Berryman, George H**, Ph D 6012 Woodlawn Ave, Chicago 37, Ill *Head of Nutrition Div, QM Food and Container Inst* (1, 1949)
- Bessey, Otto A**, Ph D Univ of Illinois College of Medicine, 1853 W Polk St, Chicago 12 *Prof and Head of Dept of Biological Chemistry* (2, 1938, 5, 1943)
- Best, Charles H**, M D, D Sc Univ of Toronto Toronto, Ontario, Canada *Dir of Banting and*

- Best Dept of Med Research and Dept of Physiology* (1, 1923, 2, 1923)
- Bethell, Frank H**, M D 409 Lenawee Drive, Ann Arbor, Mich *Prof of Internal Medicine and 1st Dir of the Thomas Henry Simpson Memorial Inst* (4, 1936)
- Bethke, Roland M**, Ph D Ohio Agricultural Exper Station, Wooster *In Charge of Nutritional Investigations* (2, 1928, 5, 1933)
- Beutner, R**, M D, Ph D 235 N 15th St, Philadelphia, Pa *Prof and Head of Dept of Pharmacology, Hahnemann Med College* (1, 1921, 3, 1921)
- Beyer, Karl H**, Ph D, M D Sharp & Dohme, Inc Med Research Div, Glenolden, Pa *Dir of Pharmacological Research* (1, 1942, 3, 1944)
- Bickford, Reginald G**, M D Mayo Clinic, Section on Physiology, Rochester, Minn *Head of Lab of Neurophysiology and Electroencephalography* (1, 1949)
- Bier, Otto**, M D Instituto Biologico, Caixa Postal 119A, São Paulo, Brazil, (6, 1947)
- Breter, Raymond N**, M D, Ph D Univ of Minnesota, Minneapolis *Prof of Pharmacology* (3, 1930)
- Bills, Charles E**, Ph D Stringtown Rd Route 12, Evansville, Ind *Independent Investigator* (2, 1928, 5, 1935)
- Bing, Franklin C**, Ph D 1135 Fullerton Ave, Chicago, Ill *Dir of American Inst of Baking, Asst Prof of Physiology, Northwestern Univ Med School* (2, 1931, 5, 1934)
- Bing, Richard J**, M D Johns Hopkins Hospital, Dept of Surgery, Baltimore 5, Md *Assoc Prof of Medicine and Surgery* (1, 1942)
- Binkley, Francis**, Ph D Univ of Utah School of Medicine, Salt Lake City *Assoc Prof* (2, 1947)
- Binkley, Stephen Bennett**, Ph D Bristol Labs, Inc, Syracuse 1, N Y *Asst Dir of Research* (2, 1911)
- Bird, Herbert R**, Ph D Bureau of Animal Industry, Agricultural Research Center, Beltsville, Md *Sr Biochemist* (5, 1947)
- Bird, Orson D**, Ph D Parke, Davis and Co, Research Labs, Detroit 32, Mich *Research Biochemist* (2, 1947)
- Bisbey, Bertha**, Ph D Univ of Missouri, Gwynn Hall, Columbia *Prof of Nutrition* (5, 1933)
- Bischoff, Fritz E**, Ph D Cottage Hospital, Santa Barbara, Calif *Dir of Research* (2, 1928, 5, 1933)
- Bishop, George H**, Ph D Washington Univ Med School Euclid and Kingshighway, St Louis, Mo *Prof of Biophysics* (1, 1923)
- Biskind, Gerson R**, M D 210 Stockton St, San Francisco, Calif *Pathologist, Mt Zion Hospital, Clin Instr in Pathology, Univ of California Med School* (4, 1914)
- Black, Alex**, Ph D Pennsylvania State College, Dept of Animal Nutrition, State College *Prof of Animal Nutrition* (5, 1947)
- Black, Edgar C**, Ph D Univ of British Columbia, Dept of Biology and Botany, Vancouver, B C, Canada (1, 1943)
- Black, Simon**, Ph D Univ of Chicago, Chicago 37, Ill *Asst Prof of Biochemistry, Dept of Medicine* (2, 1948)
- Blair, Edgar A**, Ph D Field Research Lab, Med Dept, Fort Knox, Ky *Lt Col* (1, 1936)
- Blair, Henry A**, Ph D Univ of Rochester School of Medicine and Dentistry, Rochester, N Y *Prof of Physiology and Dir of Dept of Radiation Biology* (1, 1934)
- Blake, Francis G**, M D, Sc D Yale Univ School of Medicine, New Haven, Conn *Sterling Prof of Medicine* (4, prior to 1920, 6, 1921)
- Blanchard, Ernest W**, Ph D Schieffelin and Co, 30 Cooper Sq, New York City 3 *Dir of Research* (1, 1946)
- Blankenhorn, M A**, M D Univ of Cincinnati, Cincinnati, Ohio *Prof of Medicine* (4, 1932)
- Blatherwick, Norman R**, Ph D Metropolitan Life Insurance Co, 1 Madison Ave, New York City *Dir of Biochemical Lab* (1, 1915, 2, 1915, 5, 1934)
- Blau, Nathan F**, Ph D Veterans Admin Hospital, 401 S Holyoke Ave, Wichita 8, Kan *Research Biochemist* (2, 1928)
- Blish, Morris J**, Ph D Internatl Minerals and Chemical Corp, Box G, Rossford, Ohio *Research Dir* (2, 1944)
- Bliss, Alfred**, Ph D Tufts College Med School, Boston, Mass *Assoc Prof of Physiology* (1, 1947)
- Bliss, Chester Itiner**, Ph D Conn Agricultural Exper Station, P O Box 1106, New Haven *Biometrician, Lecturer in Biometry, Yale Univ* (3, 1944)
- Bliss, Eleanor A**, Sc D Johns Hopkins Hospital, Dept of Preventive Medicine, 615 N Wolfe St, Baltimore, Md *Assoc in Preventive Medicine, Johns Hopkins Univ School of Medicine* (6, 1931)
- Bloch, Konrad**, Ph D Univ of Chicago, Dept of Biochemistry, Chicago 37, Ill *Assoc Prof of Biochemistry* (2, 1944)
- Block, Richard J**, Ph D 15 Cooper Rd, Searsdale, N Y *Dir of Research, C M Armstrong Co, Assoc Dept of Physiology and Biochemistry, New York Med College, Flower and Fifth Ave Hospital* (2, 1934, 5, 1933)
- Block, Walter D**, Ph D 813 E McCreight Ave, Springfield, Ohio, (2, 1942)
- Bloom, William**, M D 1410 E 56th St, Chicago, Ill *Prof of Anatomy, Univ of Chicago* (4, 1930)
- Bloomfield, A L**, M D Stanford Univ Hospital, San Francisco, Calif *Prof of Medicine* (3, 1927, 4, 1927)

- Bloor, W R , Ph D Univ of Rochester School of Medicine and Dentistry, Rochester 7, N Y *Prof of Biochemistry* (1R, 1915, 2, 1910)
- Blum, Harold F , Ph D Princeton Univ , Dept of Biology, Princeton, N J *Physiologist, Natl Cancer Inst , and Visiting Lecturer* (1, 1928)
- Blumberg, Harold, Sc D Research Lab , Endo Products, Inc , 84-40 101st St , Richmond Hill 18, N Y (5, 1942)
- Blumenstock, Julius, M D Veterans Admin Hospital, Sheridan, Wyo (1, 1925)
- Blumgart, Herrmann L , M D Beth Israel Hospital, 330 Brookline Ave , Boston, Mass *Physician-in-Chief, Prof of Medicine, Harvard Med School* (1, 1927)
- Blunt, Katharine, Ph D 38 Glenwood Ave , New London, Conn *Pres Emeritus, Connecticut College for Women* (2, 1921)
- Bobb, J Richard R , M D Univ of Minnesota Med School, Dept of Physiology, Minneapolis 14 *Instr in Physiology, U S Public Health Postdoctorate Research Fellow* (1, 1949)
- Bock, Joseph C , Ph D 2324 N 46th St , Milwaukee 10, Wis *Prof Emeritus of Biochemistry, Marquette Univ Med School, Biochemist, Milwaukee County Hospital* (2, 1916)
- Bodansky, Aaron, Ph D Hospital for Joint Diseases, 1919 Madison Ave , New York City *Biological Chemist* (2, 1926)
- Bodansky, Oscar, Ph D , M D Memorial Hospital Cancer Center, 444 E 68th St , New York City 21 *Chief, Clin Biochemistry, Assoc Member, Sloan-Kettering Inst for Cancer Research* (2, 1937, 3, 1942)
- Bodian, David, Ph D , M D Johns Hopkins Univ , 1901 E Madison St , Baltimore, Md *Assoc Prof of Epidemiology* (6, 1949)
- Bodine, Joseph Hall, Ph D State Univ of Iowa, Iowa City *Prof and Head of Dept of Zoology* (1, 1925)
- Boell, Edgar J , Ph D Yale Univ , Osborn Zoological Lab , New Haven, Conn *Ross G Harrison Prof of Exper Zoology* (1, 1942)
- Boger, William P , M D Sharp & Dohme, Inc , Med Research Div , Glenolden, Pa *Assoc Med Dir , Instr in Medicine, Univ of Pennsylvania School of Medicine and Grad School of Medicine* (3, 1948)
- Bogert, L Jean, Ph D Hotel Claremont, Berkeley, Calif (2, 1917)
- Bogert, Marston Taylor, Sc D 1158 Fifth Ave , New York City 29 *Prof Emeritus of Organic Chemistry, Columbia Univ , Scientific Consultant to Evans Research and Development Corp* (2, 1925)
- Bohr, David F , M D Univ of Michigan, Physiology Lab , Ann Arbor *Asst Prof* (1, 1949)
- Boivin, André, M D La Faculté de Médecine de Strasbourg, Strasbourg, France *Professor* (6, 1949)
- Bolliger, Adolph, Ph D Univ of Sydney, Gordon Craig Research Lab , Sydney, Australia *Dir of Research* (2, 1928)
- Bollman, J L , M D Mayo Clinic, Rochester, Minn *Chairman of Div of Exper Medicine, Prof of Physiology, Mayo Foundation* (4, 1927)
- Bond, Glenn C , Ph D , M D The Upjohn Co , Research Lab , Kalamazoo, Mich *Asst Dept Head of Bacteriology Research* (6, 1939)
- Bondi, Amedeo Hahnemann Med College, Philadelphia, Pa *Prof and Head of Dept of Bacteriology* (6, 1948)
- Bonner, David M , Ph D Yale Univ , Osborn Botanical Lab , New Haven, Conn *Assoc Prof and Research Assoc* (2, 1948)
- Bonnycastle, Desmond D , M D , Ph D Yale Univ School of Medicine, New Haven, Conn *Asst Prof of Pharmacology* (3, 1947)
- Bonsnes, Roy W , Ph D Cornell Univ Med College, 1300 York Ave , New York City 21 *Asst Prof of Biochemistry in Obstetrics* (2, 1947)
- Booher, Lela E , Ph D General Mills, Inc , 1081 21st Ave , S E , Minneapolis, Minn *Chief Nutritionist and Dir of Nutrition Lab* (2, 1933, 5, 1933)
- Booker, Walter M , Ph D Howard Univ School of Medicine, Washington, D C *Assoc Prof of Pharmacology* (1, 1948, 3, 1948)
- Boor, Alden K , Ph D Camp Detrick, Basic Science Div , Frederick, Md (2, 1931)
- Boothby, Walter M , M A , M D Univ of Lund, Lund, Sweden *Research Consultant Aviation Medicine Inst of Physiology, Chief Emeritus, Clin Metabolism Sect , Mayo Clinic* (1R, 1915, 2, 1920, 3R, 1923, 4R, 1924)
- Bordley, James, III, M D Mary Imogene Bassett Hospital, Cooperstown, N Y (1, 1938)
- Borek, Ernest, Ph D College of the City of New York, Convent Ave and 140th St , New York City *Asst Prof , Research Assoc in Biochemistry, Columbia Univ* (2, 1947)
- Boroff, Daniel A , M A , M S 1721 Ashland Ave , Evanston, Ill (6, 1947)
- Borsook, Henry, M D , Ph D California Inst of Technology, Pasadena 4 *Prof of Biochemistry* (2, 1931)
- Bosshardt, David K , Ph D Sharp & Dohme, Med Research Div , Glenolden, Pa *Research Biochemist* (5, 1947)
- Bostick, Warren L , M D Univ of California Med School, Dept of Pathology, San Francisco 22, *Asst Prof of Pathology* (4, 1949)
- Bosworth, Alfred Willson, A M , M D R F D 4, Circleville, Ohio *Consulting Chemist* (2, 1936, 5, 1935)
- Bott, Phyllis A , Ph D Woman's Med College of Pennsylvania, Henry Ave and Abbotsford Rd ,

- Philadelphia *Prof and Chairman of Dept of Physiological Chemistry* (2, 1938)
- Boucher, Robert V**, Ph D 303 Frear Labs, State College, Pa *Prof of Agricultural and Biological Chemistry* (5, 1945)
- Bouman, H D**, M D Univ of Wisconsin Med School, Madison *Prof of Physical Medicine* (1, 1943)
- Bourne, Wesley, M D**, M Sc McGill Univ, Montreal, Quebec, Canada *Prof of Anesthesia* (3, 1936)
- Bourque, Joseph E**, M D, Ph D Univ of Illinois College of Medicine, Dept of Physiology, 1853 W Polk St, Chicago 12 *Asst Prof of Physiology* (1, 1949)
- Bourquin, Helen**, Ph D 1331 N Tejon St, Colorado Springs, Colo (1, 1925)
- Bowen, William J**, Ph D USPHS, Inst of Exper Biology & Medicine, Bethesda 14, Md *Sr Asst Scientist* (1, 1948)
- Bowman, Donald E**, Ph D 6956 Warwick Rd, Indianapolis, Ind *Assoc Prof of Biochemistry, Indiana Univ School of Medicine* (2, 1944)
- Bowman, Katherine L**, B A 20 Plaza St, Brooklyn 17, N Y (6, 1946)
- Boxer, George E**, Ph D 605 Girard Ave, Westfield, N J *Sr Chemist, Research and Development Div, Merck & Co, Inc* (2, 1946)
- Boyd, Eldon M**, M A, M D Queen's Univ, Kingston, Ontario, Canada *Prof and Head of Dept of Pharmacology* (3, 1941)
- Boyd, M John**, Ph D Hahnemann Med College, 235 N 15th St, Philadelphia 2, Pa *Prof of Biol Chemistry* (2, 1947)
- Boyd, T E**, Ph D 9 Walworth Ave, Scarsdale, N Y (1, 1924)
- Boyd, William C**, Ph D Boston Univ School of Medicine, 80 E Concord St, Boston, Mass *Assoc Prof of Biochemistry* (6, 1933)
- Boyden, Allan A** Rutgers Univ, New Brunswick, N J *Prof of Zoology* (6, 1948)
- Boyden, Edward A**, Ph D Univ of Minnesota, Minneapolis 14 *Prof and Chairman of Dept of Anatomy* (1, 1929)
- Boyer, Paul D**, Ph D Univ of Minnesota College of Agriculture, Div of Biochemistry, St Paul 1 *Assoc Prof* (2, 1944)
- Boyle, Paul E**, D M D Univ of Pennsylvania School of Dentistry, 40th and Spruce Sts, Philadelphia 4 *Prof of Oral Pathology* (4, 1939)
- Bozicevich, John**, M A USPHS, Natl Insts of Health, Bethesda, Md *Head, Subsection of Immunology, Trop Diseases Div* (6, 1948)
- Bozler, Emil**, Ph D Ohio State Univ, Columbus *Prof of Physiology* (1, 1932)
- Bradbury, James T**, Sc D Univ of Louisville School of Medicine, 101 W Chestnut St, Louisville 2, Ky (1, 1941)
- Bradley, Harold C**, Ph D 2639 Durant Ave, Berkeley, Calif (1, 1911, 2, 1908)
- Bradley, Stanley E**, M D Columbia Univ College of Physicians and Surgeons, 620 W 168th St, New York City 32 *Asst Prof* (1, 1947)
- Bradley, William B**, Ph D American Inst of Baking, 1046 Elmwood Ave, Wilmette, Ill *Dir of Labs* (1, 1939)
- Branch, Charles F**, M D American College of Surgeons, 40 E Erie St, Chicago 11, Ill *Asst Dir* (4, 1940)
- Branch, E Arnold G**, M D Lancaster Hospital, St John, New Brunswick, Canada *Dir of Labs* (4, 1929)
- Brand, Erwin**, Ph D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 *Assoc Prof of Biological Chemistry* (2, 1929)
- Brandes, W W**, M D Roosevelt Hospital, W 59th St, New York City (4, 1931)
- Branham, Sara E**, Ph D, M D, Natl Insts of Health, Bethesda, Md *Sr Bacteriologist* (6, 1926)
- Branion, Hugh Douglas**, Ph D Ontario Agricultural College, Guelph, Canada *Prof and Head of Dept of Animal Nutrition* (5, 1933)
- Brassfield, Charles R**, Ph D Univ of Michigan, Ann Arbor *Assoc Prof of Physiology* (1, 1937)
- Bratton, Andrew Calvin, Jr**, M D, Ph D Parke, Davis and Co, Research Labs, Detroit 32, Mich *Dir of Pharmacological Research* (3, 1941)
- Brauer, Ralph W**, Ph D Louisiana State Univ School of Medicine, New Orleans *Asst Prof of Pharmacology* (3, 1948)
- Braun, Herbert A**, Ph D Fed Security Agency, Food and Drug Admin, Washington, D C *Assoc Pharmacologist* (3, 1941)
- Brazier, Mary A B**, Ph D Massachusetts General Hospital, Electroencephalographic Lab, Boston 14 *Research Assoc in Neuropathology, Harvard Med School* (1, 1947)
- Brecher, George**, M D Natl Insts of Health, Bethesda 14, Md *Chief of Unit on Morphologic Hematology* (4, 1949)
- Brecher, Gerhard A**, M D, Ph D Western Reserve Univ Med School, Dept of Physiology, Cleveland 6, Ohio *Sr Instructor* (1, 1949)
- Breedis, Charles**, M D Univ of Pennsylvania School of Medicine, Philadelphia *Instr in Pathology* (4, 1948)
- Brewer, Carl R**, Ph D Camp Detrick, Frederick, Md *Chief, Bacterial Nutrition Branch, Biological Div, Chemical Corps* (2, 1948)
- Brewer, John H**, Ph D Hynson Westcott and Dunning, Baltimore, Md *Dir of Biological Research* (6, 1948)
- Brewer, Nathan R**, Ph D Univ of Chicago, Chicago, Ill *Lecturer in Physiology* (1, 1948)
- Bridge, Edward M**, M D Univ of Buffalo School of Medicine, 24 High St, Buffalo 9, N Y *Prof of Pharmacology* (2, 1940)

- Briggs, A P , M D Univ of Georgia Med College, Augusta *Prof of Biochemistry* (2, 1923)
- Briggs, David R , Ph D Univ of Minnesota, Div of Agricultural Biochemistry, Univ Farm, St Paul *Prof of Agricultural Biochemistry, Chemist, Minn Agricultural Exper Station* (2, 1946)
- Briggs, George M , Ph D Univ of Minnesota, Univ Farm, St Paul *Asst Prof of Poultry Nutrition* (5, 1947)
- Brink, Frank Jr , Ph D Johns Hopkins Univ , Biological Labs , Baltimore 18, Md (1, 1942)
- Brinkhous, K M , M D Univ of North Carolina School of Medicine, Dept of Pathology, Chapel Hill *Prof of Pathology* (4, 1939)
- Britton, Sydney W , M D Univ of Virginia School of Medicine, Charlottesville *Prof of Physiology* (1, 1925)
- Brobeck, John R , M D , Ph D Yale Univ School of Medicine, New Haven, Conn *Asst Prof of Physiology* (1, 1943)
- Brodie, Bernard B , Ph D New York Univ Research Service, Goldwater Memorial Hospital, New York City 17 *Assoc Prof of Biochemistry* (2, 1940, 3, 1945)
- Brody, Samuel, Ph D Univ of Missouri College of Agriculture and Agricultural Experiment Station, Dairy Building, Columbia *Prof of Dairy Husbandry* (2, 1929, 5, 1933)
- Broh-Kahn, Robert H , M D May Inst for Med Research, Cincinnati, Ohio *Asst Dir* (1, 1948)
- Bromley, Reginald B , Ph D Johns Hopkins School of Medicine, Dept of Physiology, 710 N Washington St , Baltimore 5, Md *Prof of Physiology* (1, 1949)
- Bronfenbrenner, J J , Ph D , D P H Washington Univ School of Medicine, St Louis, Mo *Prof of Bacteriology and Immunology* (4, 1940, 6, 1918)
- Bronk, Detlev W , Ph D , Sc D Johns Hopkins Univ , Baltimore, Md *Pres , Chairman of Natl Research Council* (1, 1927)
- Brookes, Margaret C Hessler, Ph D Univ of Chicago, Chicago, Ill *Asst Prof , Dept of Home Economics* (5, 1935)
- Brookhart, John M , Ph D Univ of Oregon School of Medicine, Dept of Physiology, Portland *Assoc Prof* (1, 1946)
- Brooks, Chandler McCuskey, Ph D The Long Island College of Medicine, Dept of Physiology and Pharmacology, Brooklyn, N Y (1, 1934)
- Brooks, Clyde, Ph D , M D Univ Clinic, 2506 Ponce de Leon Blvd , Coral Gables, Fla (1, 1910, 3, 1912)
- Brooks, Matilda Moldenhauer, Ph D Univ of California, Dept of Physiology, Berkeley *Research Assoc in Biology* (1, 1923)
- Broun, Goronwy Owen, M D 1325 S Grand Blvd , St Louis, Mo *Prof of Internal Medicine, St Louis Univ* (4, 1927)
- Brown, Claude P , M D 1930 Chestnut St , Philadelphia Pa (6, 1913)
- Brown, Dugald E S , Ph D Bermuda Biological Station, St George's W , Bermuda (1, 1932)
- Brown, Ethan Allen, L R C P (Eng) , A R C S (London), 75 Bay State Rd , Boston, Mass *Lecturer in Medicine, Tufts College Med School, Physician-in-Chief, Allergy Clinic, Boston Dispensary* (6, 1946)
- Brown, Frank A , Jr , Ph D Northwestern Univ , Zoological Labs , Evanston, Ill *Assoc Prof of Zoology* (1, 1940)
- Brown, George B , Ph D Sloan-Kettering Inst for Cancer Research, 444 E 68th St , New York City 21 *Member, Asst Prof of Biochemistry, Cornell Univ Med College* (2, 1947)
- Brown, Gordon Campbell, Sc D School of Public Health, Ann Arbor, Mich *Assoc Prof of Epidemiology* (6, 1949)
- Brown, John B , Ph D Ohio State Univ , Columbus *Prof of Physiological Chemistry* (2, 1927, 5, 1934)
- Brown, R A , Ph D Parke Davis and Co , Research Labs , Detroit 32, Mich *Head of Div of Nutritional Research* (5, 1946)
- Brown, Rachel, Ph D 26 Buckingham Drive, Albany, N Y *Sr Biochemist, Div of Labs and Research, N Y State Dept of Health* (6, 1933)
- Brown, Robert V , Ph D Univ of Tennessee, Dept of Pharmacology, Memphis 3 *Assoc Prof* (1, 1945)
- Browne, J S L , M D , Ph D Royal Victoria Hospital, Univ Clinic, Montreal, Que , Canada *Asst Prof of Medicine, McGill Univ* (1, 1934)
- Brownell, Katharine A , Ph D Ohio State Univ , Dept of Physiology, Columbus *Research Assoc* (1, 1943)
- Brozek, Josef, Ph D Univ of Minnesota, Stadium South Tower, Minneapolis 14 *Assoc Prof , Lab of Physiological Hygiene, School of Public Health* (1, 1947)
- Brues, Austin M , M D Argonne National Labs , P O Box 5207, Chicago, Ill *Dir of Biology Div , Assoc Prof of Medicine and Instr of Radiobiology and Biophysics, Univ of Chicago* (1, 1940)
- Bruesch, S R , M D , Ph D Univ of Tennessee, Div of Anatomy, Memphis 3 *Prof of Anatomy* (1, 1949)
- Bruger, Maurice, M D , M Sc 301 E 20th St , New York City 3 *Assoc Prof of Medicine, New York Univ Post-Grad Med School, Chief, Div of Pathological Chemistry, New York University Hospital* (2, 1935, 5, 1935)
- Bruhn, John M , Ph D Med College of Alabama, Dept of Physiology, 620 S 20th St , Birmingham 5 *Prof of Physiology and Pharmacology* (1, 1939)
- Bruner, Harry Davis, M D , Ph D Med Div ORINS, Box 117, Oak Ridge, Tenn (3, 1945)
- Brunschwig, Alexander, M D Cornell Univ Med College, New York City *Prof of Clin Surgery, Attending Surgeon, Memorial Hosp* (4, 1937)

- Bryan, W Ray, Ph D Glen Rd , Rockville, Md
Principal Biologist, Natl Cancer Inst (1, 1934, 4, 1940)
- Buchanan, J William, Ph D Univ of Southern California, Alan Hancock Foundation, Los Angeles
Prof of Zoology (1, 1927)
- Buchanan, John M , Ph D Univ of Pennsylvania School of Medicine, Dept of Physiological Chemistry, Philadelphia
Asst Prof (2, 1949)
- Buchbinder, Lean, Ph D Dept of Health, 125 Worth St , New York City (6, 1934)
- Buchbinder, William C , M D 104 S Michigan Ave , Chicago, Ill
Asst Prof of Medicine, Northwestern Univ Med School, Assoc in Medicine, Michael Reese Hospital (1, 1940)
- Bucher, Gladys R , Ph D Univ of Illinois, Dept of Biology, Chicago Div , Navy Pier, Chicago 12 (1, 1946)
- Buckner, G Davis, Ph D Kentucky Agricultural Exper Station, Lexington
In Charge of Animal Nutrition (2, 1920)
- Bucy, Paul C , M D 4833 S Woodlawn Ave , Chicago, Ill
Prof of Neurology and Neurological Surgery, Univ of Illinois (1, 1933)
- Buddingh, G John, M D Louisiana State Univ School of Medicine, New Orleans
Prof of Microbiology (4, 1940)
- Bueding, Ernest, M D Western Reserve Univ School of Medicine, Dept of Pharmacology, Cleveland 6, Ohio
Assoc Prof (2, 1946, 3, 1949)
- Buell, Mary V , Ph D Univ of Wisconsin Enzyme Inst , Madison
Research Assoc (2, 1921)
- Bukantz, Samuel C , M D Washington Univ School of Medicine, St Louis 10, Mo
Research Asst in Medicine (6, 1943)
- Bulatao, Emilio, M D Univ of the Philippines, Manila, P I
Prof of Physiology and Biochemistry (1, 1924)
- Bull, Henry B , Ph D Northwestern Univ Med School, 303 E Chicago Ave , Chicago 11, Ill
Prof of Chemistry (2, 1937)
- Bullock, Theodore H , Ph D Univ of California, Los Angeles
Assoc Prof of Zoology (1, 1948)
- Bunde, Carl A , M D , Ph D Pitman-Moore Co , Indianapolis 6, Ind
Research Dir (1, 1943)
- Bunney, William Edward, Ph D E R Squibb and Sons, New Brunswick, N J
Vice Pres , Dir of Manufacturing Labs (6, 1931)
- Bunting, Charles H , M D 139 Armory St , Hamden, Conn
Prof Emeritus of Pathology, Univ of Wisconsin, Lecturer in Pathology, Yale Med School (4, 1913)
- Bunzell, H H , Ph D Box 44, General Post Office, New York City 1
Dir of Bunzell Labs (2, 1908)
- Burchell, Howard B , M D , Ph D 1506 Durand Court, Rochester, Minn
Instr in Medicine, Mayo Foundation, Consultant in Medicine, Mayo Clinic (1, 1942)
- Burdick, H O , Sc D Alfred Univ , Alfred, N Y
Prof of Biology (1, 1940)
- Burdon, Kenneth L , Ph D Baylor Univ College of Medicine, Houston, Tex
Prof of Bacteriology, Consultant, USPHS (6, 1936)
- Burk, Dean, Ph D Natl Cancer Inst , Bethesda, Md
Principal Chemist, USPHS (2, 1939)
- Burns, Edward L , M D Mercy Hospital, Toledo, Ohio
Pathologist (4, 1939)
- Burr, George O , Ph D Exper Station H S P A , Honolulu, Hawaii
Head of Dept of Biochemistry and Physiology (2, 1928, 5, 1933)
- Burris, Robert H , Ph D Univ of Wisconsin, Dept of Biochemistry, Madison 6
Assoc Prof of Biochemistry (2, 1946)
- Burrows, William, Ph D Univ of Chicago, Dept of Bacteriology and Parasitology, Chicago 37, Ill
Prof of Bacteriology (6, 1947)
- Burton, Alan C , Ph D Univ of Western Ontario, Dept of Med Research, London, Ontario, Canada
Asst Prof of Med Research (1, 1937)
- Burton-Opitz, Russell, M D , Ph D 218 Bridle Way, Palisade, N J
Attending Cardiologist, Lenox Hill Hospital, Attending Physician, Cumberland Hospital (1R, 1902, 2, 1906, 3R, 1919)
- Buschke, William H , M D Manhattan Eye, Ear and Throat Hospital, 210 E 64th St , New York City 21
Research Ophthalmologist, Head of Ayer Foundation Ophthalmic Research Lab (1, 1947)
- Bush, Milton T , Ph D Vanderbilt Univ School of Medicine, Nashville, Tenn
Assoc Prof of Pharmacology (3, 1938)
- Butler, G C , Ph D Univ of Toronto, Dept of Biochemistry, Toronto, Ont , Canada
Assoc Prof (2, 1949)
- Butler, Thomas C , M D Johns Hopkins School of Medicine, 710 N Washington St , Baltimore 5, Md
Assoc Prof of Pharmacology and Exper Therapeutics (3, 1938)
- Butt, Hugh R , M D Mayo Clinic, 102 Second Ave , S W , Rochester, Minn
Consultant in Medicine, Asst Prof of Medicine, Mayo Foundation (5, 1942)
- Butts, Joseph S , Ph D Oregon State College, Corvallis
Prof of Biochemistry and Head of Agricultural Chemistry (2, 1936, 5, 1936)
- Byers, Sanford O , Ph D 2200 Scott St , San Francisco 15, Calif
Research Biochemist, Harold Brunn Inst for Cardiovascular Research, Mount Zion Hospital (1, 1949)
- Cahen, Raymond L , Ph D , M D Maltbie Chemical Co , Research Labs , P O Box 270, Morristown, N J
Dir of Pharmacological Research (3, 1949)
- Cahill, William M , Ph D , M D 3320 Baldwin St , Los Angeles 31, Calif (2, 1940)
- Cajori, Florian A , Ph D Univ of Colorado Med School, Dept of Biochemistry, Denver 7
Asst Prof of Biochemistry (2, 1922, 5, 1933)
- Caldwell, Mary L , Ph D Columbia Univ , Dept of Chemistry, New York City
Prof of Chemistry (2, 1924, 5, 1933)

- Callison, Elizabeth C, M S U S Dept of Agriculture, Agricultural Research Station, Food and Nutrition Div, Bureau of Human Nutrition, Beltsville, Md *Nutrition Physiologist* (5, 1949)
- Calloway, Nathaniel Oglesby, Ph D, M D Univ of Illinois College of Medicine, 1819 W Polk St, Chicago 12 *Asst in Medicine* (3, 1945)
- Calvin, D Bailey, Ph D Univ of Texas Med Branch, Galveston *Prof of Biological Chemistry and Dean of Student and Curricular Affairs* (1, 1934, 2, 1939)
- Calvin, Melvin, Ph D Univ of California, Dept of Chemistry, Berkeley 4 *Prof of Chemistry* (2, 1949)
- Camunopetros, John, M D Hellenic Pasteur Inst, 103 Queen Sophie Ave, Athens, Greece *Chief of the Service of Exper Medicine* (6, 1946)
- Camp, Walter J R, M D, Ph D Univ of Illinois College of Medicine, 1853 W Polk St, Chicago, Ill *Prof of Pharmacology and Toxicology, State Toxicologist* (3, 1926)
- Campbell, Berry, Ph D Univ of Minnesota, Minneapolis 14 *Assoc Prof of Anatomy* (1, 1945)
- Campbell, Dan H, Ph D California Inst of Technology, Dept of Chemistry, Pasadena *Assoc Prof of Chemistry* (6, 1938)
- Campbell, James, Ph D Univ of Toronto, Toronto, Ont, Canada *Asst Prof of Physiology, Lt Comdr, (S B) R C N V R* (1, 1943)
- Campbell, Walter Ruggles, M D Univ of Toronto, 69 Madison Ave, Toronto, Ont, Canada *Assoc Prof of Medicine* (2, 1922)
- Cannan, R Keith, D Sc New York Univ College of Medicine, 477 First Ave, New York City *Prof of Chemistry* (2, 1931)
- Cannon, Paul R, M D, Ph D Univ of Chicago, Chicago, Ill *Prof of Pathology* (4, 1930, 6, 1929)
- Cantarow, Abraham, M D Jefferson Med College, Philadelphia 7, Pa *Prof of Biochemistry* (1, 1932, 3, 1935)
- Cantoni, G L, M D New York Univ College of Medicine, Dept of Pharmacology, New York City (3, 1945)
- Canzanelli, Attilio, M D Tufts College Med School, 416 Huntington Ave, Boston, Mass *Prof of Exper Physiology* (1, 1934)
- Carlson, A J, Ph D, M D Univ of Chicago, Hull Physiological Lab, Chicago, Ill *Prof Emeritus of Physiology* (1, 1904, 5, 1933)
- Carlson, Loren D, Ph D Univ of Washington School of Medicine, Dept of Physiology and Biophysics, Seattle 5 (1, 1945)
- Carmichael, Emmett B, Ph D Med College of Alabama, Dept of Biochemistry, Birmingham 5 *Prof and Head of Dept of Biochemistry* (1, 1931, 2, 1946)
- Carmichael, Leonard, Ph D Sc D Tufts College, Medford, Mass *Pres, Dir of Research Lab of Sensory Psychology and Physiology* (1, 1937)
- Carpenter, Frederick H, Ph D Univ of California, Dept of Biochemistry, Berkeley 4 *Asst Prof* (2, 1949)
- Carpenter, Thorne M, Ph D 27 Market St, Foxboro, Mass (1R, 1915, 2, 1909, 5, 1935)
- Carr, C Jelleff, Ph D Univ of Maryland School of Medicine, Baltimore *Assoc Prof of Pharmacology* (3, 1940)
- Carr, Jesse L, M D Univ of California Med School, Third and Parnassus Aves, San Francisco *Clin Prof of Pathology* (4, 1940)
- Carruthers, Christopher, Ph D Washington Univ School of Medicine, Dept of Anatomy, 4580 Scott Ave, St Louis 10, Mo *Research Assoc* (2, 1948)
- Carter, Herbert E, Ph D Univ of Illinois, 452 Noyes Lab, Urbana *Prof of Biochemistry* (2, 1937, 5, 1941)
- Cartland, George F, Ph D The Upjohn Co, Research Dept, Kalamazoo, Mich *Head of Antibiotics Research* (2, 1936)
- Cary, Charles A, S B U S Dept of Agriculture, Bureau of Dairy Industry, Dairy Research Lab, Beltsville, Md *Chief of Div of Nutrition and Physiology* (2, 1920)
- Casey, Albert Eugene, M D 1907 Wellington Rd, Birmingham 9, Ala *Pathologist, Baptist Hospital, Assoc Prof of Pathology, Med College of Alabama* (4, 1933)
- Cash, James Robert, M D Univ Hospital, Charlottesville, Va *Prof of Pathology, Univ of Virginia* (4, 1924)
- Cassidy, Harold G, Ph D Yale Univ, Sterling Chemical Lab, New Haven, Conn *Assoc Prof of Chemistry* (2, 1949)
- Castle, Edward S, Ph D Harvard Univ Biological Labs, Divinity Ave, Cambridge, Mass *Assoc Prof of General Physiology* (1, 1934)
- Castle, William B, M D Boston City Hospital, Boston, Mass *Prof of Medicine, Harvard Med School, Dir of Thorndike Memorial Lab* (4, 1942)
- Catchpole, Hubert Ralph, Ph D Univ of Chicago College of Medicine, 1853 W Polk St, Chicago 12, Ill *Research Assoc in Pathology* (1, 1941)
- Cathcart, E P, M D, D Sc Univ of Glasgow, Glasgow, Scotland *Dean of Univ* (5, 1935)
- Catron, Lloyd, M D City Hospital, Akron, Ohio *Pathologist* (4, 1939)
- Cattell, McKeen, Ph D, M D Cornell Univ Med College, 1300 York Ave, New York City *Prof of Pharmacology* (1, 1923, 3, 1924)
- Cavelti, Philip A, M D 672 S Westlake Ave, Los Angeles 34, Calif (6, 1947)
- Cederquist, Dena C, Ph D Michigan State College, School of Home Economics, East Lansing *Assoc Prof (Research) of Foods and Nutrition* (5, 1949)
- Cerecedo, Leopold R, Ph D Fordham Univ, New York City *Prof of Biochemistry* (2, 1931, 5, 1945)

- Chadwick, Leigh Edward**, Ph D Med Div, Army Chemical Center, Md (1, 1944)
- Chaikoff, I L**, Ph D, M D Univ of California, Berkeley *Assoc Prof of Physiology* (1, 1932)
- Chalkley, Harold W**, Ph D USPHS, Natl Insts of Health, Bethesda, Md *Sr Physiologist* (1, 1932)
- Chamberlain, W Edward**, M D Temple Univ Med School, Philadelphia, Pa *Prof of Radiology* (1, 1948)
- Chambers, Alfred H**, Ph D Univ of Vermont School of Medicine, Dept of Physiology, Burlington (1, 1946)
- Chambers, Leslie Addison**, Ph D Camp Detrick, Frederick, Md *Chief of Physical and Chemical Div* (1, 1940, 6, 1948)
- Chambers, William H**, Ph D Med Div, Army Chemical Center, Md *Chief of Toxicology Branch* (1, 1924, 5, 1933)
- Chandler, Caroline A**, M D Johns Hopkins School of Hygiene and Public Health, 615 N Wolfe St, Baltimore 5, Md *Asst Prof of Preventive Medicine* (6, 1938)
- Chandler, Joseph P**, Ph D Univ of Michigan Med School, Room G455, Univ Hosp, Ann Arbor *Asst Prof of Biological Chemistry, Biochemist, Univ Hosp* (2, 1944, 5, 1944)
- Chang, Min Cheuh**, Ph D Worcester Foundation, Shrewsbury, Mass *Assoc Fellow* (1, 1946)
- Chanutin, Alfred**, Ph D Box 1862, University Station, Charlottesville, Va *Prof of Biochemistry, Univ of Virginia* (2, 1925)
- Chapanis, Alphonse**, Ph D Johns Hopkins Univ, Baltimore, Md *Asst Prof of Psychology* (1, 1948)
- Chapman, C W**, Ph D Univ of Maryland, Baltimore *Prof of Pharmacology* (3, 1932)
- Chargaff, Erwin**, Ph D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 *Assoc Prof of Biological Chemistry* (2, 1935)
- Charipper, Harry Adolph**, Ph D Washington Square College of Arts and Sciences, 100 Washington Square E, New York City *Prof and Chairman of Dept of Biology* (1, 1941)
- Chase, Aurin M**, Ph D Princeton Univ, Dept of Biology, Princeton, N J *Research Assoc, Asst Prof* (1, 1939)
- Chase, Harold F**, M D Univ of Virginia Hospital, Charlottesville (3, 1944)
- Chase, Merrill W**, Ph D Rockefeller Inst, 66th St and York Ave, New York City *Member of Staff* (6, 1938)
- Chasis, Herbert**, M D, Med Sc D 44 E 67th St, New York City *Asst Prof of Medicine, New York Univ College of Medicine* (1, 1941)
- Chatfield, Charlotte**, BS Food and Agriculture Organization of the United Nations, Washington, D C *Nutrition Officer* (5, 1941)
- Chatterjee, Hernendra Nath**, M D Calcutta Univ, Calcutta, India *Professor* (6, 1948)
- Cheldelin, Vernon H**, Ph D Univ of Wisconsin, Enzyme Inst, Madison (2, 1947, 5, 1946)
- Chen, Graham**, Sc D, M D Parke Davis and Co, Detroit, Mich (3, 1944)
- Chen, K K**, Ph D, M D Lilly Research Labs, Indianapolis, Ind *Dir of Pharmacological Research, Prof of Pharmacology, Indiana Univ School of Medicine* (1, 1929, 3, 1942)
- Cheney, Ralph H**, Sc D Brooklyn College, Biology Dept, Bedford Ave and Ave H, Brooklyn 10, N Y (3, 1934)
- Chenoweth, Maynard Burton**, M D Univ of Michigan Med School, Dept of Pharmacology, Ann Arbor *Assoc Prof of Pharmacology* (3, 1945)
- Chesley, Leon C**, Ph D Margaret Hague Maternity Hospital, 88 Clifton Place, Jersey City 4, N J *Chief Chemist* (1, 1949)
- Chesney, Alan M**, M D Johns Hopkins Hospital, Baltimore, Md *Dean of Johns Hopkins Med School, Assoc Prof of Medicine* (4, 1925)
- Child, George P**, Ph D Albany Med College, Albany 3, N Y *Asst Prof of Pharmacology* (3, 1949)
- Choucroun, Nine**, Dept of Public Health and Preventive Medicine, 1300 York Ave, New York City 22 *Research Assoc, Cornell Med College* (6, 1949)
- Chow, Bacon F**, Ph D Johns Hopkins Univ School of Hygiene, 615 N Wolfe St, Baltimore 14, Md *Assoc Prof of Biochemistry* (2, 1940, 5, 1948, 6, 1944)
- Christensen, Halvor N**, Ph D Tufts College Med School, Boston, Mass *Prof and Head of Dept of Biochemistry* (2, 1947)
- Christensen, L Royal**, Ph D New York Univ College of Medicine, Dept of Microbiology, 477 First Ave, New York City *Asst Prof* (6, 1942)
- Christian, Henry A**, M D 20 Chapel St, Brookline, Mass *Hersey Prof Emeritus of the Theory and Practice of Physics, Harvard Univ, Physician-in-Chief, Emeritus, Peter Bent Brigham Hospital* (4, 1924)
- Christman, Adam A**, Ph D Univ of Michigan Med School, Ann Arbor *Prof of Biological Chemistry* (2, 1929)
- Chu, Wei-chang**, M D 546 W 124th St, Apt 53, New York City (3, 1945)
- Clark, Ada R**, Ph D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 *Assoc in Bacteriology, Teaching and Research* (6, 1936)
- Clark, Byron B**, Ph D Tufts College Med School, 416 Huntington Ave, Boston 15, Mass *Prof of Pharmacology* (3, 1940)
- Clark, Ernest D**, Ph D 826 Skinner Bldg, Seattle 1, Wash *Dir of the Labs, Northwest Branch, Natl Cannery Assoc* (2, 1912)

- Clark, George, Ph D Chicago Med School, Dept of Anatomy, 710 S Wolcott Ave, Chicago 12, Ill *Assoc Prof of Neuroanatomy* (1, 1943)
- Clark, Guy W, Ph D Lederle Labs, Inc, Pearl River, N Y *Tech Dir* (2, 1922)
- Clark, Janet Howell, Ph D Univ of Rochester, Anderson Hall, Rochester, N Y *Dean of the College for Women and Prof in the Div of Biological Sciences* (1, 1922)
- Clark, Paul F, Ph D Univ of Wisconsin Med School, Madison *Prof of Med Microbiology* (4, 1923, 6, 1928)
- Clark, William G, Ph D V A Center, Sawtelle and Wilshire Blvds, Los Angeles, Calif *Pharmacologist and Physiologist, Research Assoc, Univ of Calif School of Medicine* (1, 1942, 3, 1949)
- Clark, William Mansfield, Ph D Johns Hopkins Univ, Baltimore, Md *Prof of Physiological Chemistry* (2, 1920)
- Clarke, Hans Thacher, D Sc Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 *Prof of Biological Chemistry* (2, 1929)
- Clarke, Robert W Route 3, Helmwoods, Elizabethtown, Ky *Physiologist, Med Dept Field Research Lab, Fort Knox* (1, 1936)
- Clausen, Samuel Wolcott, M D Univ of Rochester School of Medicine, Rochester, N Y *Prof of Pediatrics* (2, 1922)
- Cleghorn, Robert Allen, M D, D Sc McGill Univ, Dept of Psychiatry, Montreal, Quebec, Canada (1, 1937)
- Clowes, George Henry Alexander, Ph D Eli Lilly & Co, Indianapolis 6, Ind *Dir of Research Emeritus* (2, 1914, 6, 1919)
- Coburn, Alvin F, M D Rheumatic Fever Research Inst, Chicago, Ill *Director* (6, 1948)
- Code, Charles F, Ph D, M D Mayo Foundation, Rochester, Minn *Prof of Physiology* (1, 1939)
- Coffey, Julia M, A B Div of Labs and Research, New York State Dept of Health, Albany *Assoc Bacteriologist* (6, 1937)
- Coghill, Robert D, Ph D Abbott Labs, N Chicago, Ill *Dir of Research* (2, 1932)
- Cohen, Barnett, Ph D Johns Hopkins Univ School of Medicine, 710 N Washington St, Baltimore 5, Md *Assoc Prof of Physiological Chemistry* (2, 1935)
- Cohen, Philip P, Ph D, M D Univ of Wisconsin, Service Memorial Inst, Madison *Prof of Physiological Chemistry* (2, 1941)
- Cohen, Saul L, Ph D Univ of Minnesota, 207 Millard Hall, Minneapolis *Asst Prof of Physiological Chemistry* (2, 1948)
- Cohen, Seymour S, Ph D Children's Hospital, 1840 Bainbridge St, Philadelphia, Pa *Asst Prof of Physiological Chemistry, Univ of Pennsylvania School of Medicine* (2, 1946)
- Cohen, Sophia M, B S New York State Dept of Health, Div of Labs and Research, Albany *Sr Bacteriologist* (6, 1938)
- Cohn, Alfred E, M D 300 Central Park W, New York City *Member of Rockefeller Inst for Med Research* (1R, 1911, 3, 1913)
- Cohn, Clarence, M D Michael Reese Hospital, Med Research Inst, Chicago, Ill *Dir of Dept of Biochemistry* (1, 1948)
- Cohn, Edwin J, Ph D 183 Brattle St, Cambridge, Mass *Prof of Biological Chemistry, Harvard Med School, Univ Prof, Harvard Univ* (1, 1919, 2, 1919)
- Cohn, Mildred, Ph D Washington Univ School of Medicine, Dept of Biochemistry, 4580 Scott Ave, St Louis 10, Mo *Research Assoc* (2, 1949)
- Cohn, Waldo E, Ph D Oak Ridge Natl Lab, Biology Div, Oak Ridge, Tenn *Biochemist* (2, 1944)
- Cole, Harold H, Ph D Univ of California, College of Agriculture, Div of Animal Husbandry, Davis *Professor* (1, 1947)
- Cole, Harold N, Ph B, M D 1352 Hanna Bldg, Cleveland, Ohio *Clin Prof of Dermatology and Syphilology, Western Reserve Univ* (3, 1925)
- Cole, Kenneth S, Ph D Univ of Chicago, Inst of Radiobiology and Biophysics, Chicago 37, Ill *Prof of Biophysics* (1, 1934)
- Cole, Milton B, M.D 10616 Euclid Ave, Cleveland, Ohio *Dir of Asthma, Hay Fever and Allergy Foundation* (6, 1931)
- Cole, Versa V, Ph D, M D Indiana Univ School of Medicine, 1040-1232 W Michigan St, Indianapolis *Assoc Prof of Pharmacology* (3, 1941)
- Collett, Mary Elizabeth, Ph D Western Reserve Univ, Mather College, Cleveland, Ohio *Assoc Prof of Biology* (1, 1921)
- Collier, H Bruce, Ph D Univ of Alberta, Dept of Biochemistry, Edmonton, Alta, Canada *Prof of Biochemistry* (2, 1944)
- Collings, William Doyne, Ph D Michigan State College, Dept of Physiology, East Lansing (1, 1944)
- Collins, Dean A, Ph D, M D Temple Univ School of Medicine, 3400 N Broad St, Philadelphia 40, Pa *Prof of Pharmacology* (1, 1938)
- Collins, Russell J, A M, M D St John, New Brunswick, Canada *Med Superintendent of St John Tuberculosis Hospital* (3, 1915)
- Collip, J B, Ph D M D Univ of Western Ontario, R R 3, London, Ontario, Canada *Dean of Medicine and Prof of Med Research* (1, 1920, 2, 1920)
- Colowick, Sidney P, Ph D Univ of Illinois College of Medicine, Dept of Biological Chemistry, 1853 W Polk St, Chicago 12 *Assoc Prof* (2, 1944)
- Coman, Dale R, M D Univ of Pennsylvania School of Medicine, McManes Lab of Pathol-

- ogy, Philadelphia *Assoc Prof of Pathology* (4, 1939)
- Comroe, Julius H, Jr**, M D Univ of Pennsylvania Grad School of Medicine, Philadelphia *Prof of Physiology and Pharmacology* (1, 1943, 3, 1939)
- Conant, James B**, Ph D Harvard Univ, 5 Univ Hall, Cambridge, Mass *President* (2, 1932)
- Concepcion, Isabelo**, M D 589 Zamora, Pasay, Rizal, Philippines Faculty of Medicine, Univ Santo Tomas, Manila, Philippines *Prof of Biochemistry and Nutrition* (1, 1919)
- Conklin, Ruth E**, Ph D Vassar College, Poughkeepsie, N Y *Prof of Physiology* (1, 1940)
- Conn, Jerome W**, M D Univ of Michigan Med School, Ann Arbor *Assoc Prof of Internal Med* (5, 1942)
- Consolazio, William V**, BS Office of Naval Research, 23 Argyle Ave, Garrett Park, Md *Head of Biochemistry Branch, Biological Sciences Div* (2, 1949)
- Cook, Donald Hunter**, Ph D Univ of Miami, Coral Gables 34, Fla *Prof of Chemistry* (2, 1929)
- Cooke, Robert A**, A M, M D 60 E 58th St, New York City *Dir of Dept of Allergy, Roosevelt Hospital* (6, 1920)
- Coolidge, Thomas B**, M D, Ph D Univ of Chicago, Abbot Hall, Chicago 37, Ill *Assoc Prof, Dept of Biochemistry and Walter G Zoller Memorial Dental Clinic* (2, 1942)
- Coon, Julius M**, Ph D, M D Univ of Chicago, Dept of Pharmacology, Chicago 37, Ill *Assoc Prof of Pharmacology, Dir of Toxicity Lab* (3, 1941)
- Coons, Callie Mae**, Ph D U S Dept of Agriculture, Bureau of Human Nutrition and Home Economics, Washington, D C *Asst Chief* (5, 1933)
- Cooper, Merlin L**, M D Children's Hospital Research Foundation, Elland Ave and Bethesda, Cincinnati 29, Ohio *Assoc Prof of Bacteriology, Asst Prof of Pediatrics, Univ of Cincinnati College of Medicine* (6, 1949)
- Cooper, Ruth S** Princeton Univ, Dept of Biology, Princeton, N J (6, 1949)
- Cope, Otis M**, M D Univ of Florida, School of Pharmacy, Gainesville (1, 1929)
- Copley, Alfred Lewin**, M D New York Univ, Dept of Biology, Lab of Cellular Physiology, Washington Square, New York City 3 *Research Assoc in Medicine, Asst Clin Prof, New York Med College* (1, 1944)
- Corbin, Kendall B**, M D 919 80th St, S W, Rochester, Minn *Consultant in Neurology, Mayo Clinic, Prof of Neuroanatomy, Mayo Foundation* (1, 1941)
- Corcoran, Arthur Curtis**, C M, M D Cleveland Clinic Foundation, Cleveland 6, Ohio (1, 1940)
- Corey, Edward Lyman**, Ph D Univ of Virginia School of Medicine, University, Va *Asst Prof of Physiology* (1, 1931)
- Cori, Carl F**, M D Washington Univ School of Medicine, Kingshighway and Euclid Ave, St Louis 10, Mo *Prof of Biochemistry* (2, 1925, 3, 1934)
- Cori, Gerty T**, M D Washington Univ School of Medicine, St Louis 10, Mo *Prof of Biochemistry* (2, 1927, 3, 1934)
- Corley, Ralph Conner**, Ph D Purdue Univ, Dept of Chemistry, Lafayette, Ind *Prof of Biochemistry* (2, 1927)
- Corper, Harry J**, M D, Ph D 1295 Clermont St, Denver 7, Colo *Dir of Research, Natl Jewish Hospital and Univ of Colorado School of Medicine* (2, 1912)
- Corson, Samuel A**, Ph D Howard Univ School of Medicine, Dept of Physiology, Washington, D C (1, 1943)
- Cotts, Gerhard K**, M D Lynchburg State Colony, Lynchburg, Va *Clinical Dir* (3, 1937)
- Co Tui, Frank**, M D New York Univ College of Medicine, 477 First Ave, New York City *Assoc Prof of Exper Surgery* (3, 1931)
- Cournand, Andre Frederic**, M D Chest Service, Bellevue Hospital, C D Building, 1st Ave at 28th St, New York City *Assoc Prof of Medicine, College of Physicians and Surgeons, Columbia Univ* (1, 1944)
- Cowgill, George Raymond**, Ph D Yale Univ, Nutrition Lab, 333 Cedar St, New Haven 11, Conn *Prof of Nutrition, Dept of Physiological Chemistry* (1, 1923, 2, 1922, 5, 1933)
- Cox, Alvin J, Jr**, M D Stanford Univ School of Medicine, Dept of Pathology, 2398 Sacramento St, San Francisco 15, Calif *Prof of Pathology* (4, 1949)
- Cox, Gerald J**, Ph D Univ of Pittsburgh School of Dentistry, Pittsburgh 13, Pa *Prof of Dental Research* (2, 1930, 5, 1935)
- Cox, Herald R**, Sc D Lederle Labs Div, Pearl River, N Y *Dir, Viral and Rickettsial Research* (6, 1946)
- Cox, Warren M, Jr**, Ph D Mead Johnson & Co, Evansville, Ind *Dir of Research* (2, 1935, 5, 1945)
- Craig, Francis Northrop**, Ph D Med Div, Army Chemical Center, Md *Physiologist* (1, 1946)
- Craig, L C**, Ph D Rockefeller Inst for Med Research, 66th St and York Ave, New York City 21 *Assoc Member* (2, 1938)
- Crampton, E W**, Ph D McGill Univ, Macdonald College, Montreal, Quebec, Canada *Prof of Nutrition* (5, 1940)
- Crandall, Lathan A, Jr**, M D, Ph D Miles Labs, Inc, Elkhart, Ind (1, 1930, 5, 1940)
- Cranston, Elizabeth M**, Ph D Univ of Minnesota Med School, Dept of Pharmacology, Minneapolis 14 *Instructor* (3, 1946)

- Cravens, W W , Ph D Univ of Wisconsin, Poultry Dept , Madison *Assoc Prof of Poultry Husbandry* (5, 1947)
- Craver, Bradford N , Ph D , M D Ciba Pharmaceutical Products, Inc , Lafayette Park, Summit, N J *Sr Pharmacologist* (3, 1946)
- Crawford, Madeleine Field, Ph D 9 High Rock St , Needham, Mass *Assoc in Physiology, Harvard School of Public Health* (1, 1933)
- Crescitelli, Frederick, Ph D Univ of California, Dept of Zoology, Los Angeles *Physiologist* (1, 1946)
- Cressey, Norman L , M D Veterans Hospital, Newington, Conn (6, 1943)
- Cretcher, Leonard H , Ph D Univ of Pittsburgh, Mellon Inst of Industrial Research, Pittsburgh Pa *Asst Dir and Head of Dept of Research in Pure Chemistry* (2, 1930)
- Crider, Joseph O , M D Jefferson Med College, Philadelphia, Pa *Prof of Physiology* (1, 1935)
- Crisler, George R , Ph D , M D 157 E New England Ave , Winter Park, Fla (1, 1930)
- Crismon, Jefferson Martineau, M D Stanford Univ , Calif *Assoc Prof of Physiology* (1, 1944)
- Crittenden, Phoebe J , Ph D Goucher College, Dept of Physiology and Hygiene, Towson 4, Md (1, 1937, 3, 1937)
- Crozier, William J , Ph D Harvard Univ , Biological Labs , Cambridge, Mass *Prof of General Physiology* (1, 1928)
- Csonka, F A , Ph D U S Dept of Agriculture, Bureau of Human Nutrition and Home Economics, Beltsville, Md *Sr Chemist* (2, 1924)
- Cullen, Stuart C , M D Univ Hospital, Iowa City, Iowa *Asst Prof of Surgery-Anesthesia* (3, 1944)
- Cunningham, Raymond W , Ph D Lederle Labs , Inc , Pearl River, N Y *Head, Pharmacology Research* (3, 1941)
- Cunningham, Robert Sydney, M D , Sc D Albany Med College, Albany, N Y *Prof of Anatomy and Dean* (1, 1923)
- Cureton, Thomas Kirk, Jr , Ph D Univ of Illinois, School of Physical Education, Urbana *Assoc Prof of Physical Education* (1, 1946)
- Curnen, Edward C , M D Yale Univ School of Medicine, New Haven 11, Conn (6, 1941)
- Curtis, George Morris, Ph D , M D Ohio State Univ , Kinsman Hall, Columbus *Prof of Surgery, Chairman, Dept of Research Surgery* (1, 1933, 4, 1933)
- Curtis, Howard J , Ph D Vanderbilt Univ School of Medicine, Nashville 4, Tenn *Prof of Physiology* (1, 1940)
- Cutting, Reginald A , M D , Ph D Georgetown Univ School of Medicine, 3900 Reservoir Rd , N W , Washington, D C *Prof of Physiology and Dir of the Dept* (1, 1939)
- Cutting, Windsor C , M D Stanford Univ School of Medicine, San Francisco, Calif *Prof of Therapeutics* (3, 1939)
- Daft, Floyd Shelton, Ph D Natl Insts of Health, Bethesda, Md *Chief, Biochemistry and Nutrition Lab , Asst Dir , Exper Biology and Medicine Inst* (2, 1949, 5, 1941)
- Daggs, Ray Gilbert, Ph D Med Dept , Field Research Lab , Fort Knox, Ky *Dir of Research* (1, 1935, 5, 1933)
- Dakin, Henry D , D Sc , Ph.D Scarborough-on-Hudson, N Y (2, 1906)
- Dalldorf, Gilbert, M D New York State Dept of Health, Albany *Dir , Div of Labs and Research* (4, 1947)
- Dalton, Albert J , Ph D Natl Cancer Inst , Bethesda, Md *Principal Cytologist* (4, 1942)
- Dam, Henrik, Ph D Biologisk Afdeling Danmarks Tekniske Højskole, Østervoldgade 10 L, Copenhagen, K, Denmark *Professor* (2, 1944, 5, 1943)
- D'Amour, Fred E , Ph D 2311 S Josephine St , Denver, Colo *Assoc Prof , Dept of Zoology, Univ of Denver* (1, 1934)
- D'Amour, Marie C , Ph D , M D 2311 S Josephine St , Denver, Colo (1, 1934)
- D'Angelo, Savino A , Ph D New York Univ , Dept of Biology, Washington Square, New York City 3 *Asst Prof of Biology* (1, 1947)
- Daniel, Louise J , Ph D Cornell Univ , Savage Hall, Ithaca, N Y *Asst Prof of Biochemistry* (2, 1949)
- Daniels, Amy L , Ph D 720 N Van Buren St , Iowa City, Iowa (2, 1919, 5, 1933)
- Danielson, Irvin S , Ph D Lederle Labs , Amer Cyanamid Co , 18 Bogert Ave , Pearl River, N Y *Head, Biol Production, Animal Industry Section* (2, 1937)
- Danowski, T S , M D Univ of Pittsburgh School of Medicine, Pittsburgh, Pa *Renzhausen Prof of Research Medicine* (1, 1947)
- Darby, William J , M D , Ph D Vanderbilt Univ School of Medicine, Div of Nutrition, Nashville, Tenn *Prof of Biochemistry, Asst Prof of Medicine* (2, 1949, 5, 1945)
- Darling, Robert Croly, M D 157 Glenwood Ave , Leonia, N J Columbia Univ College of Physicians and Surgeons, Dept of Medicine, New York City 32 (1, 1944)
- Darrow, Chester W , Ph D Inst for Juvenile Research, 907 S Wolcott St , Chicago, Ill *Research Psychologist, Assoc in Physiology, Univ of Illinois College of Medicine* (1, 1937)
- Darrow, Daniel Cady, M D Yale Univ School of Medicine, New Haven, Conn *Prof of Pediatrics* (2, 1936)
- Daubert, B F , Ph D Univ of Pittsburgh, 220 Alumni Hall, Dept of Chemistry, Pittsburgh 13, Pa *Research Prof and Research Admin* (2, 1947)
- Davenport, Horace Willard, Ph D Univ of Utah,

- Dept of Physiology, Salt Lake City 1 (1, 1942)
- David, Norman Austin**, M D Univ of Oregon Med School, Portland *Prof of Pharmacology* (3, 1934)
- Davidsohn, Israel**, M D Mount Sinai Hospital, Chicago, Ill *Pathologist and Dir of Labs, Chicago Med School, Prof of Pathology and Chairman of Dept* (4, 1939, 6, 1929)
- Davidson, Charles S**, M D Boston City Hospital, Thorndike Memorial Lab, Boston 18, Mass *Assoc in Medicine, Harvard Med School, Assoc Dir, 2nd and 4th Med Services, Boston City Hospital* (5, 1949)
- Davies, Philip W**, Ph D Johns Hopkins Univ, Biophysics Dept, Baltimore 18, Md (1, 1948)
- Davis, Bernard D**, M D Cornell Univ Med College, New York City *Research Assoc, Surgeon, USPHS* (6, 1948)
- Davis, George Kelso**, Ph D Nutrition Lab, Animal Industry Dept, Agricultural Exper Station, Gainesville, Fla *Nutritional Technologist and Biochemist, Prof of Nutrition, Univ of Florida, Florida Agricultural Exper Station* (5, 1944)
- Davis, Hallowell**, M D Central Inst for the Deaf, 818 S Kingshighway, St Louis 10, Mo (1, 1925)
- Davis, Harry A**, M D College of Med Evangelists, Dept of Surgery, 2007 Wilshire Blvd, Los Angeles 5, Calif (4, 1944)
- Davis, John Emerson**, Ph D Univ of Arkansas School of Medicine, Little Rock *Prof of Pharmacology and Physiology* (1, 1941, 3, 1941)
- Dawson, Charles R**, Ph D Columbia Univ, 411 Havemeyer Hall, New York City 27 *Assoc Prof of Chemistry* (2, 1946)
- Dawson, James Robertson, Jr**, M D Univ of Minnesota School of Medicine, Minneapolis 14 *Prof of Pathology* (4, 1940)
- Day, Harry G**, D Sc Indiana Univ, Dept of Chemistry, Bloomington *Assoc Prof* (2, 1948, 5, 1940)
- Day, Paul L**, Ph D Univ of Arkansas School of Medicine, Little Rock *Prof of Biochemistry* (2, 1934, 5, 1933)
- De, N N**, M B Indian Inst of Science, P O Malleswaram, Bangalore, India *Asst Prof of Pharmacology* (3, 1948)
- Dearborn, Earl H**, Ph D Johns Hopkins Univ School of Medicine, 800 N Washington St, Baltimore 5, Md *Instr in Pharmacology and Exper Therapeutics* (3, 1946)
- de Beer, Edwin J**, Ph D The Wellcome Research Labs, Tuckahoe, N Y *Asst Dir of Research* (3, 1944)
- De Bodo, Richard C**, M D 477 First Ave, New York City *Assoc Prof of Pharmacology, New York Univ College of Medicine* (1, 1932, 3, 1931)
- De Boer, Benjamin**, Ph D St Louis Univ School of Medicine, 1402 S Grand Blvd, St Louis 4, Mo *Asst Prof of Pharmacology* (1, 1947, 3, 1948)
- DeEds, Floyd**, Ph D 344 Santa Ana Ave, San Francisco, Calif *Principal Pharmacologist, Western Regional Research Lab, Albany, Calif* (2, 1937, 3, 1927)
- Defandorf, James Holmes**, Ph D Office of the Chief of the Chemical Warfare Service, Washington, D C *Colonel, Sn C* (3, 1940)
- de Gara, Paul F**, M D 200 Pinehurst Ave, New York City *Instr in Pathology, Cornell Univ Med College, Physician, New York Hospital* (6, 1941)
- DeGraff, Arthur C**, M D New York Univ College of Medicine, New York City *Prof of Therapeutics* (3, 1937)
- de Gutierrez-Mahoney, C G**, M D St Vincent's Hospital, New York City *Dir, Neurological Div and Neurosurgeon-in-Chief* (1, 1940, 4, 1941)
- Deichmann, William B**, Ph D Albany Med College, Albany, N Y *Assoc Prof and Head, Div of Pharmacology* (3, 1941)
- del Pozo, E C**, M D Medellin 196, Mexico, D.F., Mexico (1, 1943)
- Dempsey, Edward W**, Ph D Harvard Med School, Boston, Mass *Assoc Prof of Anatomy* (1, 1940)
- Denslow, J S**, D O Kirksville College of Osteopathy, Still Memorial Research Trust, Kirksville, Mo *Researcher* (1, 1949)
- Denstedt, Orville F**, Ph D McGill Univ, Dept of Biochemistry, Montreal, Que, Canada *Assoc Prof* (2, 1948)
- Derbyshire, Arthur J**, Ph D Harper Hospital, EEG Dept, Detroit, Mich (1, 1939)
- de Savitsch, Eugene**, M D Suite 24, 1150 Connecticut Ave, Washington, D C *Clinical Instr in Surgery, Georgetown Univ School of Medicine, Surgeon, Doctors Hospital* (4, 1934)
- Dettwiler, Herman A**, Ph D Eli Lilly and Co, Indianapolis, Ind *Asst Dir, Biological Div* (6, 1946)
- Deuel, Harry J, Jr**, Ph D Univ of Southern California, Los Angeles *Prof of Biochemistry and Dean of Grad School* (1, 1928, 2, 1924, 5, 1933)
- Deulofeu, Venancio D** Chem Casilla Correo 2539, Buenos Aires, Argentina *Prof of Organic Chemistry, Univ of Buenos Aires* (2, 1942)
- Deutsch, Harold F**, Ph D Univ of Wisconsin, Dept of Physiological Chemistry, Madison 6 *Assoc Prof* (2, 1948)
- Dewey, Virginia C**, Ph D Amherst College, Biological Lab, Amherst, Mass *Research Assoc, Dept of Biology* (2, 1949)

- Dey, Frederick L, Ph D, M D Box 11, Submarine Base, New London, Conn *Lt (j g)*, *U S N R* (1, 1945)
- Deyrup, Ingrith J, Ph D Barnard College, Columbia Univ, Dept of Zoology, New York City 27 *Asst Prof* (1, 1949)
- Dickison, H L, Ph D Bristol Labs, Inc, Bldg 6, Syracuse 1, N Y (3, 1946)
- Dieckmann, William J, M D The Chicago Lying-In Hospital, 5841 Maryland Ave, Chicago 37, Ill *Mary Campan Ryerson Prof and Chairman of Dept of Obstetrics and Gynecology, Univ of Chicago* (3, 1947)
- Dienes, Louis, M D Massachusetts General Hospital, Boston *Bacteriologist* (6, 1924)
- Dill, David Bruce, Ph D Med Div, Army Chemical Center, Md *Scientific Dir* (1, 1941, 2, 1927, 5, 1936)
- Dille, James M, Ph D, M D Univ of Washington School of Medicine, Seattle 5 *Prof of Pharmacology, Asst Dean* (3, 1939)
- Dillon, Robert T, Ph D G D Searle and Co, Box 5110, Chicago 80, Ill *Head, Analytical Div* (2, 1934)
- Dingle, John H, Sc D, M D Western Reserve Univ School of Medicine, Cleveland 6, Ohio *Prof of Preventive Medicine* (6, 1941)
- Di Palma, Joseph R, M D Long Island College of Medicine, 350 Henry St, Brooklyn, N Y *Assoc in Physiology* (1, 1943)
- Dische, Zacharias, M D Columbia Univ College of Physicians and Surgeons, Dept of Biochemistry, 630 W 168th St, New York City 32 (2, 1944)
- Dixon, Harold M, M D Army Inst of Pathology, Washington 25, D C (4, 1936)
- Doan, Charles A, M D Ohio State Univ College of Medicine, Columbus *Dean, Prof of Medicine* (4, 1928)
- Dobriner, Konrad, M D Sloan-Kettering Inst, 444 E 68th St, New York City 21 *Member* (2, 1946)
- Dodds, Mary L, Ph D Pennsylvania State College, State College *Prof, Foods and Nutrition Research* (5, 1948)
- Dohan, F Curtis, M D 80 Princeton Rd, Cnywyd, Pa *Fellow, George S Cox Med Research Inst, Assoc in Medicine, Univ of Pennsylvania* (1, 1941)
- Doisy Edward A, Ph D St Louis Univ School of Medicine, St Louis 4, Mo *Prof of Biological Chemistry* (2, 1920)
- Dolman, C E, D P H, Ph D Univ of British Columbia, Vancouver, B C, Canada *Head of Dept of Bacteriology and Preventive Medicine* (6, 1947)
- Dominguez, Rafael, M D Western Reserve Univ, Cleveland, Ohio *Assoc in Pathology, Dir of Labs and Research, St Luke's Hospital* (1, 1935)
- Donahue, D D, D Sc Natl Insts of Health, Div of Industrial Hygiene, Bethesda, Md *Physiologist, Toxicology Section* (3, 1941)
- Dorfman, Albert, Ph D, M D Univ of Chicago, Dept of Pediatrics, Chicago, Ill *Asst Prof* (2, 1948)
- Dorfman, Ralph I, Ph D Western Reserve Univ School of Medicine, Dept of Biochemistry, Cleveland, Ohio *Asst Prof of Biochemistry* (2, 1940)
- Dotti, Louis Basil, Ph D St Luke's Hospital, Amsterdam Ave and 113th St, New York City *Chemist, Lecturer in Physiology and Biochemistry, New York Med College* (1, 1937)
- Doty, J Roy, Ph D American Dental Assoc, 222 E Superior St, Chicago, Ill *Sr Chemist* (2, 1941)
- Doudoroff, Michael, Ph D Univ of California, Dept of Bacteriology, 3519 Life Sciences Bldg, Berkeley 4 *Assoc Prof* (2, 1946)
- Dounce, Alexander L, Ph D Strong Memorial Hospital, 260 Crittenden Blvd, Rochester N Y *Instr in Biochemistry, Univ of Rochester School of Medicine and Dentistry* (2, 1944)
- Doupe, Joseph, M D Medical College, Dept of Physiology and Med Research, Bannatyne and Emily, Winnipeg, Manitoba, Canada *Professor* (1, 1949)
- Dow, Philip, Ph D Univ of Georgia School of Medicine, Augusta *Assoc Prof of Physiology* (1, 1939)
- Dow, Robert S, M D, Ph D Univ of Oregon Med School, Portland, *Assoc Prof of Anatomy* (1, 1940)
- Downs, Ardrey W, M D, D Sc Univ of Alberta, Edmonton, Alberta, Canada *Prof of Physiology and Pharmacology* (1, 1917)
- Downs, Cora M, Ph D 1625 Alabama St, Lawrence, Kan (6, 1929)
- Doyle, William Lewis, Ph D 930 E 58th St, Chicago 37, Ill *Assoc Prof of Anatomy, Univ of Chicago* (1, 1946)
- Drabkin, David L, M D Univ of Pennsylvania Grad School of Medicine, Philadelphia 4 *Prof and Chairman of Dept of Physiological Chemistry* (2, 1928, 5, 1934)
- Dragstedt, Carl A, Ph D, M D Northwestern Univ Med School, 303 E Chicago Ave, Chicago, Ill *Prof of Pharmacology* (1, 1928, 3, 1932)
- Dragstedt, Lester R, M D, Ph D Univ of Chicago, Chicago, Ill *Prof of Surgery* (1, 1920)
- Draize, J H, Ph D Food and Drug Admin, Div of Pharmacology, Federal Security Agency, Washington, D C *Pharmacologist* (3, 1940)
- Drake, T G H, M B Univ of Toronto, Toronto, Canada *Jr Demonstrator in Pediatrics, Dept*

- of Medicine, Assoc Dir, Research Lab, Hospital for Sick Children (5, 1936)
- Draper, William B**, M Sc, M D Univ of Colorado School of Medicine, 4200 E 9th Ave, Denver Assoc Prof of Physiology and Pharmacology (1, 1947, 3, 1938)
- Dreisbach, Robert H**, Ph D, M D Stanford Univ School of Medicine, San Francisco 15, Calif Asst Prof (3, 1945)
- Dreyer, Nicholas Bernard**, M A Univ of Vermont School of Medicine, Burlington Assoc Prof of Physiology and Pharmacology (3, 1942)
- Drill, Victor Alexander**, Ph D, M D Wayne Univ College of Medicine, Detroit 26, Mich Prof of Pharmacology (1, 1943, 3, 1946)
- Drinker, Cecil K**, M D P O Box 502, Falmouth, Mass (1, 1915)
- Dripps, Robert D**, M D Univ of Pennsylvania School of Medicine, Philadelphia 4 Prof of Anesthesiology, Lecturer in Pharmacology (1, 1947, 3, 1946)
- Driver, Robert L**, M D, Ph D Humboldt, Tennessee (1, 1945, 3, 1947)
- Drury, Douglas R**, M D Univ of Southern California, Los Angeles Prof of Physiology (1, 1932)
- Dubin, Harry E**, Ph D 11 W 42nd St, New York City 18 President, H E Dubin Labs, Inc (2, 1925)
- Dubin, Isadore N**, M D Natl Cancer Inst, Bethesda, Md (4, 1947)
- Dubnoff, Jacob W**, Ph D 1201 E California St, Pasadena 4, Calif Sr Research Fellow, California Inst of Technology (2, 1946)
- DuBois, Eugene F**, M D Cornell Univ Med School, 1300 York Ave, New York City Prof and Head of Dept of Physiology and Biophysics, Attending Physician, New York Hospital (1, 1913, 3, 1921, 5, 1935)
- Du Bois, Kenneth P**, Ph D Univ of Chicago, Dept of Pharmacology, Chicago 37, Ill Asst Prof of Pharmacology (3, 1946)
- Dubos, Rene J**, Ph D, D Sc Rockefeller Inst for Med Research, 66th St and York Ave, New York City Head of Dept of Bacteriology (6, 1938)
- Dugal, L Paul**, Ph D Laval Univ Med School, Research Dept on Acclimatization, Quebec City, Canada Research Prof and Head of Dept, Assoc Dir of Inst of Hygiene (1, 1947)
- Dukes, H H**, D V.M, M S Cornell Univ, New York State Veterinary College, Ithaca Prof of Veterinary Physiology (1, 1934)
- Dulaney, Anna D**, Ph D Univ of Tennessee, Pathological Inst, Memphis Assoc Prof of Bacteriology, Med School (6, 1924)
- Dumke, Paul Rudolph**, M D Clinical Research Section, Med Research Lab, Army Chemical Center, Md Instr in Pharmacology, Univ of Pennsylvania, Captain, M C (3, 1942)
- Dunlap, Charles E**, M D Tulane Univ of Louisiana, 1430 Tulane Ave, New Orleans Prof of Pathology (4, 1942)
- Dunn, Max Shaw**, Ph D Univ of California, Los Angeles Prof of Chemistry (2, 1933)
- Dunn, Thelma Brumfield**, M D Natl Cancer Inst, Bethesda, Md Pathologist (4, 1945)
- Durlacher, Stanley H** Med Div, Army Chemical Center, Md Chief, Section on Pathology (1, 1949)
- Dury, Abraham**, Ph D Dorn Lab for Med Research, Bradford Clinic, Bennett at Pleasant St, Bradford, Pa Asst Dir of Research (1, 1948)
- Dutcher, James D**, Ph D Squibb Inst for Med Research, New Brunswick, N J Research Assoc, Div of Organic Chemistry (2, 1946)
- Dutcher, R Adams**, D Sc Pennsylvania State College, State College Prof and Head of Dept of Agricultural Biochemistry (2, 1920, 5, 1933)
- Duval, Charles Warren**, M D Sacred Heart Hospital, Pensacola, Fla Prof Emeritus of Pathology and Bacteriology, Tulane Univ (4, 1913)
- du Vigneaud, Vincent**, Ph D Cornell Univ Med College, 1300 York Ave, New York City 21 Prof of Biochemistry (2, 1929, 5, 1934)
- Dworkin, Simon**, D D S, M D McGill Univ, Biology Building, Montreal, Quebec, Canada Lecturer in Physiology, Faculty of Medicine (1, 1931)
- Dye, J A**, Ph D Cornell Univ, James Law Hall Ithaca, N Y Prof of Physiology (1, 1929)
- Dye, Marie**, Ph D Michigan State College, East Lansing Dean, School of Home Economics (2, 1929, 5, 1933)
- Dyer, Helen M**, Ph D Natl Cancer Inst Bethesda, Md Biochemist (2, 1936, 5, 1937)
- Dziemian, Arthur J**, Ph D Med Div, Army Chemical Center, Md Physiologist, Biophysics Section (1, 1948)
- Eadie, George S**, Ph D Duke Univ School of Medicine, Box 3709, Durham, N C Prof of Physiology and Pharmacology (1, 1929, 3, 1940)
- Eagle, Harry**, M D Natl Insts of Health, Bethesda, Md Chief of Section on Exper Therapeutics, Microbiological Inst (3, 1946, 4, 1936, 6, 1946)
- Eakin, Robert E**, Ph D Univ of Texas, Dept of Chemistry, Austin Assoc Prof of Chemistry (2, 1948)
- Earle, D P, Jr**, M D, Med Sc D New York Univ College of Medicine, 477 First Ave, New York City 16 Assoc Prof of Medicine (1, 1947)
- Earle, Wilton R**, Ph D Natl Cancer Inst, Bethesda, Md Cytologist, Head of Tissue Culture Unit (4, 1940)
- Eaton, Monroe D**, M D Harvard Med School, Boston, Mass Assoc Prof of Bacteriology and Immunology (6, 1937)

- Eckenhoff, James E, M D Hospital of the Univ of Pennsylvania, Philadelphia *Assoc in Clinical Pharmacology and Anesthesiology* (1, 1948)
- Ecker, E E, Ph D Western Reserve Univ School of Medicine, 2085 Adelbert Rd, Cleveland, Ohio *Prof of Immunology* (4, 1925, 6, 1947)
- Eckstein, Gustav, M D Univ of Cincinnati College of Medicine, Cincinnati, Ohio *Assoc Prof of Physiology* (1, 1948)
- Eckstein, Henry C, Ph D Univ of Michigan, 320 W Med Building, Ann Arbor *Assoc Prof of Biological Chemistry* (2, 1925)
- Eckstein, R W, M A, M D Western Reserve Univ, Dept of Medicine, Cleveland, Ohio *Sr Instr, in charge of Cardiovascular Exper Med Research Lab* (1, 1947)
- Eddy, Bernice E, Ph D Natl Insts of Health, Inst of Microbiology, Bethesda 14, Md *Sr Bacteriologist* (6, 1949)
- Eddy, Nathan B, M D Natl Insts of Health, Bethesda, Md *Principal Pharmacologist* (3, 1929)
- Eddy, Walter H, Ph D Box 969 Lake Worth, Fla *Scientific Dir, American Chlorophyll, Inc* (2, 1913, 5, 1933)
- Edelmann, Abraham, Ph D Brookhaven Natl Labs, Upton, L I, N Y *Physiologist* (1, 1948)
- Ederstrom, Helge E, Ph D St Louis Univ School of Medicine, Dept of Physiology, 1402 S Grand Blvd, St Louis 4, Mo *Asst Prof* (1, 1949)
- Edholm, O G, M B Univ of Western Ontario Med School, London, Ontario, Canada *Head, Dept of Physiology* (1, 1948)
- Edsall, Geoffrey, M D Boston Univ School of Medicine, 80 E Concord St, Boston 18, Mass *Prof of Bacteriology* (6, 1943)
- Edsall, John Tileston, M D Harvard Med School, Boston, Mass *Assoc Prof of Biological Chemistry, Tutor in Biochemical Sciences* (2, 1931)
- Edwards, Dayton J, Ph D Cornell Univ Med College, 1300 York Ave, New York City *Assoc Prof of Physiology, Asst Dean* (1, 1921)
- Edwards, Jesse E, M D Mayo Clinic, Rochester 4, Minn *Asst Prof of Pathologic Anatomy, Mayo Foundation* (4, 1941)
- Edwards, J Graham, Ph D 24 High St, Buffalo, N Y *Assoc Prof of Anatomy, Univ of Buffalo* (1, 1932)
- Eggerth, Arnold H, Ph D Hoagland Lab, 335 Henry St, Brooklyn, N Y *Assoc Prof of Bacteriology, Long Island College of Medicine* (4, 1925)
- Ehrenstein, Maximilian R, Ph D Univ of Pennsylvania Hospital, 806 Maloney Clinic, 36th and Spruce Sts, Philadelphia 4 *Assoc Prof of Physiological Chemistry assigned to Medicine, Univ of Pennsylvania School of Medicine* (2, 1942)
- Ehrlich, William E, M D Univ of Pennsylvania, Philadelphia *Prof of Pathology, Grad School of Medicine, Chief, Div of Pathology, Philadelphia General Hospital* (4, 1945)
- Eichelberger, Lillian, Ph D Univ of Chicago, Dept of Surgery, Chicago, Ill *Assoc Prof of Biochemistry* (2, 1937)
- Eichna, Ludwig W, M D New York Univ College of Medicine, Dept of Medicine, 477 First Ave, New York City 16 *Assoc Prof of Medicine, Visiting Physician, Bellevue Hospital* (1, 1949)
- Eiler, John J, Ph D Univ of California Med Center, San Francisco 22 *Assoc Prof of Biochemistry and Pharmacology, Univ of California College of Pharmacy* (2, 1948)
- Eisenman, Anna J, Ph D U S Public Health Service Hospital, Lexington, Ky *Biological Chemist* (2, 1930)
- Elberg, Sanford S, Ph D Univ of California, Berkeley *Assoc Prof of Bacteriology* (6, 1948)
- Elderfield, Robert C, Ph D Columbia Univ, New York City *Prof of Chemistry* (2, 1934)
- Elftman, Herbert, Ph D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City *Assoc Prof in Anatomy*, (1, 1940)
- Eliot, Martha M, M D World Health Organization, Palais des Nations, Geneva, Switzerland. *Asst Dir General* (5, 1933)
- Elkinton, J Russell, M D Univ of Pennsylvania Hospital, Dept of Medicine Philadelphia (1, 1947)
- Elliott, Henry W, Ph D Univ of California Med School, San Francisco *Instr in Pharmacology and Lecturer in Dental Pharmacology and Toxicology* (3, 1948)
- Elliott, K Allan C, Ph D Montreal Neurological Inst, 3801 Univ St, Montreal, Que, Canada *Asst Prof of Exper Neurology and Biochemistry* (2, 1937)
- Ellis, C H, Ph D Labs of Gordon A Alles, 770 S Arroyo Parkway, Pasadena 5, Calif *Research Assoc in Physiology* (1, 1947)
- Ellis, Fred W, Ph D Univ of North Carolina, Chapel Hill *Asst Prof of Pharmacology* (3, 1945)
- Ellis, Lillian N, Ph D Adelphi College, Garden City, N Y *Instr in Chemistry* (5, 1940)
- Ellis, Max Mapes, Ph D Univ of Missouri Med School, Columbia *Prof of Physiology and Pharmacology* (1, 1923)
- Ellis, N R, M S U S Dept of Agriculture, Bureau of Animal Industry, Beltsville, Md *1st Chief, Animal Husbandry Div* (2, 1928, 5, 1933)

- Ellis, Sidney, Ph D Temple Univ Med School, Dept of Pharmacology Philadelphia, Pa (3, 1947)
- Elrod, Ralph P, Ph D Univ of South Dakota Med School, Vermillion *Chairman of Dept of Microbiology* (6, 1947)
- Elser, William J, M D Kent, Conn (6, 1920)
- Elvehjem, Conrad Arnold, Ph D Univ of Wisconsin, Biochemistry Bldg, Madison *Chairman, Dept of Biochemistry, Dean of Grad School* (2, 1931, 5, 1933)
- Embree, Norris Dean, Ph D Distillation Products, Inc, 755 Ridge Rd W, Rochester 13, N Y *Dir of Research* (2, 1946)
- Emerson, George A, Ph D Univ of Texas, Med Branch, Galveston *Prof of Pharmacology* (3, 1935)
- Emerson, Gladys A, Ph D Merck Inst of Therapeutic Research, Rahway, N J *Nutritionist* (5, 1942)
- Emerson, Oliver H, Ph D U S Dept of Agriculture, Western Regional Research Lab, Albany 6, Calif *Assoc Chemist* (2, 1938)
- Emery, Frederick E, D V M, Ph D Univ of Arkansas School of Medicine, Little Rock *Prof of Physiology and Pharmacology* (1, 1930)
- Emmart, Emily W, Ph D Natl Insts of Health, Bethesda 14, Md (3, 1947)
- Emmett, Arthur D, Ph D Star Route 1, Lewiston, Mich (2, 1908, 5, 1933)
- Enders, John F, Ph D Harvard Univ Med School, Dept of Bacteriology, Boston, Mass *Assoc Prof of Bacteriology and Immunology, Chief, Research Div of Infectious Diseases, Children's Hospital* (6, 1936)
- Endicott, Kenneth M, M D USPHS, Natl Insts of Health, Bethesda 14, Md *Chief of Section on Metabolic and Degenerative Disease* (4, 1947)
- Engel, Frank L, M D Duke Univ School of Medicine, Durham, N C *Asst Prof of Medicine* (1, 1947)
- Engel, Lewis L, Ph D, Massachusetts General Hospital, Huntington Labs, Boston *Research Assoc in Biological Chemistry, Harvard Med School, Tutor in Biological Sciences, Harvard Univ* (2, 1949)
- Engel, Ruben W, Ph D Alabama Polytechnic Inst, Lab of Animal Nutrition, Auburn *Animal Nutritionist* (5, 1948)
- Engle, Earl Theron, Ph D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City *Prof of Anatomy* (1, 1930)
- English, James, Jr, Ph D Yale Univ, Sterling Chemistry Lab, New Haven, Conn *Asst Prof of Chemistry* (2, 1946)
- Enright, John B, Ph D State of California Dept of Public Health, Berkeley *Supervising Bacteriologist* (6, 1948)
- Epstein, Albert A, M D 1111 Madison Ave, New York City *Physician, Beth Israel Hospital and Hospital for Joint Diseases* (2, 1912)
- Ercoli, N, M D, Ph D Warner Inst for Therapeutic Research, New York City 11 *Dir of Pharmacological Research* (3, 1947)
- Erickson, Cyrus C, M D Duke Univ School of Medicine, Durham, N C *Assoc Prof of Pathology* (4, 1941)
- Erickson, John Otto, Ph D Veterans Admin Center, Radioisotope Lab, Wilshire and Sawtelle Blvds, Los Angeles 25, Calif (6, 1946)
- Eschenbrenner, Allen B, M D Natl Insts of Health, Bethesda 14, Md (4, 1946)
- Espe, Dwight L, Ph D State College Station, Dept of Dairy Husbandry, Fargo, N D (1, 1940)
- Essex, Hiram E, Ph D Mayo Foundation, Inst of Exper Medicine, Rochester, Minn *Prof of Physiology* (1, 1932, 3, 1940)
- Ettlinger, C H, M D, C M Queen's Univ, Kingston, Ontario, Canada *Prof of Physiology, Asst Dir, Div of Med Research, Natl Research Council, Ottawa* (1, 1943)
- Evans, Earl Alison, Jr, Ph D Univ of Chicago, Dept of Biochemistry, Chicago, Ill *Prof and Chairman of Dept* (2, 1939)
- Evans, Everett Idris, M D, Ph D Med College of Virginia, Richmond *Prof of Surgery, Dir, Lab of Surgical Research* (1, 1935)
- Evans, Gerald Taylor, M D, Ph D Univ of Minnesota Hospitals, Minneapolis *Dir of Lab Service, Assoc Prof of Medicine, Univ of Minnesota* (1, 1942)
- Evans, Herbert M, M D Univ of California, Berkeley *Prof of Anatomy and Dir of Inst of Exper Biology* (1, 1919)
- Evans, Titus C, Ph D State Univ of Iowa, College of Medicine, Iowa City *Research Prof, Head of Radiation Research Lab* (1, 1949)
- Eveleth, D F, Ph D, D V M North Dakota Agricultural College, Fargo *Prof of Veterinary Science, North Dakota Agricultural Exper Station* (2, 1939)
- Everett, Mark R, Ph D Univ of Oklahoma School of Medicine, 800 N E 13th St, Oklahoma City 4 *Dean, School of Medicine, Supt of Univ Hospitals* (2, 1929)
- Everson, Gladys, Ph D Iowa State College, Ames *Assoc Prof of Nutrition* (5, 1948)
- Ewing, P L, Ph D Univ of Texas School of Medicine, Galveston *Assoc Prof of Pharmacology* (3, 1938)
- Eyster, John A English, M D Univ of Wisconsin, Madison *Prof of Physiology* (1, 1906, 3R, 1908)
- Fahr, George, M D Univ of Minnesota, 102 Millard Hall, Minneapolis *Prof of Clinical Medicine* (1, 1913, 3, 1940)

- Failey, Crawford F, Ph D 416 S 6th St, Terre Haute, Ind *Prof of Biochemistry, Univ of Chicago* (2, 1933)
- Fairhall, Lawrence T, Ph D USPHS, Washington, D C *Principal Industrial Toxicologist* (2, 1924)
- Falk, Carolyn R, B A 40 E 66th St, New York City *Bacteriologist, Bureau of Labs, Dept of Health* (6, 1943)
- Falk, K George, Ph D 40 E 66th St, New York City *Dir, Lab of Industrial Hygiene* (2, 1913)
- Farah, Alfred E, M D Univ of Washington School of Medicine, Seattle *Asst Prof of Pharmacology* (3, 1948)
- Farber, Sidney, M D Harvard Med School, 25 Shattuck St, Boston, Mass *Asst Prof of Pathology* (4, 1934)
- Farmer, Chester J, A M Northwestern Univ Med School, 303 E Chicago Ave, Chicago, Ill *Prof of Chemistry* (2, 1935)
- Farr, Lee E, M D Brookhaven Natl Lab, Upton, L I, N Y *Dir, Dept of Med Sciences, Physician-in-Chief, Brookhaven Natl Lab Hospital* (4, 1941)
- Farrar, George E, Jr, M D Wyeth Inc, 1600 Arch St, Philadelphia 3, Pa *Med Dir* (3, 1947)
- Farrell, James L, Ph D, M D 636 Church St, Evanston, Ill (1, 1938)
- Fassett, David W, M D Eastman Kodak Co, Lab of Industrial Medicine, Kodak Park Works, Rochester, N Y (3, 1942)
- Fay, Marion, Ph D Woman's Med College of Pennsylvania, Philadelphia 29 *Dean, Prof of Physiological Chemistry* (2, 1937)
- Featherstone, Robert M, Ph D State Univ of Iowa, College of Medicine, Iowa City *Asst Prof of Pharmacology* (3, 1947)
- Feigen, George A, Ph D Stanford Univ, Dept of Physiology, Stanford, Calif *Instructor* (1, 1948)
- Feldman, Harry A, M D 704 Crawford Ave, Syracuse 10, N Y (6, 1943)
- Feldman, William H, D V M, M S Mayo Foundation, Rochester, Minn *Prof in the Div of Exper Surgery and Pathology* (4, 1934)
- Fell, Norbert, Ph D Camp Detrick, Frederick, Md *Chief, Pilot Plant Div* (6, 1944)
- Feller, A E, M D Western Reserve School of Medicine, Cleveland 6, Ohio *Assoc Prof of Preventive Medicine* (6, 1943)
- Fellows, Edwin J, Ph D Temple Univ School of Medicine, Philadelphia, Pa *Asst Prof of Pharmacology* (3, 1939)
- Felsenfeld, Oscar, M D, D T M & H Hektoen Research Inst, 629 South Wood St, Chicago 12, Ill *Dir of Bacteriology* (6, 1949)
- Felton, Lloyd D, M D, D Sc USPHS, Natl Insts of Health, Bethesda, Md *Med Dir* (6, 1926)
- Fenn, Wallace Osgood, Ph D Univ of Rochester School of Medicine and Dentistry, 260 Crittenden Blvd, Rochester, N Y *Prof of Physiology* (1, 1924)
- Fenning, Con, M D Univ of Utah School of Medicine, Salt Lake City *Prof of Pharmacology and Physiology* (1, 1942)
- Fenton, P F, Ph D Brown Univ, Providence, R I *Assoc Prof of Biology* (1, 1947, 5, 1949)
- Ferguson, Frederick P, Ph D Univ of Maryland School of Medicine, Dept of Physiology, Baltimore 1 (1, 1949)
- Ferguson, James Kenneth Wallace, M A, M D 76 Kilbarry Rd, Toronto, Ont, Canada *Prof of Pharmacology, Univ of Toronto, Wing Comdr, R C A F* (1, 1933, 3, 1941)
- Ferguson, John, Ph D Creighton Univ School of Medicine, Omaha 2, Neb *Asst Prof of Physiology* (1, 1949)
- Ferguson, John Howard, M A, M D Univ of North Carolina School of Medicine, Dept of Physiology, Chapel Hill *Prof of Physiology and Acting Prof of Pharmacology* (1, 1933)
- Ferguson, L Kraeer, M D 133 S 36th St, Philadelphia 4, Pa *Prof of Surgery, Grad School, Univ of Pennsylvania, Surgeon, Doctors' Hosp and Woman's Med College, Philadelphia General Hosp* (4, 1935)
- Ferguson, Ralph L, M D Univ of South Dakota Med School, Dept of Pathology, Vermillion *Prof of Pathology* (4, 1949)
- Ferry, John Douglass, Ph D Univ of Wisconsin, Dept of Chemistry, Madison *Prof of Chemistry* (2, 1941)
- Ferry, Ronald M, M D 966 Memorial Drive, Cambridge, Mass *Assoc Prof of Biochemistry, Harvard Univ* (2, 1924)
- Fetcher, Edwin S, Ph D Steamboat Springs, Colo (1, 1944)
- Fetter, Dorothy, Ph D Brooklyn College, Dept of Hygiene, Brooklyn, N Y *Instr in Physiology* (1, 1944)
- Fevold, Harry L, Ph D Quartermaster Food and Container Inst, 1849 W Pershing Rd, Chicago 9, Ill *Chief, Food Research Div* (2, 1942)
- Field, John, II, Ph D Navy Dept Office of Naval Research, Med Sciences Div, Washington, D C *Head of Ecology Branch* (1, 1930)
- Fincke, Margaret L, Ph D Oregon State College, Corvallis *Assoc Prof of Foods and Nutrition, School of Home Economics* (5, 1940)
- Findley, Thomas, Jr, M D Ochsner Clinic, 3503 Prytania, New Orleans, La *Head of Dept of Internal Medicine, Asst Prof of Clinical Medicine, Tulane Univ School of Medicine* (1, 1938)

- Finerty, John C**, Ph D Univ of Texas Med Branch, Dept of Anatomy, Galveston Assoc Prof of Anatomy (1, 1947)
- Finland, Maxwell, M D** Boston City Hospital, Boston, Mass Asst Prof of Medicine, Harvard Med School (6, 1941)
- Finnegan, J K**, Ph D Med College of Virginia, Richmond 19 Asst Prof of Pharmacology (3, 1947)
- Firor, Warfield Monroe, M D** Johns Hopkins Hospital, Baltimore, Md Assoc Prof of Surgery, Johns Hopkins Univ (1, 1932)
- Fischel, Edward E**, M D, D Med Sc 23 Haven Ave, New York City 32 Fellow in Medicine, Columbia Univ (6, 1948)
- Fischer, Ernst, M D** Med College of Virginia, Richmond Prof of Physiology (1, 1936)
- Fischer, Hermann O L**, Ph D Univ of California, Virus Lab, Forestry Bldg, Berkeley 4 Lecturer, Dept of Biochemistry (2, 1940)
- Fischer, Martin H**, M D Univ of Cincinnati College of Medicine, Eden Ave, Cincinnati 19, Ohio Prof of Physiology (1, 1901, 2, 1919)
- Fishberg, Ella H**, M A, M D Beth Israel Hospital, Stuyvesant Park E, New York City Biochemist (2, 1931)
- Fisher, Alpert Madden, Ph D** Univ of Toronto, Connaught Med Research Labs, Toronto 5, Ont, Canada Research and Admin Assoc (2, 1944)
- Fisher, Kenneth C**, Ph D Univ of Toronto, Toronto, Ont, Canada Asst Prof of Physiological Zoology (1, 1940)
- Fishman, William H**, Ph D Tufts College Med School, 30 Bennet St, Boston 15, Mass Research Prof of Biochemistry (2, 1947)
- Fiske, Cyrus H**, M D Harvard Med School, Boston, Mass Prof of Biological Chemistry (2, 1914)
- Fitzhugh, O Garth, Ph D** Federal Security Agency, Food and Drug Admin, Div of Pharmacology, Washington, D C Pharmacologist (3, 1940)
- Fleischmann, Walter, M D**, Ph D Med Div, Army Chemical Center, Md Chief, Physiology Section, Instr in Pediatrics, Johns Hopkins Univ (1, 1940)
- Fleisher, Moyer S**, M D Jewish Hospital, St Louis, Mo Research Bacteriologist (4, 1924, 6, 1932)
- Flexner, Louis B**, M D Carnegie Labs, Carnegie Inst of Washington, Wolfe and Madison Sts, Baltimore 5, Md Staff member, Dept of Embryology (1, 1933, 2, 1948)
- Flick, John A** Univ of Pennsylvania School of Medicine, Dept of Bacteriology, Philadelphia 4 (6, 1949)
- Flock, Eunice V**, Ph D Mayo Clinic, Rochester, Minn Assoc Prof in Exper Medicine, Mayo Foundation, Univ of Minnesota (2, 1940)
- Florman, Alfred L**, M D Mt Sinai Hospital, New York City Investigator in Virology (6, 1942)
- Flosdorf, Earl W**, Ph D Forest Grove, Pa Dir of Research, Univ of Pennsylvania School of Medicine (6, 1941)
- Floyd, Cleveland, M D**, Sc D 246 Marlborough St, Boston, Mass Chief Examiner, Boston Health Dept (6, 1916)
- Foa, Piero Pio, Ph D** 710 S Wolcott St, Chicago, Ill Assoc Prof of Physiology and Pharmacology, Chicago Med School (1, 1944)
- Folch, Jordi, M D** McLean Hospital, Waverly, Mass Asst Prof of Biological Chemistry, Harvard Med School, Dir of Scientific Research, McLean Hospital (2, 1941)
- Folkers, Karl, Ph D** Merck and Co, Inc, Rahway, N J Dir of Organic and Biochemical Research (2, 1947)
- Follensby, Edna M**, Ph D 80 E Concord St, Boston, Mass Research Asst, Evans Memorial, Special Instr in Biology, Simmons College (6, 1933)
- Follis, Richard H, Jr, M D** Johns Hopkins Univ School of Medicine, Baltimore, Md Assoc Prof of Pathology (4, 1942)
- Fontaine, Thomas Davis, Ph D** U S Dept of Agriculture, Bureau of Agric and Indust Chemistry, Beltsville, Md Biochemist, Head, Div of Biologically Active Compounds (2, 1946)
- Foot, Nathan Chandler, M D** Cornell Univ Med College, New York City Prof Emeritus of Surgical Pathology (4, 1924)
- Forbes, Alexander, A.M**, M D Harvard University, Biological Labs, Divinity Ave, Boston, Mass (1, 1910)
- Forbes, Ernest B**, Ph D State College, Pa Prof Emeritus of Animal Nutrition (1R, 1917, 5, 1935)
- Forbes, John C**, Ph D Med College of Virginia, Richmond Research Prof of Biochemistry (2, 1937)
- Forbes, William H**, Ph D Harvard Univ, Boston, Mass Asst Dir, Fatigue Lab, Asst Prof of Industrial Physiology (1, 1943)
- Forster, Francis M**, M D Jefferson Med College, Philadelphia, Pa Asst Prof of Neurology (1, 1948)
- Fosdick, Leonard S**, Ph D 311 E Chicago Ave, Chicago, Ill Prof of Chemistry, Northwestern Univ (2, 1944)
- Foster, G L**, Ph D Columbia Univ, College of Physicians and Surgeons, 630 W 168th St, New York City Prof of Biological Chemistry (2, 1923)
- Foster, Harry E**, M D Fairmont Hotel, San Francisco, Calif (6, 1913)

- Foster, Jackson W, Ph D Univ of Texas, Dept of Bacteriology, Austin 12 *Prof of Bacteriology* (2, 1946)
- Foster, Robert H K, Ph D, M D St Louis Univ School of Medicine, St Louis, Mo *Prof and Dir of Dept of Pharmacology* (1, 1940, 3, 1944)
- Foster, Ruth A C, Ph D Princeton Univ, Princeton, N J (6, 1943)
- Fothergill, LeRoy D, M D Camp Detrick, Frederick, Md *Asst Technical Dir* (6, 1936)
- Fox, Sidney W, Ph D Iowa State College, Chemistry Dept, Ames *Prof of Chemistry, Prof in charge, Chemistry Section, Iowa Agricultural Exper Station* (2, 1946)
- Fraenkel, Gottfried S, Ph D Univ of Illinois, Urbana *Prof of Entomology* (5, 1949)
- Fraenkel-Conrat, Heinz, M D, Ph D U S Dept of Agriculture, Western Regional Research Lab, Albany 6, Calif *Sr Chemist* (2, 1942)
- Francis, Thomas, Jr, M D Univ of Michigan School of Public Health, Ann Arbor *Henry Sewall Prof of Epidemiology* (4, 1940, 6, 1930)
- Franke, Florent E, M D 9 Sylvester Ave, Webster Groves, Mo *Asst Prof of Physiology, St Louis Univ School of Medicine* (1, 1934)
- Frankel, Edward M, Ph D 214 River Rd, Nyack 9, N Y *Consulting Chemist* (2, 1916)
- Fraps, R M, Ph D Bureau of Animal Husbandry, Beltsville, Md *Sr Physiologist* (1, 1947)
- Fraser, Donald T, M B, D P H Univ of Toronto, Connaught Labs, Toronto 5, Canada *Prof of Hygiene and Preventive Medicine* (6, 1935)
- Frear, Donald E H, Ph D Pennsylvania State College, Dept of Agricultural and Biological Chemistry, State College *Prof of Agricultural and Biological Chemistry* (2, 1946)
- Fredette, Victorian Univ of Montreal, Montreal, Que, Canada *Asst Prof of Bacteriology, Assoc Dir, Inst of Microbiology* (6, 1948)
- Free, Alfred H, Ph D Miles Labs, Inc, Research Lab, Elkhart, Ind *Head of Biochemistry Section* (2, 1946, 5, 1944)
- Freeman, Harry, M D Worcester State Hospital, Worcester, Mass *Internist, Research Service* (1, 1939)
- Freeman, Leslie Willard, Ph D, M D Univ of Indiana, Dept of Surgery, Indianapolis *Asst Prof of Surgery, Dir of Surgical Research* (1, 1944)
- Freeman, Norman E, M D Univ of California Med School, Dept of Surgery, San Francisco 22 (1, 1936)
- Freeman, Smuth, M D, Ph D Northwestern Univ School of Medicine, 303 E Chicago Ave, Chicago, Ill *Prof and Chairman of Dept of Exper Medicine* (1, 1937)
- French, Cyrus E, Ph D Pennsylvania State College, State College *Assoc Prof of Animal Nutrition* (5, 1948)
- French, C Stacy, Ph D Carnegie Institution of Washington, Stanford Univ, Calif *Dir, Div of Plant Biology* (1, 1947, 2, 1946)
- Freund, Jules, M D Public Health Research Inst of the City of New York, New York City *Chief of the Div of Applied Immunology* (6, 1924)
- Frey, Charles N, Ph D Standard Brands Inc, 595 Madison Ave at 57th St, New York City 22 *Dir, Scientific Relations* (5, 1948)
- Fried, Josef, Ph D Squibb Inst for Med Research, New Brunswick, N J *Assoc Member, Div of Organic Chemistry* (2, 1948)
- Friedemann, Theodore E, Ph D Northwestern Univ Med School, 303 E Chicago Ave, Chicago, Ill *Assoc Prof of Physiology* (2, 1925)
- Friedemann, Ulrich, M D Jewish Hospital of Brooklyn, Dept of Bacteriology, Classon and St Marks Ave, Brooklyn, N Y (6, 1938)
- Friedenwald, Jonas S, M A, M D 1212 Eutaw Place, Baltimore 17, Md *Assoc Prof of Ophthalmology, Johns Hopkins Hospital* (1, 1947)
- Friedewald, William F, M D Emory Univ School of Medicine, Atlanta, Ga *Prof of Bacteriology, Assoc Prof of Medicine* (4, 1941)
- Friedgood, Harry B, M D Univ of California, Los Angeles *Assoc Clinical Prof of Medicine, Pres and Chairman, California Inst of Cancer Research* (1, 1936)
- Friedman, Maurice H, Ph D, M D 2040 Belmont Rd, Washington 9, D C (1, 1929)
- Friedman, Meyer, M D Harold Brunn Inst for Cardiovascular Research, Mt Zion Hospital, 2200 Post St, San Francisco, Calif *Director* (1, 1947)
- Friedman, M H F, Ph D Jefferson Med College of Philadelphia, 1025 Walnut St, Philadelphia, Pa *Assoc Prof of Physiology* (1, 1941)
- Friedman, Nathan B, M D Cedars of Lebanon Hospital, 4833 Fountain Ave, Los Angeles, Calif (4, 1942)
- Friedman, Sydney M, M D, Ph D McGill Univ, Dept of Anatomy, Montreal 2, Que, Canada *Asst Prof of Anatomy* (1, 1947)
- Friedman, Townsend B Children's Memorial Hospital, Chicago, Ill *Attending Allergist* (6, 1948)
- Frost, Douglas Van Anden, Ph D Abbott Labs, N Chicago, Ill *Head of Nutritional Research* (2, 1946, 5, 1947)
- Fruton, J S, Ph D Yale School of Medicine, 333 Cedar St, New Haven, Conn *Assoc Prof of Physiological Chemistry* (2, 1938)

- Fugo, Nicholas W**, Ph D Univ of Chicago, Dept of Obstetrics and Gynecology, Chicago, Ill (3, 1944)
- Fuhrman, Frederick A**, Ph D Stanford Univ, Dept of Physiology, Stanford Univ, Calif *Asst Prof of Physiology* (1, 1946)
- Fulton, John Farquhar**, Ph D, M D Yale Univ School of Medicine, New Haven, Conn *Sterling Prof of Physiology* (1, 1925)
- Funk, Casimir, D Sc**, Ph D 186 Riverside Drive, New York City 24 (2, 1921)
- Furchgott, Robert F**, Ph D Washington Univ School of Medicine, Euclid Ave and Kingshighway, St Louis 10, Mo *Asst Prof of Pharmacology* (2, 1948)
- Furth, Jacob**, M D Oak Ridge Natl Lab, Oak Ridge, Tenn *Chief of Pathology Section, Div of Biology* (4, 1932, 6, 1930)
- Gaebler, Oliver H**, Ph D, M D Henry Ford Hospital, Detroit, Mich *Head, Dept of Biochemistry, Edsel B Ford Inst for Med Research* (2, 1927)
- Gaffron, Hans**, Ph D Univ of Chicago, Dept of Chemistry, 5747 Ellis Ave, Chicago 37, Ill *Assoc Prof of Biochemistry, Member, Inst of Radio Biology and Biophysics* (2, 1941)
- Gagge, Adolf Pharo**, Ph D Aeromedical Research Lab, Wright Field, Dayton, Ohio *Lt Col, Chief, Biophysics Branch, Air Corps, U S Army* (1, 1939)
- Galambos, Robert**, Ph D, M D Psycho-Acoustic Lab, Cambridge, Mass *Research Fellow, Harvard Univ* (1, 1942)
- Gall, Edward A**, M D Cincinnati General Hospital, Cincinnati, Ohio *Mary M Emery Prof of Pathology, Univ of Cincinnati College of Medicine* (4, 1941)
- Gallagher, Thomas F**, Ph D Sloan-Kettering Inst, 444 E 68th St, New York City 21 *Member* (2, 1932)
- Gallup, Willis D**, Ph D Oklahoma Agricultural and Mechanical College, Stillwater *Chemist and Prof of Agricultural Chemistry* (2, 1932)
- Gamble, James L**, M D, S M 33 Edgehill Rd, Brookline, Mass *Prof of Pediatrics, Harvard Med School* (2, 1922, 5, 1933)
- Gantt, W Horsley**, M D Johns Hopkins Hospital, Phipps Psychiatric Clinic, Baltimore, Md *Assoc in Psychiatry* (1, 1935)
- Garbat, Abraham L**, M D 103 E 78th St, New York City *Dir, Med Service, Lenox Hill Hospital, Clinical Prof of Medicine, New York Univ Med School* (6, 1913)
- Gardner, Ernest**, M D Wayne Univ College of Medicine, Dept of Anatomy, 1512 St Antoine St, Detroit 26, Mich *Asst Prof of Anatomy* (1, 1949)
- Garner, Raymond L**, Ph D Univ of Michigan Med School, 218 W Med Bldg, Ann Arbor *Asst Prof of Biological Chemistry* (2, 1947)
- Garrey, Walter Eugene**, Ph D, M D Vanderbilt Univ School of Medicine, Nashville, Tenn *Prof Emeritus of Physiology* (1R, 1910, 2, 1906)
- Gasser, Herbert S**, A M, M D Rockefeller Inst for Med Research, 66th St and York Ave, New York City *Dir of Labs* (1, 1915, 3, 1924)
- Gassner, Frank X**, M S, D V M Colorado A and M College, Fort Collins *Assoc Prof of Physiology, Colorado State College, Assoc Pathologist, Colorado State Exper Station* (1, 1947)
- Gates, Olive**, M D Harvard Med School, 25 Shattuck St, Boston, Mass *Assoc Pathologist* (4, 1940)
- Gaunt, Robert**, Ph D Syracuse Univ, Syracuse, N Y *Prof and Chairman of Dept of Zoology* (1, 1939)
- Gay, Leslie N**, M D 1114 St Paul St, Baltimore, Md *Dir of Allergy Clinic, Johns Hopkins Hospital, Assoc Prof of Medicine, Johns Hopkins Univ* (6, 1927)
- Geiling, E M K**, M D, Ph D Univ of Chicago, Chicago 37, Ill *Frank P Hixon Distinguished Service Prof of Pharmacology and Chairman of Dept* (1, 1933, 2, 1927, 3, 1925)
- Gelfan, Samuel**, Ph D Yale Univ School of Medicine, 333 Cedar St, New Haven 11, Conn *Asst Prof of Physiology* (1, 1930)
- Gellhorn, Alfred**, M D Columbia Univ College of Physicians and Surgeons, Dept of Pharmacology, 630 W 168th St, New York City 32 *Assoc Prof of Pharmacology* (3, 1946)
- Gellhorn, Ernst**, M D, Ph D Univ of Minnesota, Room 116, Med Sciences, Minneapolis *Prof of Neurophysiology* (1, 1930)
- Gemmell, Chalmers L**, M D Univ of Virginia Med School, Charlottesville *Prof of Pharmacology* (1, 1928, 2, 1935, 3, 1946)
- Gerard, R W**, Ph D, M D Univ of Chicago, Chicago, Ill *Prof of Physiology* (1, 1927)
- Gersh, Isadore**, Ph D Univ of Chicago, Dept of Anatomy, Chicago 37, Ill *Assoc Prof of Anatomy* (4, 1947)
- Gerstenberger, Henry John**, M D Western Reserve Univ School of Medicine, Cleveland, Ohio *Prof Emeritus of Pediatrics, Dir of Pediatrics, Babies and Children's Hospital* (5, 1938)
- Gesell, Robert**, M D Univ of Michigan, Ann Arbor *Prof of Physiology* (1, 1913)
- Gettler, Alexander O**, Ph D New York Univ, 29 Washington Square, New York City *Prof of Chemistry and Toxicology, Toxicologist to Chief Med Examiner's Office* (2, 1916)
- Gey, George Otto**, M D Johns Hopkins Univ, Baltimore, Md *Instr in Surgery* (1, 1940)
- Gibbard, J**, M Sc Dept of Natl Health and Welfare, Ottawa, Ont, Canada *Chief, Lab of Hygiene* (6, 1946)
- Gibbs, Frederick Andrews**, M D 720 N Michigan Ave, Suite 610 Chicago Ill (1 1935)

- Gibbs, Owen Stanley, M D P O Box 166, White haven, Tenn *Dir*, *Gibbs Med Research Lab* (1, 1935, 3, 1930)
- Gibson, Robert Banks, Ph D Univ Hospital, Iowa City, Iowa *Assoc Prof of Biochemistry, State Univ of Iowa* (1, 1907, 2, 1906)
- Gies, William John, Ph D Columbia Univ, 632 W 168th St, New York City *Prof of Biological Chemistry* (1R, 1898, 2, 1906, 3R, 1909)
- Gilbert, Ruth, A M, M D R F D 2, Altamont, N Y *Asst Dir*, *Diagnostic Labs*, New York *State Dept of Health*, Albany (6, 1920)
- Gilman, Alfred, Ph D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 *Prof of Pharmacology* (1, 1935, 3, 1934)
- Gilson, Arthur S, Jr, Ph D Washington Univ Med School, St Louis, Mo *Assoc Prof of Physiology* (1, 1927)
- Givens, Maurice H, Ph D Box 5116, Biltmore, N C (1, 1917, 2, 1915)
- Gjessing, Erland C, Ph D Univ of Virginia, Box 1062, Univ Station, Charlottesville *Asst Prof of Biochemistry* (2, 1948)
- Glass, Howard G, Ph D 514 Wesley Ave, Oak Park, Ill (3, 1947)
- Glazko, Anthony J, Ph D Parke, Davis and Co, Research Labs, Detroit 32, Mich *Research Biochemist* (1, 1942)
- Glick, David, Ph D Univ of Minnesota, 225 Med Sciences Bldg, Minneapolis 14 *Assoc Prof of Physiological Chemistry* (2, 1936)
- Glickman, Nathaniel, M S Univ of Illinois College of Medicine, Dept of Medicine, 1853 W Polk St, Chicago 12 *Asst Prof of Medicine and Research Physiologist* (1, 1947)
- Goebel, Walther F, Ph D Rockefeller Inst for Med Research, 66th St and York Ave, New York City *Member* (2, 1929, 6, 1937)
- Goerner, Alfred, Pharm D, M D 366 Sterling Place, Brooklyn, N Y *Asst Clinical Prof of Medicine*, Long Island College of Medicine (2, 1939)
- Goettsch, Marianne, Ph D School of Tropical Medicine of Columbia Univ, San Juan, Puerto Rico *Asst Prof of Chemistry* (2, 1933, 5, 1941)
- Goetzl, Franz R, Ph D, M D Permanente Foundation, Dept of Med Research, Oakland 11, Calif *Director* (1, 1947)
- Gold, Harry, M.D Cornell Univ Med College, New York City *Prof of Clinical Pharmacology, Attending Physician-in-Charge, Cardiovascular Research Unit, Beth Israel Hospital* (3, 1927)
- Goldblatt, Harry, M D Cedars of Lebanon Hospital, Los Angeles, Calif *Dir*, *Inst for Med Research* (1, 1945, 4, 1927)
- Golden, Alfred, M D Veterans Admin Hosp, Buffalo, N Y *Chief of Lab Service*, Univ of Buffalo, *Dept of Physiology* (4, 1947)
- Goldfarb, Walter, M D 25 E 86th St, New York City (1, 1938)
- Goldforb, A J, Ph D College of the City of New York, New York City *Prof of Biology* (1, 1930)
- Goldie, Horace, M D, D T M 7910 Lynnbrook Drive, Bethesda, Md (6, 1943)
- Goldring, William, M D New York Univ College of Medicine, 477 First Ave, New York City *Assoc Prof of Medicine* (1, 1939)
- Goldschmidt, Samuel, Ph D Univ of Pennsylvania Med School, Philadelphia *Prof of Physiology* (1, 1919, 2, 1915)
- Goldsmith, Grace A, M D Tulane Univ of Louisiana, New Orleans *Asst Prof of Medicine* (5, 1943)
- Goldstein, Avram, M D Harvard Med School, Boston, Mass *Instr in Pharmacology* (3, 1948)
- Golub, Orville Joseph, Ph D Bio-Service Labs, Inc, 10717 Venice Blvd, Los Angeles 24, Calif *Associate* (6, 1944)
- Gomori, George, M D, Ph D Univ of Chicago, Chicago, Ill *Prof of Medicine* (4, 1948)
- Goodale, Walter T, M D 79 Webster Rd, Weston 93, Mass *Research Fellow*, *Harvard Med School*, *Asst in Medicine*, *Peter Bent Brigham Hospital* (1, 1949)
- Goodman, Louis Sanford, M S, M D Univ of Utah School of Medicine, Salt Lake City *Prof of Pharmacology and Chairman of Dept of Pharmacology* (1, 1946, 3, 1937)
- Goodner, Kenneth, Ph D Jefferson Med College, Philadelphia, Pennsylvania *Prof of Bacteriology* (6, 1932)
- Goodpasture, Ernest William, M D Vanderbilt Univ Med School, Nashville, Tenn *Prof of Pathology and Dean* (4, 1923)
- Gordon, Albert S, Ph D New York Univ, Washington Square College of Arts and Sciences, New York City *Assoc Prof of Biology* (1, 1942)
- Gordon, Francis B, M D Camp Detrick, Biology Dept, Frederick, Md *Chief of MV Div* (4, 1947)
- Gordon, Harry H, M D 4200 E 9th Ave, Denver, Colo *Prof of Pediatrics*, *Univ of Colorado Med School*, *Pediatrician in Chief*, *Colorado Gen Hospital* (5, 1940)
- Gordon, Irving, M D New York State Dept of Health, Div of Labs and Research, New Scotland Ave, Albany 1 *Assoc Med Bacteriologist*, *Assoc Prof of Medicine and Bacteriology*, *Albany Med College* (6, 1943)
- Gordon, William G, Ph D U S Dept of Agriculture, Eastern Regional Research Lab, Philadelphia 18, Pa *Sr Chemist* (2, 1939)
- Gortner, Ross Aiken, Jr, Ph D Wesleyan Univ, Shanklin Lab, Middletown, Conn *Assoc Prof of Biochemistry* (5, 1947)
- Gortner, Willis A, Ph D Pineapple Research Inst of Hawaii, Honolulu, T H *Head of Dept of Chemistry* (2, 1947)

- Goss, Harold, Ph D Univ of California College of Agriculture, Davis *Prof of Animal Husbandry* (2, 1936, 5, 1933)
- Goth, Andres, M D Southwestern Med College, 2211 Oak Lawn Ave, Dallas 4, Tex *Assoc Prof of Pharmacology* (3, 1947)
- Gottschall, Russell Y, Ph D Michigan Dept of Health, Bureau of Labs, Lansing *Bacteriologist* (6, 1939)
- Goudsmit, Arnoldus, Jr, M D, Ph D 4141 Windsor Rd, Youngstown 7, Ohio (1, 1940)
- Gould, R Gordon, Ph D Presbyterian Hospital, 1753 W Congress St, Chicago 12, Ill *Assoc Attending Biochemist, Assoc Prof of Biological Chemistry, Univ of Illinois College of Medicine* (2, 1949)
- Govier, William M, M D Upjohn Co, Research Div, Kalamazoo 99, Mich (3, 1944)
- Grabfield, G Philip, M D 27 Forest St, Milton, Mass *Assoc in Medicine and Pharmacology, Harvard Med School, Col M C, U S A* (3, 1923)
- Grady, Hugh G, M D Armed Forces Inst of Pathology, Washington, D C *Scientific Dir, Am Registry of Pathology* (4, 1940)
- Graef, Irving, M D New York Univ College of Medicine, New York City *Asst Prof of Clin Medicine* (4, 1941)
- Graham, Claire E, Ph D Wilson Labs, 4221 S Western Ave, Chicago, Ill *Dir of Research* (2, 1948)
- Graham, Clarence H, Ph D Columbia Univ, New York City 27 *Prof of Psychology* (1, 1933)
- Graham, Helen Tredway, Ph D Euclid Ave and Kingshighway, St Louis, Mo *Assoc Prof of Pharmacology, Washington Univ School of Medicine* (1, 1933, 3, 1931)
- Granick, S, Ph D Rockefeller Inst for Med Research, York Ave at 66th St, New York City 21 *Associate* (2, 1949)
- Grant, E Rhoda, Ph D Univ of Illinois Med College, Clinical Science Dept, 1853 West Polk St, Chicago 12, Ill *Researcher in Clin Science* (1, 1949)
- Grant, R Lorimer, Ph D Federal Security Agency, Food and Drug Admin, Div of Pharmacology, Washington 25, D C *Pharmacologist* (2, 1938)
- Grant, Wilson Clark, Ph D 65 Beucler Place, Bergenfield, N J *Asst Prof of Physiology, Columbia Univ College of Physicians and Surgeons* (1, 1949)
- Grau, Charles R, Ph D Univ of California, Div of Poultry Husbandry, Berkeley 4 *Asst Prof of Poultry Husbandry and Asst Poultry Husbandman in Exper Station* (5, 1949)
- Graubard, Mark, Ph D Univ of Minnesota, Dept of Physiology, Minneapolis (1, 1940)
- Grauer, Robert C, M.D Allegheny General Hospital, Pittsburgh, Pa *Dir of Research, and Head of Dept of Endocrinology and Metabolism, William H Singer Memorial Research Lab, Prof of Medicine, School of Medicine, Univ of Pittsburgh* (4, 1941)
- Gray, John S, Ph D, M D Northwestern Univ Med School, 303 E Chicago Ave, Chicago 11, Ill *Prof and Chairman of Dept of Physiology* (1, 1937)
- Gray, M Geneva, Ph D Labs of Arthur D Little Inc, Cambridge, Mass *Dir of Pharmacological Research* (3, 1946)
- Gray, Samuel H, M D Jewish Hospital of St Louis, Kingshighway and Forest Park Blvd, St Louis, Mo *Dir of Labs, Assoc Prof of Pathology, Washington Univ* (4, 1939)
- Gray, Stephen W, Ph D Emory Univ School of Medicine, Emory Univ, Ga *Asst Prof of Anatomy* (1, 1948)
- Graybiel, Ashton, M D U S Naval School of Aviation Medicine and Research, Naval Air Station, Pensacola, Fla *Coordinator of Research* (1, 1948)
- Greaves, J D, Ph D U S Dept of Agriculture, Western Regional Research Lab, 800 Buchanan St, Albany 6, Calif *Biochemist* (2, 1938)
- Greaves, Joseph E, Ph D Utah State Agricultural College, Dept of Bacteriology and Biochemistry, Logan (2, 1940)
- Greeley, Paul O, Ph D, M D Univ of Southern California Med School, Dept of Aviation Medicine, Univ Park, Los Angeles (1, 1940)
- Green, Arda Alden, M D Cleveland Clinic, Research Div, Euclid and E 93rd St, Cleveland 6, Ohio (2, 1932)
- Green, Daniel M, M D G D Searle Co, Chicago 80, Ill *Dir of Biological Research* (1, 1948, 3, 1942)
- Green, David E, Ph D Univ of Wisconsin, Enzyme Inst, P O Box 2066 Madison 5 *Prof of Enzyme Chemistry* (2, 1941)
- Green, Harold David, M D Wake Forest College, Bowman Gray School of Medicine, Winston-Salem 7, N C *Prof of Physiology and Pharmacology* (1, 1936, 3, 1945)
- Green, Robert Holt Yale Univ School of Medicine, 333 Cedar St, New Haven, Conn (6, 1949)
- Greenberg, David Morris, Ph D Univ of California, Berkeley 4 *Prof and Chairman, Div of Biochemistry* (2, 1927, 5, 1946)
- Greenberg, Louis D, Ph D Univ of California Med Center, 3rd and Parnassus Aves, San Francisco 22 *Asst Prof of Pathology, Univ of California Med School* (2, 1946)
- Greene, Carl Hartley, Ph D, M D 401 Clinton Ave, Brooklyn 5, N Y *Assoc Clin Prof of Medicine, New York Univ Post-Grad Med School* (1, 1921, 2, 1922, 4, 1924)

- Greene, Harry S N, M D, C M Yale Univ School of Medicine, Dept of Pathology, New Haven, Conn *Prof of Pathology* (4, 1937)
- Greene, James Alexander, M D Baylor Univ College of Medicine, Buffalo Drive, Houston, Tex *Prof and Chairman of Dept of Internal Medicine and Dean of Clin Faculty* (1, 1939)
- Greene, Ronald R, M D Northwestern Univ Med School, 303 E Chicago Ave, Chicago, Ill *Instr in Physiology, Instr in Obstetrics and Gynecology* (1, 1941)
- Greengard, Harry, Ph D, M D Northwestern Univ Med School, 303 E Chicago Ave, Chicago, Ill *Asst Prof of Physiology* (1, 1939)
- Greenstein, Jesse P, Ph D Natl Cancer Inst, Bethesda, Md *Chief Biochemist* (2, 1935)
- Greenwald, Isidor, Ph D New York Univ College of Medicine, 477 First Ave, New York City *Assoc Prof of Chemistry* (2, 1912, 5, 1933)
- Greep, Roy O, Ph D Harvard School of Dental Medicine, 25 Shattuck St, Boston 15, Mass *Assoc Prof of Dental Science* (1, 1940)
- Greer, C M, M S Vanderbilt Univ School of Medicine, Nashville, Tenn *Research Assoc in Pharmacology* (3, 1938)
- Gregersen Magnus I, Ph D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 *Prof of Physiology* (1, 1933)
- Gregg, Donald Eaton, Ph D, M D Field Research Lab, Med Dept, Fort Knox, Ky *Chief Research Physician* (1, 1933)
- Gregory, John E, Hahnemann Med College, Philadelphia, Pa *Prof of Pathology* (6, 1948)
- Gregory, Raymond L, Ph D, M D Univ of Texas School of Medicine, 1419 24th St, Galveston *Prof of Medicine* (1, 1945)
- Greig, Margaret E, Ph D Vanderbilt Univ School of Medicine, Nashville 4, Tenn *Assoc Prof in Pharmacology* (3, 1946)
- Greisheimer, Esther M, Ph D M D Temple Univ Med School, 3400 N Broad St, Philadelphia, Pa *Prof of Physiology* (1, 1925)
- Grenell, Robert G, Ph D Johns Hopkins Univ, Dept of Biophysics, Baltimore, Md (1, 1945)
- Griffin, Angus, Ph D George Washington Univ School of Medicine, Dept of Bacteriology, 1335 H St, N W, Washington, D C *Assoc Prof of Bacteriology* (6, 1940)
- Griffith, Fred R, Jr, Ph D Univ of Buffalo Med School, 24 High St, Buffalo, N Y *Prof of Physiology* (1, 1923, 5, 1933)
- Griffith, Wendell H, Ph D Univ of Texas Med School, Galveston *Prof and Chairman of Dept of Biochemistry and Nutrition* (2, 1923, 5, 1934)
- Grimson, Keith S, M D Duke Univ School of Medicine, Durham, N C *Prof of Surgery* (1, 1943, 3, 1949)
- Grindlay, John H, M D Mayo Clinic, Rochester, Minn (1, 1945)
- Groat, Richard A, Ph D Wake Forest College, Bowman Gray School of Medicine, Winston-Salem 7, N C *Assoc Prof of Anatomy* (1, 1945)
- Groat, William A, M D 713 E Genesee St, Syracuse, N Y *Prof of Clinical Pathology, Syracuse Univ College of Medicine* (6, 1917)
- Grodkins, Fred S, Ph D, M D Northwestern Univ Med School, 303 E Chicago Ave, Chicago 11, Ill *Assoc Prof of Physiology* (1, 1945)
- Groedel, Franz M, M D 829 Park Ave, New York City *Research Fellow, Fordham Univ, Biology Dept* (1, 1949)
- Grollman, Arthur, M D, Ph D Southwestern Med College, 2211 Oak Lawn Ave, Dallas, Tex *Prof of Medicine and Chairman of Dept of Exper Medicine, Prof of Pharmacology and Chairman of Dept of Physiology and Pharmacology* (1, 1928, 3, 1933)
- Gross, Erwin G, Ph D, M D State Univ of Iowa, Med Labs, Iowa City *Prof of Pharmacology* (1, 1927, 2, 1923, 3, 1927)
- Gross, Robert E, M D Children's Hosp, 300 Longwood Ave, Boston 15, Mass *Surgeon-in-Chief, Ladd Prof of Children's Surgery* (4, 1940)
- Grossman, Morton Irvin, Ph D, M D Univ of Illinois College of Medicine, Chicago 12 *Assoc Prof of Physiology* (1, 1946)
- Groupé, Vincent, Ph D Rutgers Univ, Dept of Microbiology, New Jersey Agricul Exper Station, New Brunswick *Assoc Prof in Animal Diseases* (6, 1946)
- Gruber, Charles M, M D, Ph D Jefferson Med College, 1025 Walnut St, Philadelphia, Pa *Prof of Pharmacology*, (1, 1914, 3, 1919)
- Gruber, Charles M, Jr, M D Jefferson Med College Hospital, Philadelphia, Pa *Research Fellow in Hematology* (3, 1948)
- Gruhzit, Oswald M, M D Parke, Davis and Co, Research Labs, Detroit, Mich *Research in Pathology and Pharmacology* (4, 1928)
- Grundfest, Harry, Ph D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 *Assoc in Neurology* (1, 1932)
- Gudernatsch, F, Ph D 41 Fifth Ave, New York City 3 (1, 1930)
- Guerra, Francisco, M Sc, M D Facultad de Medicina, Univ Nacional de Mexico, Mexico, D F *Prof of Pharmacology* (3, 1947)
- Guerrant, N B, Ph D Pennsylvania State College, State College *Prof of Biological Chemistry* (2, 1934, 5, 1933)
- Guest, George Martin, M S, M D Children's Hospital, Cincinnati 29, Ohio *Prof of Pediatrics, Univ of Cincinnati College of Medicine and Grad School* (2, 1933)

- Guest, Maurice Mason, Ph D Wayne Univ College of Medicine, Dept of Physiology, Detroit 26, Mich *Assoc Prof of Physiology* (1, 1946)
- Gulick, Addison, Ph D 308 Westmount Ave, Columbia, Mo *Prof of Physiological Chemistry, Univ of Missouri* (1, 1915, 5, 1933)
- Gunn, Francis D, M D, Ph D Univ of Utah School of Medicine, Salt Lake City *Prof and Head of Dept of Pathology* (4, 1938)
- Gunsalus, Irwin C, Ph D Indiana Univ, Bloomington *Prof of Bacteriology* (2, 1946)
- Gurin, Samuel, Ph D Univ of Pennsylvania School of Medicine, Philadelphia *Prof of Physiological Chemistry* (2, 1938)
- Gustavson, R G, Ph D Univ of Nebraska, Lincoln *Chancellor* (2, 1927)
- Gustus, Edwin L, Ph D Bjorksten Research Lab, 13791 Ave O, Chicago 33, Ill *Vice President* (2, 1934)
- Guterman, Henry S, M D Michael Reese Hospital, Chicago 16, Ill *Asst Dir, Dept of Metabolic and Endocrine Research* (1, 1949)
- Guthrie, Charles Claude, M D, Ph D Univ of Pittsburgh Med School, Pittsburgh, Pa *Prof of Physiology and Pharmacology* (1, 1905, 3, 1909)
- Gutierrez-Noriega, Carlos, M D Facultad de Medicina, Av Grau, Lima, Peru *Prof of Pharmacology* (3, 1948)
- Gutman, Alexander B, M D Columbia Research Service, Welfare Island, New York City 17 *Director* (2, 1947)
- Guttman, Rita M, Ph D Brooklyn College, Brooklyn, N Y *Instr in Physiology* (1, 1946)
- Guyton, Arthur C, M D Univ of Mississippi School of Medicine, Dept of Physiology, University *Acting Chairman, Dept of Physiology* (1, 1949)
- Gyorgy, Paul, M D Univ of Pennsylvania School of Medicine, 3400 Spruce St Philadelphia 4 *Prof of Clinical Pediatrics* (2, 1938, 5, 1939)
- Haag, Harvey B, M D Med College of Virginia, Richmond *Prof of Pharmacology* (3, 1934)
- Haag, J R, Ph D Oregon Agric Exper Station, Corvallis *Chemist* (2, 1947, 5, 1941)
- Haas, Erwin, Ph D Cedars of Lebanon Hospital, Inst for Med Research, Los Angeles 27, Calif *Research Assoc* (2, 1946)
- Haberman, Sol, Ph.D Wm Buchanan Blood, Plasma and Serum Center, Baylor Hospital, Dallas, Tex *Chief of Bacteriology and Serology Services* (6, 1944)
- Hadidian, Zareh, Ph D Tufts College Med School, Boston, Mass (1, 1945)
- Hafkesbring, H Roberta, Ph D Woman's Med College of Pennsylvania, East Falls, Philadelphia *Prof of Physiology* (1, 1931)
- Haggard, Howard W, M D 4 Hillhouse Ave, New Haven, Conn *Dir of Lab of Applied Physiology, Yale Univ* (1, 1919, 2, 1920)
- Hahn, Paul F, Ph D Meharry Med College, Nashville, Tenn *Dir of Cancer Research Labs* (1, 1946, 4, 1939)
- Haig, Charles, Ph D New York Med College, Fifth Ave at 105th St, New York City *Assoc Prof of Physiology and Biochemistry* (1, 1942)
- Haist, Reginald E, M D, Ph D Univ of Toronto, Toronto, Ontario, Canada *Assoc Prof of Physiology* (1, 1943)
- Halbert, Seymour P, M D Columbia Univ College of Physicians and Surgeons, Dept of Ophthalmology, 630 W 168th St, New York City 32 (6, 1947)
- Haldi, John, Ph D Emory Univ School of Dentistry, Emory University, Ga *Prof of Physiology* (1, 1928)
- Hale, Wm M, M D Brookhaven Natl Lab, Upton, L I, N Y *Head, Div of Bacteriology and Virology, Bacteriologist-in-Chief, Brookhaven Natl Lab Hosp* (4, 1941, 6, 1935)
- Haley, Thomas J, Ph D 4133 W 22nd Place, Los Angeles 16, Calif *Chief, Div of Pharmacology and Toxicology, A E Project, Med School, Univ of California* (3, 1949)
- Hall, Charles E, Ph D Univ of Texas, Med Branch, Dept of Physiology, Galveston *Asst Prof* (1, 1949)
- Hall, F G, Ph D Duke Univ School of Medicine, Dept of Physiology and Pharmacology, Durham, N C (1, 1937)
- Hall, George Edward, M D, Ph D Univ of Western Ontario, Ottaway Ave and Waterloo St, London, Ontario, Canada *Dean of the Faculty of Medicine* (1, 1938)
- Hall, Victor E, M A, M D Stanford Univ, Dept of Physiology, Stanford University, Calif *Prof of Physiology* (1, 1934)
- Hall, W Knowlton, Ph D Univ of Georgia School of Medicine, Augusta *Assoc Prof of Biochemistry* (2, 1948)
- Hallenbeck, George Aaron, Ph D, M D Mayo Clinic, Rochester, Minn *Research Assoc* (1, 1946)
- Halliday, Nellie, Ph D Laguna Honda Home, Lab of Exper Oncology, San Francisco, Calif (5, 1933)
- Halpert, Béla, M D Veterans Admin Hospital, 2002 Holcombe Blvd, Houston 4, Texas
- Halstead, Ward C, Ph D, Univ of Chicago, Dept of Medicine, Chicago, Ill *Assoc Prof of Exper Psychology, Div of Psychiatry* (1, 1942)
- Ham, Arthur W, M B Univ of Toronto, Toronto 5, Ontario, Canada *Prof of Anatomy in charge of Histology* (4, 1939)

- Hambourger, Walter E , Ph D , M D G D Searle and Co , P O Box 5110, Chicago, Ill *Chief Pharmacologist* (3, 1934)
- Hamilton, Bengt L K , M D U S Marine Hospital, Staten Island 4, N Y *Med Dir , USPHS* (2, 1925)
- Hamilton, James B , Ph D Long Island College of Medicine, Dept of Anatomy, 350 Henry St Brooklyn 2, N Y (1, 1938)
- Hamilton, Paul B , M D , Ph D Alfred I duPont Inst , Nemours Foundation, Rockland Rd , Wilmington 99, Del *Chief of Biochemistry* (2, 1946)
- Hamilton, Tom S , Ph D Univ of Illinois, 1513 W Clark St , Champaign *Prof and Chief in Animal Nutrition* (2, 1937, 5, 1938)
- Hamilton, W F , Ph D Univ of Georgia School of Medicine, Augusta *Prof of Physiology* (1, 1924)
- Hammett, Frederick S , Ph D 493 Commercial St , Provincetown, Mass *Sr Member Emeritus, Lakenau Hospital Research Inst* (1R, 1920, 2, 1917)
- Hammon, William McD , M D , D P H Univ of Pittsburgh, Pittsburgh, Pa *Prof of Epidemiology, Graduate School of Public Health* (4, 1944, 6, 1946)
- Hampel, C W , Ph D New York Univ College of Medicine, New York City *Assoc Prof of Physiology* (1, 1936)
- Hamre, Dorothy, Ph D Squibb Inst for Med Research, New Brunswick, N J *Research Assoc* (6, 1948)
- Hand, David B , Ph D New York State Agricultural Exper Station, Geneva *Head, Div of Food Science and Technology and Prof of Biochemistry* (2, 1947)
- Handler, Philip, Ph D Duke Univ School of Medicine, Durham, N C *Assoc Prof of Biochemistry and Nutrition* (2, 1944, 5, 1946)
- Handley, Carroll A , Ph D Baylor Univ College of Medicine, 1200 M D Anderson Blvd , Houston 5, Tex *Prof of Physiology and Pharmacology* (3, 1942)
- Haney, Hance F , Ph D , M D Univ of Oregon Med School, Portland *Asst Prof of Medicine* (1, 1939)
- Hanger, Franklin, M D Columbia Univ College of Physicians and Surgeons, 630 W 168th St , New York City 32 *Assoc Prof of Medicine* (6, 1930)
- Hanke, Martin E , Ph D Univ of Chicago, Chicago, Ill *Assoc Prof of Biochemistry* (2, 1925)
- Hanke, Milton Theo, Ph D 7550 S Green St , Chicago, Ill *Research Consultant, Biochemistry and Nutrition* (2, 1919)
- Hanks, John H , Ph D Harvard Univ Med School, Boston, Mass *Dept of Bacteriology, Bacteriologist of the Leonard Wood Memorial* (6, 1935)
- Hansen, Arild E , M D Univ of Texas Med School, Galveston *Prof and Chairman of Dept of Pediatrics, Dir of Univ of Texas Child Health Program* (4, 1941, 5, 1942)
- Hanzal, Ramón F , Ph D Killian Research Labs , 49 W 45th St , New York City *Biochemist* (2, 1935)
- Hanzlik, Paul J , M D Stanford Univ School of Medicine, Sacramento and Webster Sts , San Francisco, Calif *Prof of Pharmacology* (1, 1912, 3, 1912)
- Hardy, James Daniel, Ph D Russell Sage Inst of Pathology, 525 E 68th St , New York City *Research Assoc* (1, 1939)
- Hardy, Mary, D Sc The Brearley School, 610 E 83rd St , New York City *Teacher of Science* (1, 1933)
- Hare, Kendrick, Ph D Children's Hospital, 219 Bryant St , Buffalo 9, N Y (1, 1938)
- Harford, Carl G , M D Washington Univ School of Medicine, St Louis, Mo *Asst Prof of Medicine and Preventive Medicine* (6, 1948)
- Harger, R N , Ph D Indiana Univ School of Medicine, Indianapolis *Prof and Chairman of Dept of Biochemistry and Toxicology* (2, 1938, 3, 1949)
- Harkins, Henry N , Ph D , M D Univ of Washington School of Medicine, Seattle 5 (1, 1942)
- Harmon, Paul M , Ph D Indiana Univ , Bloomington *Prof of Physiology* (1, 1930)
- Harne, O G Univ of Maryland School of Medicine, Baltimore *Assoc Prof of Histology* (1, 1935)
- Harned, Ben King, Ph D Lederle Labs , Pearl River, N Y *Assoc Dir , Pharmacology Research* (2, 1931, 3, 1941)
- Harris, Albert H , M D N Y State Dept of Health, Div of Labs and Research, New Scotland Ave , Albany 1 *Asst Dir for Local Labs* (6, 1937)
- Harris, Albert Sidney, Ph D Baylor Univ College of Medicine, Houston 5, Texas (1, 1939)
- Harris, Milton, Ph D Harris Research Labs , 1246 Taylor St , N W , Washington 11, D C *President* (2, 1939)
- Harris, Paul N , M D Eli Lilly and Company, Indianapolis, Ind *Pathologist, Div of Pharmacology* (3, 1948)
- Harris, Philip L , Ph D Distillation Products, Inc , 755 Ridge Rd W , Rochester 13, N Y *Head of Biochemistry Research Dept , Assoc in Physiology, Univ of Rochester School of Medicine and Dentistry* (2, 1946, 5, 1945)
- Harris, Robert S , Ph D Massachusetts Inst of Technology, Cambridge *Prof of Nutritional Biochemistry* (5, 1941)

- Harris, S C , Ph D Northwestern Univ , Dept of Physiology and Pharmacology, Chicago, Ill *Chairman of Dept* (1, 1947)
- Harris, T N , M D Children's Hospital of Philadelphia, Philadelphia, Pa *Assoc in Pediatrics, Univ of Pennsylvania* (6, 1946)
- Harris, William H , M D Tulane Univ School of Medicine, New Orleans, La *Asst Prof of Pathology and Bacteriology* (4, 1925)
- Harrison, Frank, Ph D Univ of Tennessee College of Medicine, Memphis *Prof and Chief, Div of Anatomy* (1, 1941)
- Harrison, James A , Ph D Temple Univ , Philadelphia 22, Pa *Prof of Biology* (6, 1946)
- Harrison, Ross Granville, M D , Ph D Osborn Zoological Lab , New Haven, Conn *Sterling Prof Emeritus of Biology, Yale Univ* (1, 1906)
- Harrison, R Wendell, M D , Ph D Ellis Ave at 58th St , Chicago 37, Ill *Prof of Bacteriology, Dean of Faculties and Vice Pres , Univ of Chicago* (6, 1934)
- Harrow, Benjamin, Ph D College of the City of New York, Convent Ave and 139th St , New York City *Prof of Chemistry* (2, 1927)
- Hart, E B , B S Univ of Wisconsin Agricultural College, Madison *Prof of Biochemistry* (2, 1910, 5, 1933)
- Hart, E Ross, Ph D Medical Division, Army Chemical Center, Md (3, 1944)
- Hart, William M , Ph D Temple Med School, Broad and Ontario Sts , Philadelphia 40, Pa *Asst Prof of Physiological Chemistry* (1, 1945)
- Hartline, H K , M D Johns Hopkins Univ , Dept of Biophysics, Baltimore 18, Md (1, 1929)
- Hartman, Carl G , Ph D Ortho Research Foundation, Raritan, N J *Dir of Physiology* (1, 1921)
- Hartman, Frank Alexander, Ph D Ohio State Univ , Dept of Physiology, Columbus *Research Prof of Physiology* (1, 1916)
- Hartman, F W , M D Henry Ford Hospital, Detroit, Mich *Pathologist* (4, 1927)
- Hartmann, Alexis F , M D 500 S Kingshighway, St Louis, Mo *Prof of Pediatrics, Washington Univ School of Medicine* (2, 1932)
- Harvey, A McGhee, M D Johns Hopkins Hospital, Baltimore 5, Md *Physician-in-Chief, Prof of Medicine, Johns Hopkins Univ Med School* (1, 1946, 3, 1946)
- Harvey, E Newton, Ph D Princeton Univ , Guyot Hall, Princeton, N J *Henry Fairfield Osborn Prof of Biology* (1, 1914, 2, 1916)
- Hass, George, M D Presbyterian Hospital of Chicago, Chicago, Ill *Prof and Chairman of Dept of Pathology, Univ of Illinois College of Medicine* (4, 1939)
- Hassid, William Z , Ph D , Univ of California, Div of Plant Nutrition, Berkeley *Prof of Plant Nutrition* (2, 1946)
- Hastings, A Baird, Ph D Harvard Med School, 25 Shattuck St , Boston 15, Mass *Hamilton Kuhn Prof of Biological Chemistry* (1, 1927, 2, 1921, 5, 1940)
- Hathaway, Millicent L , Ph D Agricultural Research Administration, Bureau of Human Nutrition and Home Economics, Food and Nutrition Div , Washington 25, D C (5, 1945)
- Hauck, Hazel M , Ph D Cornell Univ , Ithaca, N Y *Prof of Home Economics* (5, 1941)
- Hauge, Siegfred M , Ph D Purdue Univ Agricultural Exper Station, Lafayette, Ind *Research Assoc in Biochemistry* (5, 1933)
- Haurowitz, Felix, M D , D Sc Indiana Univ , Bloomington *Prof of Biochemistry* (2, 1949, 6, 1948)
- Haury, Victor G , M D Box 206, Wellsville, Kansas (3, 1939)
- Haven, Frances L , Ph D Univ of Rochester School of Medicine and Dentistry, 260 Crittenden Blvd , Rochester, N Y *Assoc in Biochemistry* (2, 1941)
- Hawk, Philip B , Ph D Food, Drug and Cosmetic Consultants, 30 Rockefeller Plaza, Room 4631, New York City 20 *Pres of Food Research Labs , Inc* (1, 1903, 2, 1906)
- Hawkins, J E , Jr , Ph D Merck Inst for Therapeutic Research, Rahway, N J *Physiologist* (1, 1943)
- Hawkins, William Bruce, M D Univ of Rochester School of Medicine and Dentistry, 260 Crittenden Blvd , Rochester, N Y *Assoc Prof of Pathology* (4, 1933)
- Hawley, Estelle E , Ph D Univ of Rochester School of Medicine and Dentistry, Rochester, N Y *Research Fellow in Pediatrics* (5, 1935)
- Hawn, Clinton van Zandt, M D Mary Imogene Bassett Hospital, Cooperstown, N Y *Pathologist, Dir of Otsego County Labs* (4, 1948)
- Hay, Eleanor Clarke, Ph D 7 Greenhill Rd , Madison, N J (1, 1945)
- Hayman, J M , Jr , M D Lakeside Hospital, Cleveland, Ohio *Prof of Clinical Medicine and Therapeutics, Western Reserve Univ* (1, 1928, 3, 1932)
- Haynes, Florence W , Ph D Harvard Med School, 25 Shattuck St , Boston, Mass *Research Assoc in Medicine* (1, 1937)
- Hays, Edwin Everett, Ph.D Armour Labs , 1425 W 42nd St , Chicago 9, Ill *Dir of Biochemical Research* (2, 1946)
- Hays, Harry W , Ph D Wayne Univ College of Medicine, 1512 St Antoine St , Detroit 26, Mich *Assoc Prof of Physiology and Pharmacology* (3, 1949)
- Haythorn, Samuel R , M D Allegheny General Hospital, 320 E North Ave , Pittsburgh, Pa *Dir of William H Singer Memorial Lab* (4, 1925)

- Haywood, Charlotte, Ph D Mount Holyoke College, South Hadley, Mass *Prof of Physiology* (1, 1939)
- Hazen, Elizabeth L, Ph D New York State Dept of Health Labs, 339 E 25th St, New York City *Sr Bacteriologist* (6, 1931)
- Hazleton, Lloyd W, Ph D Box 333, Falls Church, Va *Research Consultant* (3, 1944)
- Hechter, Oscar M, Ph D Worcester Foundation for Exper Biology, 222 Maple Ave, Shrewsbury, Mass (1, 1945)
- Hegnauer, Albert H, Ph D Boston Univ School of Medicine, Boston, Mass *Assoc Prof of Physiology* (1, 1937)
- Hegsted, David Mark, Ph D Harvard School of Public Health, Boston, Mass *Prof of Nutrition* (5, 1944)
- Heidelberger, Michael, Ph D Columbia Univ, 620 W 168th St, New York City 32 *Prof of Immunochemistry, Chemist to the Presbyterian Hospital* (2, 1927, 6, 1935)
- Heilbrunn, Lewis Victor, Ph D Univ of Pennsylvania, Philadelphia *Prof of Zoology* (1, 1930)
- Helm, J William, Ph D 1 Yale Ave, Dayton 6, Ohio Aero-Medical Lab, Army Air Forces, Wright Field *Principal Research Physiologist, Asst in Physiology, Harvard School of Public Health* (1, 1936)
- Heinbecker, Peter, M D Washington Univ Med School, St Louis, Mo *Assoc Prof of Clinical Surgery* (1, 1930)
- Heinle, Robert W, M D Western Reserve Univ, Cleveland, Ohio *Asst Prof of Medicine* (5, 1948)
- Helff, O M, Ph D New York Univ, University Heights, New York City *Assoc Prof of Biology* (1, 1932)
- Hellbaum, Arthur A, Ph D, M D Univ of Oklahoma School of Medicine, Oklahoma City *Prof of Pharmacology* (1, 1937, 3, 1945)
- Hellebrandt, Frances Anna, M D Med College of Virginia, Richmond *Prof of Physical Medicine* (1, 1933)
- Heller, Carl G, M D, Ph D Univ of Oregon Med School, Portland 1 *Assoc Prof of Physiology and Medicine* (1, 1945)
- Heller, Victor G, Ph D Oklahoma A & M College, Stillwater *Head of Dept of Agricultural Chemistry Research* (2, 1935, 5, 1935)
- Hellerman, Leslie, Ph D Johns Hopkins Univ School of Medicine, 710 N Washington St, Baltimore 5, Md *Assoc Prof of Physiological Chemistry* (2, 1935)
- Helmer, Oscar Marvin, Ph D Indianapolis General Hospital, Indianapolis, Ind *Head of Dept of Physiological Chemistry* (2, 1935)
- Hemingway, Allan, Ph D 241 Cecil St, S E, Minneapolis, Minn *Assoc Prof of Physiology, Univ of Minnesota* (1, 1933)
- Henderson, LaVell M, Ph D Univ of Illinois, W A Noyes Lab, Urbana *Asst Prof of Biochemistry* (2, 1949)
- Hendley, Charles D, Ph D Ohio State Univ Dept of Physiology, Hamilton Hall, Columbus 10 *Asst Prof of Physiology* (1, 1949)
- Hendrix, Byron M, Ph D Univ of Texas School of Medicine, Galveston *Prof of Biochemistry* (2, 1920)
- Hendrix, James Paisley, M A, M D Duke Hospital, Durham, N C *Assoc in Medicine (in charge of Therapeutics), Assoc in Physiology and Pharmacology, Duke Univ School of Medicine* (3, 1942)
- Hendry, Jessie L, M A Div of Labs and Research, New York State Dept of Health, New Scotland Ave, Albany *Sr Bacteriologist* (6, 1938)
- Henle, Werner, M D 1740 Bainbridge St, Philadelphia 46, Pa *Assoc Prof of Virology in Pediatrics* (6, 1938)
- Henry, James P, M A, M Sc Aero-Med Lab, Wright Field, Dayton, Ohio On leave of absence from the Univ of Southern California, Los Angeles (1, 1947)
- Henschel, Austin F, Ph D Univ of Minnesota, Minneapolis *Asst Prof of Physiological Hygiene* (1, 1944)
- Hepburn, Joseph Samuel, Ph D, M D Chem D 235 N 15th St, Philadelphia 2, Pa *Prof of Chemistry and Research Assoc in Gastro-enterology, Registrar, Hahnemann Med College and Hospital* (2, 1915)
- Hepler, Opal E, Ph D, M D Northwestern Univ Med School, 303 E Chicago Ave, Chicago, Ill *Assoc Prof of Pathology* (4, 1939)
- Heppel, Leon A, Ph D, M D Natl Insts of Health, 4511 Maple Ave, Bethesda 14, Md *Surgeon, USPHS* (2, 1949)
- Herbst, Robert M, Ph D Kedzie Chemical Lab, Michigan State College, East Lansing *Assoc Prof of Chemistry* (2, 1938)
- Herget, Carl M, Ph D Med Div, Army Chemical Center, Md *Chief, Biophysics Section* (1, 1948)
- Herrick, Julia F, Ph D Mayo Foundation, Rochester, Minn *Assoc Prof of Exper Medicine* (1, 1933)
- Herrin, Raymond C, Ph D, M D Univ of Wisconsin Med School, Madison *Prof of Physiology* (1, 1932)
- Herrington, Lovic P, Ph D 290 Congress Ave, New Haven, Conn *Assoc Dir, John B Pierce Lab of Hygiene, Research Assoc Prof, Dept of Public Health, Yale Med School* (1, 1942)
- Herriott, Roger M, Ph D Johns Hopkins School of Hygiene and Public Health, 615 N Wolfe St, Baltimore 5, Md *Prof of Biochemistry* (2, 1940)

- Hermann, Julian B , Ph B , M D 1185 Park Ave , New York City (3, 1941)
- Herrmann, George K , Ph D , M D Univ of Texas Med Branch, Galveston *Prof of Medicine* (4, 1925)
- Herrmann, Louis George, M D Cincinnati General Hospital, Cincinnati 29, Ohio *Attending Surgeon, Assoc Prof of Surgery, Univ of Cincinnati College of Medicine* (4, 1933)
- Hershey, A D , Ph D Washington Univ School of Medicine, St Louis, Mo *Assoc Prof of Bacteriology and Immunology* (6, 1942)
- Hertz, Roy, Ph D , M D Natl Insts of Health, Bethesda 14, Md *Public Health Surgeon (R), Div of Physiology* (1, 1945)
- Hertz, Saul, M D Radioactive Isotope Foundation, 270 Commonwealth Ave , Boston, Mass *Dir , Instr , Harvard Med School* (4, 1935)
- Hertzman, Alrick B , Ph D St Louis Univ School of Medicine, St Louis, Mo *Prof of Physiology and Dir of Dept* (1, 1925)
- Herwick, Robert P , Ph D , M D American Home Products Corp , 22 E 40th St , New York City *Med Dir , Whitehall Div* (3, 1938)
- Hess, Charles L , M S , M D 308 Davidson Bldg , Bry City, Mich (1, 1916)
- Hess, Walter C , Ph D Georgetown Med School, 39th St and Reservoir Rd , N W , Washington 7, D C *Prof of Biological Chemistry* (2, 1935)
- Hetherington, Albert W , Ph D School of Aviation Medicine, Randolph Field, Texas (1, 1943)
- Hewetson, Jean Hawks, M D 115 E York St , Biglerville, Pa (5, 1944)
- Hewitt, Earl Albon, Ph D Iowa State College, Ames *Assoc Prof of Veterinary Physiology* (1, 1932)
- Hewitt, Julia A W , B A % Emerson H Lillibridge, 8046 Woodlawn Drive, Minneapolis 21, Minn *Sr Technician, Huntington Hospital Lab , Huntington, N Y* (6, 1921)
- Heyroth, Francis F , M D , Ph D Univ of Cincinnati, Cincinnati, Ohio *Assoc Prof of Ind Toxicology and Asst Dir , Kettering Lab , Assoc Prof of Biological Chemistry, College of Medicine* (2, 1935)
- Hiatt, Edwin P , Ph D Univ of North Carolina School of Medicine, Chapel Hill *Assoc Prof of Physiology* (1, 1942)
- Hickman, Kenneth C D , Ph D 56 Thackeray Rd , Rochester 10, N Y *Consultant, Arthur D Little, Inc* (2, 1944)
- Hiestand, William A , Ph D Purdue Univ , Dept of Biology, Lafayette, Ind *Prof of Physiology* (1, 1947)
- Higgins, George M , Ph D Mayo Clinic, Rochester, Minn *Prof of Exper Biology, Mayo Foundation* (5, 1948)
- Higgins, Harold Leonard, M D 322 Franklin St , Newton, Mass (1, 1914, 5, 1933)
- Highman, Benjamin, M D Natl Insts of Health, Bethesda, Md *Surgeon, USPHS, Lab of Pathology and Pharmacology* (4, 1947)
- Hill, Edgar S , Ph D Washington Univ College of Dentistry, St Louis, Mo *Assoc Prof of Biological Chemistry and Physiology* (2, 1936)
- Hill, Robert M , Ph D Univ of Colorado Med Center, 4200 E 9th Ave , Denver *Prof of Biochemistry* (2, 1933)
- Hill, Samuel E , Ph D 18 Collins Ave , Troy, N Y *Research Worker, The Behr-Manning Corp* (1, 1934)
- Hilleman, Maurice, Ph D Army Med Center, AMDR & GS, Dept of Virus and Rickettsial Diseases, Washington 12, D C *Med Bacteriologist* (6, 1949)
- Hiller, Alma, Ph D Presbyterian Hospital, 1753 W Congress St, Chicago 12, Ill *Assoc , Rush Dept of Biochemistry* (2, 1929)
- Himwich, Harold E , M D Fallston, Md *Chief, Clinical Research Branch, Med Div , Army Chem Center, Md* (1, 1925, 5, 1933)
- Himwich, Williamina A , Ph D Johns Hopkins Univ , Welch Med Library, Baltimore, Md (1, 1947)
- Hine, Charles H , Ph D , M D Univ of California Med School, Pharmacology and Toxicology Dept , Med Center, San Francisco 22 *Lecturer in Toxicology, Consulting Pharmacologist and Toxicologist, Shell Development Co* (3, 1947)
- Hines, Harry M , Ph D State Univ of Iowa, Iowa City *Prof of Physiology* (1, 1928)
- Hines, Marion, Ph D Emory Univ Dept of Anatomy, Emory University, Ga *Prof of Exper Anatomy* (1, 1932)
- Hinrichs, Marie, Ph D , M D Southern Illinois Normal Univ , Carbondale *Prof of Physiology, Dir of Student Health Service* (1, 1928).
- Hinsey, Joseph C , Ph D Cornell Univ Med College, 1300 York Ave , New York City *Prof of Anatomy and Dean of the Med College* (1, 1929)
- Hirschmann, Hans, M D , Ph D Lakeside Hospital, Cleveland, Ohio *Asst Prof of Biochemistry, Dept of Medicine, Western Reserve Univ* (2, 1946)
- Hirszfeld, Ludwik, M D Univ of Wroclaw, Wroclaw, Ul Chaubinskiego 4, Poland *Prof of Microbiology, Dir of Med Microbiology* (6, 1946)
- Hisaw, Frederick L , Ph D Harvard Univ Biological Labs , Cambridge, Mass *Prof of Zoology* (1, 1932)
- Hitchcock, David I , Ph D Yale Univ , 333 Cedar St , New Haven 11, Conn *Assoc Prof of Physiology* (2, 1930)
- Hitchcock, Fred A , Ph D Ohio State Univ , Columbus *Prof of Physiology* (1, 1927, 5, 1933)

- Hitchcock, Philip**, Ph D Med College of Alabama, Dept of Physiology and Pharmacology, Birmingham 5 *Asst Prof of Physiology and Pharmacology* (3, 1946)
- Hitchings, George H**, Ph D 50 Primrose Ave, Tuckahoe 7, N Y, *Chief Biochemist, Wellcome Research Labs* (2, 1942)
- Hjort, Axel M**, M D, Ph D P O Box 281, 14 Fern Way, Scarsdale, N Y *Adjunct Physician, Grasslands Hospital, Valhalla, N Y* (2, 1925)
- Hoagland, Hudson**, Ph D 222 Maple Ave, Shrewsbury, Mass *Exec Dir, Worcester Foundation for Exper Biology, Neurophysiologist, Worcester State Hospital* (1, 1932)
- Hobby, Gladys L**, Ph D 11 Bartlett St, Brooklyn 6, N Y *Research Bacteriologist in charge of Biological Control, Chas Pfizer & Co* (6, 1946)
- Hoberman, Henry D**, Ph D, M D Yale Univ School of Medicine, Dept of Physiological Chemistry, 333 Cedar St, New Haven 11, Conn *Asst Prof of Physiological Chemistry* (2, 1949)
- Hodes, Robert**, Ph D Tulane Univ School of Medicine, Dept of Psychiatry, New Orleans 12, La *Prof of Exper Neurology and Neurophysiology* (1, 1941)
- Hodge, Harold Carpenter**, Ph D Univ of Rochester School of Medicine and Dentistry, 260 Crittenden Blvd, Rochester 7, N Y *Prof of Pharmacology and Toxicology* (2, 1937, 3, 1948)
- Hoefler, Paul F A**, Ph D, M D Neurological Inst of New York, 710 W 168th St, New York City 32 *Assoc Prof of Neurology* (1, 1945)
- Hoff, Ebbe Curtis**, Ph D Med College of Virginia, Richmond 19 *Assoc Prof* (1, 1933)
- Hoff, Hebbel E**, Ph D McGill Univ, Montreal, Quebec, Canada *Prof of Physiology* (1, 1933)
- Hoffman, William Samuel**, Ph D, M D Hektoen Inst for Med Research, 629 S Wood St, Chicago 12, Ill *Dir of Biochemistry, Cook County Hospital* (2, 1935)
- Hofmann, Klaus**, Ph D Univ of Pittsburgh Dept of Chemistry, Pittsburgh, Pa *Research Prof* (2, 1947)
- Hogan, Albert G**, Ph D Univ of Missouri, 105 Schweitzer Hall, Columbia *Prof of Animal Nutrition* (2, 1916, 5, 1933)
- Hogness, Thorfin R**, Ph D Univ of Chicago, Chicago Ill *Dir, Inst of Radiobiology and Biophysics* (2, 1941)
- Holck, Harald G O**, Ph D Univ of Nebraska, College of Pharmacy, Lincoln *Prof of Pharmacology* (1, 1935, 3, 1938)
- Hollaender, Alexander**, Ph D Oak Ridge Natl Lab, Oak Ridge, Tenn (1, 1947)
- Hollander, Franklin**, Ph D Mount Sinai Hospital, Fifth Ave and 100th St, New York City *Assoc in Physiology, Head, Gastro-enterology Research Lab* (1, 1942, 2, 1932)
- Hollinshead, W Henry**, Ph D Mayo Clinic, Section on Anatomy, Rochester, Minn *Head of Section on Anatomy, Prof of Anatomy, Mayo Foundation* (1, 1949)
- Holm, August**, Sc D E R Squibb & Sons, New Brunswick, N J *Research Assoc* (6, 1946)
- Holman, Ralph Theodore**, Ph D A and M College of Texas, College Station *Assoc Prof of Biochemistry and Nutrition* (2, 1948)
- Holman, Russell Lowell**, M D Louisiana State Univ School of Medicine, New Orleans *Prof of Pathology* (4, 1940)
- Holmes, Arthur Dunham**, Ph D Univ of Massachusetts, Amherst *Research Prof of Chemistry* (2, 1931, 5, 1933)
- Holmes, Joseph H**, M D, D Med Sc Univ of Colorado School of Medicine, 4200 E Ninth Ave, Denver 7 *Assoc Prof of Medicine* (1, 1947)
- Holmes, Julia O**, Ph D Univ of Massachusetts, 15 Sunset, Amherst *Research Asst of Chemistry Dept* (2, 1942, 5, 1936)
- Holt, Joseph Paynter**, M D, Ph D Standard Oil Co, Room 2400, 30 Rockefeller Plaza, New York City 20 *Dir of Med Research* (1, 1942)
- Holt, L Emmett, Jr**, M D 477 First Ave, New York City 16 *Prof of Pediatrics, New York Univ College of Medicine* (2, 1930, 5, 1946)
- Hoobler, Icie Macy**, Ph D 660 Frederick St, Detroit 2, Mich *Dir, Research Lab Children's Fund of Michigan* (2, 1925, 5, 1933)
- Hoobler, Sibley W**, M D Univ of Michigan Hospital, Dept of Medicine and Surgery, Ann Arbor *Asst Prof of Medicine* (1, 1949)
- Hooker, Davenport**, Ph D Univ of Pittsburgh School of Medicine, Pittsburgh, Pa *Prof of Anatomy* (1, 1920)
- Hooker, Sanford B**, A M, M D 80 E Concord St, Boston, Mass *Member Evans Memorial* (6, 1918)
- Hoover, Sam R**, Ph D 7815 Linden Rd, Philadelphia 18, Pa *Sr Chemist Eastern Regional Research Lab, U S Dept of Agriculture* (2, 1946)
- Hoppert, C A**, Ph D Michigan State College, Box 626, East Lansing *Prof of Biological Chemistry* (5, 1935)
- Hopps, Howard C**, M D Univ of Oklahoma School of Medicine, Dept of Pathology, 801 E 13th St, Oklahoma City *Prof and Chairman of Dept of Pathology* (4, 1947, 6, 1946)
- Horecker, Bernard L**, Ph D Natl Insts of Health, Bethesda 14, Md *Sr Biochemist* (2, 1947)
- Horn, Millard J**, Ph D 1811 Varum St, N E, Washington 18, D C *U.S Dept of Agriculture, Chemist* (2, 1949)
- Horowitz, Norman H**, Ph D California Inst of Technology, Pasadena *Assoc Prof of Biology* (2, 1946)

- Horsfall, Frank L, Jr, M D C M Rockefeller Inst for Med Research, 66th St and York Ave, New York City *Member, Physician to the Hospital of the Rockefeller Inst for Med Research* (6, 1937)
- Horton, Richard G, Ph D Med Div, Army Chemical Center, Md *Acting Chief, Toxicology Section* (1, 1948)
- Horvath, Steven M, Ph D State Univ of Iowa School of Medicine, Dept of Physiology, Iowa City (1, 1943)
- Horwitt, M K, Ph D Biochemical Research Lab, Elgin State Hospital, Elgin, Ill *Dir, Asst Prof, Biological Chemistry, Univ of Illinois College of Medicine* (2, 1941, 5, 1949)
- Hotchkiss, Rollin D, Ph D Rockefeller Inst for Med Research, 66th St and York Ave, New York City *Associate* (2, 1941)
- Hou, H C, M D Inst of Nutrition, Nanking, China *Director*, (5, 1949)
- Houck, C Riley, Ph D Univ of Tennessee Med School, Memphis 3 *Asst Prof in Physiology* (1, 1947)
- Hove, Edwin L, Ph D Alabama Polytechnic Inst, Lab of Animal Nutrition, Auburn *Research Biochemist* (5, 1946)
- Howard, Evelyn, Ph D Johns Hopkins School of Medicine, Baltimore, Md *Asst Prof of Physiology* (1, 1933)
- Howard, John Eager, A B, M D Johns Hopkins Hospital, Baltimore 5, Md *Asst Prof of Medicine* (1, 1946)
- Howard, Marion E, M D New Haven Hospital, New Haven, Conn *Assoc Clinical Prof of Medicine Yale School of Medicine, Assoc Physician, New Haven Hospital and New Haven Dispensary* (4, 1939, 6, 1937)
- Howe, Howard A, Ph D Johns Hopkins Univ, 1901 East Madison St, Baltimore 5, Md *Adjunct Prof of Epidemiology* (6, 1949)
- Howe, Paul E, Ph D U S Dept of Agriculture, Bureau of Animal Ind, Washington 25, D C, *Nutrition Consultant* (1, 1913, 2, 1909, 5, 1933)
- Howe, Percy R, M D, D D S Forsyth Dental Infirmary, Harvard Med School, Boston, Mass *Dir, Prof of Dental Sciences* (5, 1935)
- Howell, Katherine M, M D 6830 S Merrill Ave, Chicago, Ill *Head of Depts of Bacteriology and Serology* (6, 1940)
- Howell, Stacy F, Ph D V D Research Lab, U S Marine Hospital, Stapleton, Staten Island, N Y *Chemist, USPHS* (2, 1940)
- Howland, Joe W, M D, Ph D Strong Memorial Hospital, 260 Crittenden Blvd, Rochester 7, N Y *Asst Prof of Radiation Biology, Univ of Rochester, Chief, Div of Med Services* (1, 1949)
- Hubbard, Roger Sanford, Ph D Univ of Buffalo School of Medicine, 24 High St, Buffalo 2, N Y *Prof of Pharmacology* (1, 1922, 2, 1920)
- Hubbell, Rebecca B, Ph D Connecticut Agricultural Experiment Station, New Haven *Asst Biochemist* (2, 1937, 5, 1935)
- Hudack, Stephen Sylvester, M D 180 Fort Washington Ave, New York City *Assoc Prof of Orthopedic Surgery, Columbia Univ* (4, 1933)
- Huddleston, Ora L, M D, Ph D Kabat-Kaiser Inst, 1815 Ocean Front, Santa Monica, Calif *Med Dir* (1, 1936)
- Hueper, Wilhelm C, M D Natl Cancer Inst, Bethesda, Md *Chief, Cancerogenic Studies Section* (4, 1940)
- Huf, Ernst G, Ph D, M D 3500 Carolina Ave, Richmond, Va *Asst Prof of Physiology, Med College of Virginia* (1, 1949)
- Huff, Jesse W, Ph D Sharp & Dohme, Inc, Glenolden, Pa *Research Biochemist* (2, 1949)
- Huffman, C F, Ph D Michigan State College, East Lansing *Research Prof and Prof in Dairy Husbandry* (5, 1937)
- Huffman, Max N, Ph D Southwestern Med College, 2211 Oak Lawn, Dallas, Tex *Research Prof of Biochemistry* (2, 1947)
- Huggins, Charles Brenton, M D Univ of Chicago, Chicago, Ill *Prof of Surgery* (1, 1932)
- Huggins, Joseph, M D 111 N 49th St, Philadelphia, Pa *Asst Prof of Exper Neurology, Grad School of Medicine, Univ of Pennsylvania, Dir of Lab, Pennsylvania Hospital for Mental Diseases* (1, 1936)
- Huggins, Russell A, Ph D Baylor Univ College of Medicine, 1200 M D Anderson Blvd, Houston 5, Tex *Assoc Prof of Pharmacology* (3, 1949)
- Hughes, Hettie B, Ph D Christ Hospital, Cincinnati 19, Ohio *Research Assoc* (2, 1946)
- Hughes, Josiah Simpson, Ph D Kansas State College, Manhattan *Prof of Chemistry* (2, 1931, 5, 1939)
- Hughes, Thomas P, Ph D Johns Hopkins Univ School of Public Health, Baltimore, Md (6, 1934)
- Hulpieu, Harold R, Ph D Indiana Univ School of Medicine, Indianapolis *Prof of Pharmacology* (3, 1939)
- Hunscher, Helen A, Ph D Western Reserve Univ, 2023 Adelbert Rd, Cleveland 6, Ohio *Head of Dept of Home Economics* (5, 1934)
- Hunter, Andrew, C B E, M B Hospital for Sick Children, Toronto, Ontario, Canada *Consulting Biochemist, Prof Emeritus of Pathological Chemistry, Univ of Toronto* (2, 1908)
- Hunter, Francis Edmund, Jr, Ph D Washington Univ Med School, Dept of Pharmacology, St Louis 10, Mo *Asst Prof of Pharmacology* (2, 1946)
- Hunter, George, M A, D Sc Univ of Alberta, Edmonton, Canada *Prof of Biochemistry* (2, 1924)

- Hunter, Jesse E , Ph D Allied Mills, Inc , 7500 S Adams St , Peoria, Ill *Dir of Research* (5, 1936)
- Hunter, John, Ph D Univ of Michigan, Ann Arbor *Instr in Physiology* (1, 1948)
- Hussey, Raymond, M D Wayne Univ Med Science Center, 1547 Penobscot Building, Detroit 26, Mich *Dir , Inst for Occupational Health Research, Dean and Prof of Occupational Health, School of Occupational Health* (4, 1927)
- Hutchens, John O , Ph D Univ of Chicago, Dept of Physiology, Chicago 37, Ill *Assoc Prof and Chairman of Dept of Physiology, Dir of Toxicity Lab* (1, 1947)
- Hutchings, Brian L , Ph D Lederle Labs Div , American Cyanamid Co , Pearl River, N Y *Research Chemist* (2, 1949)
- Hyman, Chester, Ph D Univ of Southern California School of Medicine, Los Angeles *Asst Prof of Physiology* (1, 1948)
- Ingalls, Mabel S , Ph D Salisbury Mills, Orange County, N Y (6, 1940)
- Ingle, Dwight J , Ph D Upjohn Research Labs , Kalamazoo, Mich *Sr Research Scientist* (1, 1939)
- Ingraham, Raymond Clifford, Ph D Univ of Illinois College of Medicine, 1853 W Polk St , Chicago *Asst Prof in Physiology* (1, 1938)
- Ingram, W R , Ph D State Univ of Iowa College of Medicine, Iowa City *Prof and Head of Dept of Anatomy* (1, 1936)
- Irish, Don D , Ph D Dow Chemical Co , Midland, Mich *Dir , Biochemical Research Lab* (3, 1948)
- Irvin, J Logan, Ph D Johns Hopkins Univ School of Medicine, 710 N Washington St , Baltimore, Md *Asst Prof of Physiological Chemistry* (2, 1942)
- Irving, George Washington, Jr , Ph D U S Dept of Agriculture, Bureau of Agricultural and Industrial Chemistry, South Bldg , Washington 25, D C *Asst Chief* (2, 1946)
- Irving, Laurence, Ph D Swarthmore College, Swarthmore, Pa *Lt Col , A C , Prof of Exper Biology* (1, 1927)
- Irwin, M R , Ph D Univ of Wisconsin, Dept of Genetics, Madison *Prof of Genetics* (6, 1936)
- Isaacs, Raphael, M D 104 S Michigan Ave , Suite 630, Chicago 3, Ill *Attending Physician, Dept of Hematology, Michael Reese Hospital* (4, 1928)
- Isbell, Harris, M D USPHS Hospital, Lexington, Ky *Med Dir , USPHS, Dir , Research Div* (3, 1949)
- Isenberger, R M , M A , M D Univ of Kansas School of Medicine, Kansas City *Prof of Pharmacology* (3, 1937)
- Ivy, A C , Ph D , M D Univ of Illinois, Chicago Professional Colleges, Chicago, Ill *Vice-Pres , Distinguished Prof of Physiology* (1, 1919, 5, 1933)
- Iwamoto, Harry K , Ph D Univ of Maryland School of Medicine, Baltimore *Asst Prof of Pharmacology* (3, 1948)
- Izquierdo, J Joaquin, M D Natl School of Medicine, Mexico City *Prof of Physiology in Natl School of Medicine and the Escuela Medico Militar of Mexico* (1, 1928)
- Jackson, Elizabeth B , A B Army Med Center—AMDR & GS, Dept of Virus and Rickettsial Diseases, Washington 12, D C *Bacteriologist* (6, 1949)
- Jackson, Eugene L , Ph D 1322 W Broad St , Richmond 20, Va *Med Dir , A H Robins Co* (3, 1942)
- Jackson, Richard W , Ph D U S Dept of Agriculture, Northern Regional Research Lab , 825 N University St , Peoria 5, Ill *Head of Fermentation Div* (2, 1930, 5, 1933)
- Jacobs, Merkel Henry, Ph D Univ of Pennsylvania, Philadelphia *Prof of General Physiology* (1, 1919)
- Jacobs, Walter A , Ph D Rockefeller Inst for Med Research, 66th St and York Ave , New York City 21 *Member Emeritus* (2, 1908, 3, 1913)
- Jacobson, Edmund, Ph D , M D Lab for Clinical Physiology, 55 E Washington St , Chicago, Ill (1, 1929)
- Jaffe, Henry L , M D Hospital for Joint Diseases, 1919 Madison Ave , New York City *Dir of Labs* (4, 1925)
- Jahn, Theodore Louis, Ph D Univ of California, Los Angeles 24 *Prof of Zoology* (1, 1944)
- Jailer, Joseph W , Ph D Columbia Univ College of Physicians and Surgeons, New York City *Instr in Medicine* (1, 1948)
- Jamieson, Walter A , Sc D Green Braes, R F D 2, Indianapolis, Ind *Dir , Biological Div , Eli Lilly & Co* (6, 1927)
- Jandorf, Bernard J , Ph D Med Div , Army Chemical Center, Md *Research Biochemist, Biochemistry Section, Lecturer in Preventive Medicine, Johns Hopkins Univ School of Medicine* (2, 1946)
- Janes, Ralph G , Ph D State Univ of Iowa College of Medicine, Iowa City *Assoc Prof of Anatomy* (1, 1948)
- Jansen, Eugene F , B A Enzyme Research Lab , Western Regional Research Lab , Albany 6, Calif *Sr Chemist, Bureau of Agricultural and Industrial Chemistry, U S Dept of Agriculture* (2, 1947)
- Jaques, L B , Ph D Univ of Saskatchewan, Saskatoon Canada *Prof of Physiology* (1, 1943)
- Jasper, Herbert H , Ph D , D s Sci Montreal Neurological Inst , 3801 University St , Mon-

- treal, Quebec, Canada *Lecturer in Neuro-electrography and Dir of Dept of Electrophysiology* (1, 1940)
- Jeans, P C**, M D State Univ of Iowa, Iowa City *Prof of Pediatrics* (5, 1937)
- Jensen, Hans F**, Ph D Med Dept Field Research Lab, Fort Knox, Ky *Chief Biochemist* (2, 1929)
- Jochum, Kenneth E**, Ph D Univ of Kansas, Dept of Physiology, Lawrence (1, 1942)
- Johlin, J M**, Ph D Vanderbilt Univ School of Medicine, Nashville, Tenn *Assoc Prof of Biochemistry* (2, 1928)
- Johnson, B Conner**, Ph D Univ of Illinois, Div of Animal Nutrition, 554 Davenport Hall, Urbana *Assoc Prof* (2, 1947, 5, 1947)
- Johnson, Frank H**, Ph D Princeton Univ, Princeton, N J *Asst Prof, Dept of Biology* (1, 1942)
- Johnson, Joseph L**, Ph D, M D Howard Univ School of Medicine, Washington, D C *Prof and Head of Dept of Physiology* (1, 1934)
- Johnson, J Raymond**, Ph D Univ of Ottawa, Faculty of Medicine, Ottawa, Ontario, Canada (1, 1938)
- Johnson, Marvin J**, Ph D Univ of Wisconsin, Biochemistry Bldg, Madison *Prof of Biochemistry* (2, 1941)
- Johnson, Robert E**, M D, Ph D Univ of Illinois College of Liberal Arts and Sciences, Dept of Physiology, Urbana *Head of Dept* (1, 1944, 2, 1939, 5, 1946)
- Johnson, S R**, Ph D Missouri Farmers Assoc Milling Co, Springfield *Dir of Nutrition* (5, 1947)
- Johnson, Victor**, Ph D, M D Mayo Foundation, Rochester, Minn (1, 1933)
- Johnston, Charles G**, M S, M D Wayne Univ College of Medicine, Detroit, Mich *Prof of Surgery* (1, 1933)
- Johnston, Frances A**, Ph D Cornell Univ, Ithaca, N Y *Asst Prof of Foods and Nutrition* (5, 1948)
- Johnston, Margaret W**, Ph D Box 452, Univ Hospital, Ann Arbor, Mich *Research Assoc in Internal Medicine* (2, 1930, 5, 1938)
- Jolliffe, Norman**, M D 39 75th St, New York City (1, 1932)
- Jones, D Breese**, Ph D U S Dept of Agriculture, Bureau of Human Nutrition and Home Economics, Agricultural Research Admin, Beltsville, Md *Protein Chemist* (2, 1920, 5, 1935)
- Jones, Hardin B**, Ph D Univ of California, Div of Med Physics, Berkeley 4 *Asst Prof of Med Physics and Physiology, Asst Dir of Donner Lab* (1, 1949)
- Jones, James H**, Ph D Univ of Pennsylvania School of Veterinary Medicine, Philadelphia 4 *Prof of Physiological Chemistry* (2, 1928, 5, 1933)
- Jones, Kenneth K**, Ph D Northwestern Univ Med School, 303 E Chicago Ave, Chicago, Ill *Assoc Prof of Physiology and Pharmacology* (1, 1936)
- Joseph, Norman R**, Ph D Univ of Illinois, 1853 W Polk St Chicago 12 *Asst Prof of Chemistry* (2, 1947)
- Joslin, Elliott P**, M A, M D New England Deaconess Hospital, 81 Bay State Rd, Boston, Mass *Dir, George F Baler Clinic* (5, 1933)
- Jukes, Thomas Hughes**, Ph D Lederle Labs, Pearl River, N Y *Head, Dept of Nutrition and Physiology Research* (2, 1935, 5, 1938)
- Jung, Frederic Theodore**, Ph D, M D American Med Assoc, Chicago, Ill *Asst Secretary* (1, 1930)
- Jungeblut, Claus W**, M D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 *Prof of Bacteriology* 4, 1929, 6, 1926)
- Kabat, Elvin A**, Ph D Columbia Univ College of Physicians and Surgeons, 710 W 168th St, New York City 32 *Assoc Prof of Bacteriology* (2, 1940, 6, 1943)
- Kabat, Herman**, Ph D, M D 2633 16th St, N W, Washington, D C *Consultant in Neurology, Health Dept District of Columbia* (1, 1941)
- Kahn, Reuben L**, Sc D, Univ of Michigan Hospital, Ann Arbor *Assoc Prof of Serology of Syphilis and Chief of Serology Lab* (4, 1934, 6, 1919)
- Kalckar, Herman M**, M D, Ph D Univ of Copenhagen, Inst for Enzyme Research, 28 Juliane Maries Vej, Copenhagen, Denmark *Research Prof* (2, 1942)
- Kalnitsky, George**, Ph D State Univ of Iowa, Iowa City *Asst Prof of Biochemistry* (2, 1948)
- Kamen, Martin D**, Ph D Washington Univ Med School, 510 S Kingshighway, St Louis 10, Mo *Assoc Prof of Chemistry* (2, 1946)
- Kamm, Oliver**, Ph D 365 Lakeshore Drive, Detroit 30, Mich *Research Consultant, Parke, Davis and Co* (2, 1928)
- Kaplan, Nathan O**, Ph D Univ of Illinois College of Medicine Dept of Biological Chemistry, 1853 W Polk St, Chicago 12 *Asst Prof* (2, 1949)
- Karel, Leonard**, Ph D Natl Insts of Health, Div of Research and Fellowships, Bethesda 14, Md *Exec Sec, Hematology and Pharmacology Study Sections, Univ of Maryland School of Medicine, Lecturer in Pharmacology* (3, 1947)
- Karpovich, Peter V**, M D, M P E Springfield College, Springfield, Mass *Prof of Physiology* (1, 1942)
- Karshan, Maxwell**, Ph D Columbia Univ, Dept of Biological Chemistry, 630 W 168th St, New

- York City 32 *Assoc Prof of Biochemistry* (2, 1939)
- Karsner, Howard T, M D Dept of the Navy, Bureau of Medicine and Surgery, Research Div, Washington 25, D C *Med Research Advisor* (4, 1913)
- Katz, Louis Nelson, M A, M D 2900 Ellis Ave, Chicago, Ill *Dir of Cardiovascular Research, Michael Reese Hospital, Professorial Lecturer in Physiology, Univ of Chicago* (1, 1924)
- Katzman, Philip A, Ph D St Louis Univ School of Medicine, 1402 S Grand Blvd, St Louis 4, Mo *Assoc Prof of Biochemistry* (2, 1935)
- Kaulbersz, Jerzy, Ph D M D Collegium Medica, Grzegorzeczka 16, Cracow, Poland *Prof of Physiology* (1, 1944)
- Kaunitz, Hans, M D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 *Research Assoc in Pathology* (4, 1947)
- Kay, Herbert Davenport, Ph D Natl Inst for Research in Dairying, Shinfield, near Reading, England *Dir, Research Prof of Biochemistry, Univ of Reading* (2, 1930)
- Kazal, Louis Anthony, Ph D Sharp & Dohme, Inc, Med Research Div, Glenolden, Pa *Research Biochemist* (2, 1947)
- Keeton, Robert W, M S, M D Univ of Illinois College of Medicine, 1817 W Polk St, Chicago *Prof of Medicine* (1, 1916, 3, 1924)
- Kehoe, Robert A, M D Univ of Cincinnati College of Medicine, Kettering Lab of Applied Physiology, Eden Ave, Cincinnati, Ohio *Research Prof of Physiology* (1, 1940)
- Keith, Norman M, M D Mayo Clinic, Rochester, Minn *Consulting Physician, Div of Medicine, Mayo Clinic, Prof of Medicine, Mayo Foundation, Univ of Minnesota* (1, 1920, 3, 1932, 4, 1924)
- Keith, T B, Ph D Dept of Animal Husbandry, Univ of Idaho, Moscow, Idaho (5, 1941)
- Kellaway, Peter E, Ph D Baylor Univ College of Medicine, Houston, Texas *Assoc Prof of Neuropsychiatry and Physiology, Dir of Lab of Clinical Electrophysiology* (1, 1947)
- Keller, Allen D, Ph D Field Research Lab, Fort Knox, Ky *Med Dept* (1, 1931)
- Kellner, Aaron, M D Cornell Univ Med College, Dept of Pathology, 1300 York Ave, New York City 21 *Research Assoc in Pathology, Dir of Central Labs, New York Hospital and Cornell Med Center* (4, 1949)
- Kelser, Raymond A, D V M, Ph D The Wyndon, Apt A-105, Wynnewood, Pa *Prof of Bacteriology and Dean of Faculty, School of Veterinary Medicine, Univ of Pennsylvania, Philadelphia* (4, 1932)
- Kelsey, F Ellis, Ph D Univ of Chicago Chicago, Ill *Assoc Prof of Pharmacology* (3, 1941)
- Kelsey, Frances Kathleen O, Ph D Univ of Chicago, Chicago, Ill *Research Assoc (Instr) in Pharmacology* (3, 1941)
- Kemmerer, A R, Ph D Univ of Arizona, Tucson *Head of Dept of Human Nutrition* (5, 1946)
- Kempner, Walter, M D Duke Univ School of Medicine, Durham, N C *Asst Prof of Medicine* (1, 1940)
- Kendall, Edward C, Ph D, 627 Eighth Ave, S W, Rochester, Minn *Prof of Biochemistry, Mayo Foundation, Univ of Minnesota* (1, 1916, 2, 1913, 4, prior to 1920)
- Kendall, Forrest E, Ph D 240-06-53rd Ave, Douglaston, Long Island, N Y *Asst Prof of Biochemistry, Research Service, Columbia Div, Goldwater Memorial Hospital Welfare Island* (6, 1943)
- Kennard, Margaret A, M D Univ of Oregon Med School, Dept of Surgery, Portland *Assoc Prof of Exper Surgery* (1, 1934)
- Kennedy, Cornelia, Ph D 2700 W Robbins St, Minneapolis 10, Minn *Prof Emeritus of Agricultural Biochemistry, Univ of Minnesota* (2, 1924, 5, 1934)
- Kensler, Charles J, Ph D Cornell Univ Med College, 1300 York Ave, New York City 21 *Instr, Dept of Pharmacology, Sr Research Fellow, Natl Research Council* (3, 1949)
- Kent, John F, A M Army Med Center Washington 12, D C *Scientific Dir, Dept of Serology, Army Med Dept Research and Grad School*, (6, 1947)
- Kenton, Harold B, Ph D New England Deaconess Hospital, Boston, Mass *Bacteriologist and Dir of the Blood Bank* (6, 1934)
- Kenyon, Allan T, M D Univ of Chicago, Div of Biological Sciences, 950 E 59th St, Chicago, Ill *Asst Prof of Medicine* (3, 1940)
- Keresztesy, John C, Ph D Natl Insts of Health, Bethesda 14, Md *Scientist Officer Div of Physiology* (2, 1941, 5, 1945)
- Kerr, Stanley E, Ph D American Univ of Beirut, Beirut, Lebanon, Syria *Prof of Biological Chemistry* (2, 1937)
- Kerr, Wm J, M D Univ of California Hospital, 3rd and Parnassus Aves, San Francisco *Physician-in-Chief, Prof of Medicine, Univ of California* (3, 1930)
- Kesten, Homer D, M D Columbia Univ College of Physicians and Surgeons, New York City 32 *Assoc Prof of Pathology* (4, 1931)
- Kety, Seymour S, M D Univ of Pennsylvania Grad School of Medicine, Dept of Physiology and Pharmacology, Philadelphia 4 *Prof of Clinical Physiology* (1, 1949, 3, 1945)
- Keys, Ancel, Ph D Univ of Minnesota, Stadium South Tower, Minneapolis *Prof and Dir of Lab of Physiological Hygiene* (1, 1939, 2, 1936)

- Kidd, John G**, M D Cornell Univ Med College, 1300 York Ave, New York City 32 *Prof of Pathology, Pathologist, New York Hospital* (4, 1937, 6, 1937)
- Kidder, George W**, Ph D Amherst College, Dept of Biology, Amherst, Mass *Prof of Biology* (2, 1949)
- Kies, Marian W**, Ph D Agricultural Research Center, North Bldg, Beltsville, Md *Biochemist, Bur Agri and Indust Chemistry* (2, 1948)
- Kik, M G**, Ph D Univ of Arkansas College of Agriculture, Fayetteville *Assoc Prof of Agricultural Chemistry* (5, 1942)
- Kilborn, Leslie G**, M D, Ph D 47 Warren Rd, Toronto, Ontario, Canada At present West China Union Univ, Chengtu, Sze, China (1, 1928)
- Killian, John Allen**, Ph D Killian Research Labs, Inc, 49 W 45th St, New York City (2, 1921)
- Kinard, F W**, Ph D, M D Med College of the State of South Carolina, Charleston 16 *Assoc Prof of Physiology* (1, 1947)
- King, Barry G**, Ph D Dept of Commerce, Civil Aeronautics Admin, Med Service, Safety Regulations, Washington, D C *Chief, Aeromedical Design and Material Div* (1, 1938)
- King, Charles Edwin**, Ph D Vanderbilt Univ, Nashville, Tenn *Assoc Prof of Physiology* (1, 1916)
- King, Charles Glen**, Ph D Nutrition Foundation Inc, Chrysler Building, New York City *Scientific Dir, Prof of Chemistry, Columbia Univ* 2, 1931, 5, 1933)
- King, Joseph T**, M D, Ph D Univ of Minnesota Med School, 314 Millard Hall, Minneapolis *Assoc Prof of Physiology* (1, 1931)
- Kirch, Ernst R**, Ph D Univ of Illinois College of Pharmacy, 808 S Wood St, Chicago 12, Ill *Assoc Prof of Chemistry* (2, 1948)
- Kirchhof, Anton C**, M S, M D Univ of Oregon Med School, Dept of Pharmacology, Portland 1 *Research Assoc* (3, 1947)
- Kirk, Paul L**, Ph D Univ of California, Berkeley *Prof of Biochemistry* (2, 1933)
- Kirkbride, Mary B**, Sc D 314 State St, Albany 6, N Y (6, 1921)
- Kirschbaum, Arthur**, M D, Ph D Univ of Minnesota Med School, Minneapolis *Assoc Prof of Anatomy* (4, 1948)
- Kisch, Bruno**, M D Yeshiva Univ, New York City *Prof of Biochemistry* (1, 1943)
- Kleiber, M**, D Sc Univ of California, Davis *Prof of Animal Husbandry* (1, 1943, 5, 1933)
- Klein, J Raymond**, Ph D Brookhaven Natl Lab, Upton, L I, N Y *Sr Biochemist, Dept of Biology* (2, 1941)
- Kleiner, Israel Simon**, Ph D New York Med College and Flower Hospital, New York City 29 *Prof of Physiology and Biochemistry* (1, 1911, 2, 1912, 3R, 1912, 5, 1933)
- Kleitman, Nathaniel**, Ph D Univ of Chicago, Chicago, Ill *Assoc Prof of Physiology* (1, 1923)
- Klemperer, Friedrich Wilhelm**, M D Trudeau Foundation, Trudeau, N Y *Head of Dept of Biochemistry* (2, 1941)
- Kletzien, Seymour W**, Ph D 330 S Ninth St, Philadelphia, Pa Nutrition Research Clinic, Philadelphia Lying-In and Pennsylvania Hospitals *Biochemist* (5, 1933)
- Kline, O L**, Ph D Federal Security Agency, Food and Drug Admin, Washington, D C *Biochemist* (5, 1936)
- Kline, Raymond F**, M S R F D 1, Charlottesville, Va (1, 1946)
- Klotz, Irving M**, Ph D Northwestern Univ, Dept of Chemistry, Evanston, Ill *Assoc Prof of Chemistry* (2, 1947)
- Kluver, Heinrich**, Ph D Univ of Chicago, Chicago, Ill *Prof of Exper Psychology* (1, 1935)
- Knight, C Arthur**, Ph D Univ of California, Virus Lab, Berkeley 4 *Assoc Prof* (2, 1946)
- Knisely, Melvin H**, Ph D Med College of South Carolina, Dept of Anatomy, Charleston *Chairman of Dept of Anatomy* (1, 1949)
- Knoefel, Peter K**, M A, M D Univ of Louisville, 101 W Chestnut St, Louisville, Ky *Prof of Pharmacology* (3, 1934)
- Knowlton, G Clinton**, Ph D Emory Univ, Room 101, Physiology Bldg, Emory University, Ga (1, 1938)
- Knox, Eugene W**, M D 3026 S California Ave, Chicago 8, Ill *Research Associate, Rheumatic Fever Research Inst, Northwestern Univ Med School* (2, 1948)
- Knudson, Arthur**, Ph D Albany Med College, New Scotland Ave, Albany, N Y *Prof of Biochemistry and Assoc Dean* (2, 1919, 5, 1936)
- Knutti, Ralph Eddy**, M D Children's Hospital, Los Angeles, Calif *Dir of Labs, Asst Prof of Pathology, Univ of Southern California* (4, 1933)
- Kober, Philip A**, B S Sherman Labs, Detroit, Mich *Dir of Research* (2, 1912)
- Kobrak, Heinrich G**, Ph D Univ of Chicago, Chicago, Ill *Asst Prof of Surgery* (1, 1948)
- Kochakian, Charles D**, Ph D Univ of Rochester School of Medicine and Dentistry, Rochester 7, N Y *Assoc Prof of Physiology* (1, 1942, 2, 1948)
- Kocher, Rudolph Alfred**, M D Box 936, Carmel, Calif *Dir of Velie Metabolic Clinic* (2, 1915)
- Koehler, Alfred E**, M D, Ph D 317 W Pueblo St, Santa Barbara, Calif *Physician, Sansum Clinic, Santa Barbara Cottage Hospital* (2, 1924)
- Koehn, Carl J**, Ph D Surgeon General's Office, Preventive Medicine Div, Washington, D C *Chief, Nutrition Branch* (5, 1949)

- Koehn, Martha, Ph D 285 15th Ave, Apt 22, Columbus, Ohio *Nutritionist, Ohio State Dept of Health* (5, 1933)
- Koelle, George B, Ph D Johns Hopkins Hospital, Wilmer Inst, Baltimore, Md *Chalfont Fellow in Ophthalmology* (3, 1947)
- Koepf, George F, M D 537 Delaware Ave, Buffalo 2, N Y *Assoc in Physiology, Univ of Buffalo* (1, 1942)
- Koerber, Walter L, Ph D E R Squibb and Sons, New Brunswick, N J *Asst Dept Head* (6, 1943)
- Kohlstaedt, Kenneth G, M D Lilly Lab for Clinical Research, Indianapolis General Hospital, Indianapolis 7, Ind *Director* (1, 1947)
- Kohn, Henry I, Ph D, M D Univ of California Hospital, Radiology Dept, San Francisco *Surgeon, USPHS* (1, 1940)
- Kolmer, John A, M S, M D, D P H 1 Montgomery Ave, Bala-Cynwyd, Pa *Prof of Medicine, Temple Univ, Dir, Research Inst of Cutaneous Medicine* (6, 1913)
- Komarov, Simon A, M D, Ph D S S Fels Fund Med Research Lab, 255 S 17th St, Philadelphia, Pa *Dir of Dept of Biochemistry, Temple Univ School of Medicine* (1, 1933)
- Kopeloff, Nicholas, Ph D New York State Psychiatric Inst, 722 W 168th St, New York City *Principal Research Bacteriologist, New York State Psychiatric Inst and Hospital* (6, 1937)
- Koppanyi, Theodore, Ph D Georgetown Univ, Washington D C *Prof of Pharmacology* (1, 1924, 3, 1935)
- Koprowski, Hilary, M D Lederle Labs Div, American Cyanamid Co, Pearl River, N Y *Asst Dir, Viral and Rickettsial Research* (6, 1946)
- Kornberg, Arthur, M D Natl Insts of Health, Bethesda 14, Md *Sr Surgeon* (2, 1949)
- Korr, Irwin M, Ph D Kirksville College of Osteopathy and Surgery, Kirksville, Mo *Prof of Physiology* (1, 1939)
- Kosman, A J, Ph D Northwestern Univ Med School, Dept of Physiology, 303 E Chicago Ave, Chicago 11, Ill *Asst Prof* (1, 1949)
- Koster, Rudolf, Ph D Tulane Univ, Dept of Pharmacology, Station 20, New Orleans, La *Instr, Dept of Pharmacology, School of Medicine* (3, 1949)
- Kottke, Frederic J, Ph D, M D Univ of Minnesota, Minneapolis *Baruch Fellow in Physical Medicine* (1, 1947)
- Kozelka, Frank L, Ph D Univ of Wisconsin, Dept of Pharmacology and Toxicology, Madison *Assoc Prof of Toxicology* (3, 1939)
- Krahl, Maurice E, Ph D Washington Univ School of Medicine, St Louis 10, Mo *Asst Prof of Pharmacology* (2, 1939, 3, 1949)
- Krakower, Cecil Alexander, M D Univ of Illinois College of Medicine, 1853 West Polk St, Chicago *Prof of Pathology* (4, 1945)
- Kramer, Benjamin, A M, M D 60 Plaza St, Brooklyn 17, N Y *Pediatrician-in-Chief, Brooklyn Jewish Hospital, Prof of Clin Pediatrics, Long Island College Med School* (1, 1915, 2, 1914)
- Kramer, Martha, Ph D Kansas State College, Manhattan *Asst Dean, School of Home Economics* (5, 1933)
- Kramer, S D, M D, Ph D 192 Washington Sq, Salem, Mass *Virologist* (6, 1944)
- Krampitz, Lester O, Ph D Western Reserve Univ, 2109 Adelbert Rd, Cleveland 6, Ohio *Head, Dept of Microbiology* (2, 1946, 6, 1949)
- Krantz, John C, Jr, Ph D Univ of Maryland Med School, Baltimore *Prof of Pharmacology* (3, 1937)
- Kratzer, F H, Ph D Univ of California, Div of Poultry Husbandry, Davis *Asst Prof* (5, 1949)
- Krauss, William E, Ph D Ohio Agricultural Exper Station, Wooster *Assoc Dir* (2, 1932, 5, 1933)
- Kraybill, Henry R, Ph D 5720 Woodlawn Ave, Chicago 37, Ill *Professorial Lecturer, Dept of Biochemistry, Univ of Chicago, Dir, Dept of Scientific Research, American Meat Inst Foundation* (2, 1942)
- Krayer, Otto, M D Harvard Med School, 25 Shattuck St, Boston, Mass *Assoc Prof of Comparative Pharmacology* (3, 1938)
- Kreezer, George L, Ph D Washington Univ, St Louis 10, Mo *Assoc Prof of Psychology* (1, 1948)
- Krehl, Willard A, Ph D Yale Univ, 333 Cedar St, New Haven, Conn *Asst Prof of Nutrition* (2, 1947, 5, 1949)
- Krop, Stephen, Ph D Warner Inst, Dept of Pharmacology, 113 W 18th St, New York City (1, 1949, 3, 1944)
- Krueger, Albert Paul, M D Univ of California, Berkeley *Prof and Chairman, Dept of Bacteriology, Dir, Office of Naval Research Task V* (4, 1930, 6, 1937)
- Krueger, Hugo M, Ph D Oregon State College, Dept of Zoology, Corvallis *Prof of Physiology* (1, 1931, 3, 1935)
- Krumbhaar, Edward B, M D, Ph D Univ of Pennsylvania Med School, Philadelphia *Prof Emeritus of Pathology* (1R, 1914, 4, prior to 1920)
- Kruse, Harry Dayton, M D, Sc D Milbank Memorial Fund, 40 Wall St, New York City (2, 1933)
- Kruse, Theophile K, Ph D Univ of Pittsburgh Med School, Pittsburgh, Pa *Prof of Physiology and Pharmacology* (1, 1919, 3, 1920)
- Kubicek, William G, Ph D Univ of Minnesota Hospitals, Div of Physical Medicine, Minneapolis *Assoc Prof* (1, 1947)

- Kubie, Lawrence S**, M D 7½ E 81st St, New York City 29 *Clin Prof of Psychiatry and Mental Hygiene, Yale Univ School of Medicine* (4, 1928)
- Kuffler, Stephen W**, M D Johns Hopkins Med School, Wilmer Inst, Baltimore 5, Md *Assoc Prof of Physiological Optics* (1, 1949)
- Kuhn, Harry A**, M S 3915 Fulton St N W, Washington, D C *Colonel, C W S, War Dept, Executive Officer, C W Procurement District* (3, 1927)
- Kuhn, L Roland**, Ph D Army Medical Center, AMDR GS, Washington 12, D C *Chief, Dept of Bacteriology* (6, 1939)
- Kuizenga, Marvin H**, Ph D Upjohn Company, Kalamazoo, Mich *Head of Dept of Pharmacology-Endocrinology* (2, 1947)
- Kun, Ernest**, M D Tulane Univ, Dept of Medicine, 1430 Tulane Ave, New Orleans 12, La *Asst Prof* (3, 1949)
- Kunde, Margarete M**, Ph D, M D 30 N Michigan Ave, Suite 1308, Chicago, Ill *Instr in Medicine, Northwestern Univ Med School, Clin Asst in Endocrinology, Cook County Hospital* (1, 1924)
- Kuntz, Moses**, Ph D Rockefeller Inst for Med Research, Princeton, N J *Assoc Member* (2, 1947)
- Kurotchkin, Timothy J**, M D 156 Forest Ave, Pearl River, N Y *Bacteriologist* (6, 1946)
- Kurtz, Alton C**, Ph D Univ of Oklahoma Med School, Dept of Biochemistry, Oklahoma City *Assoc Prof* (2, 1942)
- Kuyper, Adrian C**, Ph D Wayne Univ College of Medicine, Detroit 26, Mich *Asst Prof of Physiological Chemistry* (2, 1946)
- Kydd, David M**, M D Yale Univ School of Medicine, New Haven, Conn *Assoc Prof of Medicine* (5, 1934)
- Kyker, Granvil C**, Ph D Univ of North Carolina School of Medicine, Dept of Biological Chemistry and Nutrition, Chapel Hill *Assoc Prof* (2, 1947)
- LaBelle, Annette, B A** Grasslands Hospital, Valhalla, New York (1, 1948)
- Lackey, Robert W**, Ph D Southwestern Med College, Dept of Physiology and Pharmacology, Dallas 4, Texas *Prof of Physiology* (1, 1947)
- Lacorte, Jose G**, M D Instituto Oswaldo Cruz, Rio de Janeiro, Brazil, S A *Dir, Virus Section, Oswaldo Cruz Inst* (6, 1946)
- Lacy, G R**, M D Univ of Pittsburgh, Pittsburgh, Pa *Prof of Bacteriology and Immunology* (4, 1927)
- Lahch, Joseph J**, M D Univ of Wisconsin, Dept of Pathology, 426 N Charter St, Madison 6 *Asst Prof of Pathology* (4, 1946)
- Lamb, Alvin R**, Ph D Hawaiian Sugar Planters' Assoc, Exper Station, Honolulu *Research Assoc* (2, 1923, 5, 1934)
- Lambert, Edward H**, Ph D, M D Mayo Foundation, Rochester, Minn *Asst Prof of Physiology* (1, 1945)
- Lambert, Robert A**, M D Bricks Springs, Greensboro, Ala *Consultant for Fellowship Section, Pan American Sanitary Bureau, Regional Office of World Health Organization* (4, 1922)
- Lambertsen, Christian J**, M D Univ of Pennsylvania, Philadelphia, Pa *Asst Prof of Pharmacology, Assoc in Medicine* (3, 1948)
- Lampen, J Oliver**, Ph D 1530 Rydalmount Rd, Cleveland Heights, Ohio *Assoc Prof of Microbiology, Western Reserve Univ School of Medicine* (2, 1947)
- Lamport, Harold, M D** Yale Univ School of Medicine, New Haven, Conn *Assoc Prof of Physiology* (1, 1943)
- Lamson, Paul Dudley**, M D Vanderbilt Univ Med School, Nashville, Tenn *Prof of Pharmacology* (1, 1921, 3, 1915)
- Lancefield, Rebecca C**, Ph D 66th St and York Ave, New York City 21 *Assoc Member, Rockefeller Inst for Med Research* (6, 1933)
- Landis, Carney**, Ph D Columbia Univ, Psychiatric Inst, 722 W 168th St, New York City *Principal Research Psychologist and Prof of Psychology* (1, 1939)
- Landis, Eugene Markley**, Ph D, M D Harvard Med School, Dept of Physiology, 25 Shattuck St, Boston, Mass *George Higginson Prof of Physiology* (1, 1928)
- Landowne, Milton**, M D 6101 Stewart Ave, Baltimore 9, Md (1, 1947)
- Lands, Alonzo, M**, Ph D Sterling-Winthrop Research Inst, Rensselaer, N Y *Head, Pharmacology Section* (1, 1942, 3, 1947)
- Lange, Carl**, M D 371 Morris St, Albany, N Y *Assoc Bacteriologist, Divs of Labs and Public Health, N Y State Dept of Health* (6, 1938)
- Langley, Wilson D**, Ph D Univ of Buffalo Med School, 24 High St, Buffalo, N Y *Prof of Biochemistry* (2, 1937)
- Langworthy, Orthello R**, M A, M D Johns Hopkins Hospital, Baltimore, Md *Assoc Prof of Psychiatry, Johns Hopkins Univ* (1, 1928)
- Lanni, Frank**, Ph D Duke Univ School of Medicine, Durham, N C *Research Assoc* (6, 1949)
- Lardy, Henry A**, Ph D Univ of Wisconsin, Dept of Biochemistry, Madison 6 *Assoc Prof* (2, 1946, 5, 1949)
- Larrabee, Martin G**, Ph D Johns Hopkins Univ, Biophysics Dept, Baltimore 18, Md (1, 1940)
- Larsen, Eleanor M**, Ph D Univ of Wisconsin, Dept of Physiology, S M I, Madison 6 *Instr in Physiology* (1, 1949)
- Larson, Carl L**, M D Natl Insts of Health, Div of Infectious Diseases, Bethesda, Md *Surgeon, USPHS* (6, 1948)

- Larson, Edward, Ph D Univ of Miami, Miami, Fla *Prof of Physiology and Pharmacology* (1, 1929, 3, 1937)
- Larson, Hardy W, Ph D Metropolitan Life Insurance Co, Biochemical Lab, 1 Madison Ave, New York City *Research Chemist* (2, 1937)
- Larson, Paul S, Ph D Med College of Virginia, Richmond *Assoc Prof of Research Pharmacology* (1, 1939, 3, 1947)
- Lashley, K S, Ph D Yerkes Labs, Orange Park, Fla *Research Prof of Neuropsychology, Harvard Univ, Dir, Yerkes Labs of Primate Biology, Inc* (1, 1923)
- Laskowski, M, Ph D Marquette Univ Med School, Milwaukee 3, Wis *Assoc Prof of Biochemistry* (2, 1944)
- Last, Jules H, Ph D, M D Univ of Illinois College of Medicine, Chicago *Asst Prof of Pharmacology* (3, 1948)
- Lauffer, Max A, Jr, Ph D Univ of Pittsburgh, Pittsburgh 13, Pa *Research Prof, Dept of Physics and Physiological Chemistry* (2, 1946)
- Laug, E P, Ph D Food and Drug Admin, Div of Pharmacology, 12th and C Sts S W, Washington 25, D C *Sr Pharmacologist* (2, 1938, 3, 1947)
- Lauson, Henry D, Ph D, M D Rockefeller Inst, 66th St and York Ave, New York City 21 *Associate* (1, 1946)
- Lavine, T F, Ph D Lankenau Hospital Research Inst, Philadelphia, Pa *Research Chemist* (2, 1938)
- Lawrence, W Sherwood, M D 906 Hazel St, Gridley, Calif (3, 1944)
- Lawson, Hampden, M D, Ph D Univ of Louisville, Louisville, Ky *Prof of Physiology* (1, 1933)
- Lawton, Alfred H, M D, Ph D Veterans Admin, Bureau of Medicine and Surgery, Washington, D C *Chief, Research Div* (1, 1949, 3, 1948)
- Leake, Chauncey D, Ph D Univ of Texas Med Branch, Galveston, *Vice-Pres of Univ of Texas in Charge of Med Program* (1, 1923, 3, 1924)
- Leatham, James H, Ph D Rutgers Univ, New Brunswick, N J *Prof of Zoology* (1, 1945)
- Leathes, John Beresford, M A, M B Westfield Ware Lane, Lyme Regis, Dorset, England (2, 1909)
- Lederer, Ludwig George, Ph D, M D Pennsylvania Central Airlines, Natl Airport, Washington, D C, *Med Dir* (1, 1940)
- Lee, Douglas H K, M D Johns Hopkins Univ, I Bowman School of Geography, Baltimore 18, Md *Prof of Physiological Climatology, Lecturer in Physiological Hygiene* (1, 1949)
- Lee, Milton O, Ph D 2101 Constitution Ave, Washington 25, D C *Exec Sec and Managing Editor, American Physiological Society, Federation Sec* (1, 1927, 5, 1933)
- Leese, Chester E, Ph D George Washington Univ School of Medicine, Washington, D C *Assoc Prof of Physiology* (1, 1934)
- Lehman, Arnold J, Ph D, M D Food and Drug Admin, Washington 25, D C *Chief, Div of Pharmacology* (3, 1937)
- Lehman, Robert A, Ph D New York Univ College of Medicine, 477 First Ave, New York City *Instr in Therapeutics* (3, 1942)
- Lehmann, Gerhard, M D, Dr Ing Scientific Dept, Hoffmann-LaRoche, Nutley 10, N J *Pharmacologist* (3, 1939)
- Lehninger, Albert L, Ph D Univ of Chicago Med School, 950 E 59th St, Chicago 37, Ill *Asst Prof of Biochemistry in Depts of Biochemistry and Surgery* (2, 1946)
- Lehr, David, M D New York Med College, Flower and Fifth Ave Hospitals, Fifth Ave at 105th St, New York City 29 *Asst Prof of Pharmacology* (3, 1947)
- Leichsenring, Jane M, Ph D Univ of Minnesota, Univ Farm, St Paul *Prof of Nutrition* (5, 1948)
- Leimdorfer, Alfred, M D Univ of Illinois College of Medicine, Dept of Psychiatry, Chicago 12 *Assoc Prof* (1, 1947)
- Lein, Allen, Ph D Northwestern Univ Med School, Dept of Physiology, Chicago 11, Ill *Physiologist* (1, 1946)
- Lenhart, Carl H, M D Lakeside Hospital, 2065 Adelbert Rd, Cleveland, Ohio *Oliver H Payne Prof of Surgery, Western Reserve Univ* (1, 1921)
- Lennette, Edwin H, Ph D, M D California State Dept of Public Health, Berkeley *Dir, Viral and Rickettsial Disease Lab* (4, 1941, 6, 1947)
- Leonard, Clifford Shattuck, Ph D 87 Oxford Rd, Longmeadow, Springfield, Mass, Yale Univ Med School, New Haven, Conn *Technical Assoc, Chemical-Biological Coordination Center, Natl Research Council* (3, 1927)
- Leonards, Jack Ralph, Ph D Western Reserve Univ, Cleveland, Ohio *Asst Prof of Biochemistry* (2, 1948)
- Le Page, G A, Ph D Univ of Wisconsin, McArdle Memorial Lab for Cancer Research, Madison 6 *Asst Prof of Oncology* (2, 1949)
- Lepkovsky, Samuel, Ph D Univ of California, Poultry Div, Berkeley 4 *Prof of Poultry Husbandry* (2, 1933, 5, 1933)
- L'Esperance, Elise L, M D 2 East 61st St, New York City *Dir, Strong Cancer Precaution Clinic, Memorial Hospital, and New York Infirmary* (6, 1920)
- Leverton, Ruth M, Ph D Univ of Nebraska, Dept of Home Economics, Lincoln *Assoc Prof of Human Nutrition Research* (5, 1942)
- Levin, Louis, Ph D Columbia Univ, College of Physicians and Surgeons, 630 W 168th St,

- New York City 32 *Asst Prof of Anatomy* (2, 1939)
- Levine, Harold**, Ph D 941 E Sylvan Ave, Milwaukee 11, Wis (2, 1933, 5, 1933)
- Levine, Milton**, Ph D Inst of Exper Medicine, College of Med Evangelists, 312 N Boyle Ave, Los Angeles, Calif *Assoc Dir, Inst of Exper Medicine* (6, 1942)
- Levine, Philip**, M D Ortho Research Foundation, Raritan, N J *Dir, Biologic Div* (6, 1925)
- Levine, Rachmiel**, M D Michael Reese Hospital, Chicago, Ill *Acting Dir, Dept of Metabolic Research, Professorial Lecturer in Physiology, Univ of Chicago* (1, 1942)
- Levine, Samuel Z**, M D, New York Hospital, 525 E 68th St, New York City *Prof of Pediatrics, Cornell Univ Med College, Pediatrician-in-Chief, New York Hospital* (5, 1933)
- Levine, Victor Emanuel**, Ph D, M D Creighton Univ School of Medicine, Omaha, Nebr *Prof of Biological Chemistry and Nutrition* (2, 1936)
- Levinson, Samuel A**, Ph D, M D Univ of Illinois College of Medicine, 808 S Wood St, Chicago *Prof of Pathology, Dir of Labs, Research and Educational Hospital* (4, 1938)
- Levison, Louis A**, M D 421 Michigan St, Toledo, Ohio *Physician to Toledo and St Vincent Hospitals* (6, 1916)
- Levy, Milton**, Ph D New York Univ College of Medicine, 477 First Ave, New York City *Assoc Prof of Chemistry* (2, 1933)
- Levy, Robert L**, M D 730 Park Ave, New York City *Prof of Clin Medicine, Columbia Univ College of Physicians and Surgeons* (3, 1915)
- Lewey, F H**, M D 3400 Spruce St, Philadelphia 4, Pa *Prof of Neuroanatomy, Univ of Pennsylvania Grad School of Medicine, Assoc in Neuropathology and Neurosurgery, Med School* (1, 1937)
- Lewis, George T**, Ph D Emory Univ School of Medicine, Dept of Biochemistry, Emory Univ, Ga *Assoc Prof* (2, 1949)
- Lewis, Gladys Kinsman**, Ph D 401 S Lafayette St, Denver 9, Colo (5, 1944)
- Lewis, Howard Bishop**, Ph D Univ of Michigan Med School, Ann Arbor *John Jacob Abel Univ Prof of Biological Chemistry* (1, 1925, 2, 1913, 5, 1933)
- Lewis, James C**, Ph D U S Dept of Agriculture, Western Regional Research Lab, Albany 6, Calif *Biochemist* (2, 1946)
- Lewis, Jessica H**, M D Univ of North Carolina School of Medicine, Dept of Physiology, Chapel Hill *Research Assoc* (1, 1949)
- Lewis, Julian Herman**, M D 4750 Champlain Ave, Chicago, Ill *Assoc Prof of Pathology, Univ of Chicago, Member, Otho S A Sprague Memorial Inst* (4, 1924)
- Lewis, Lena A**, Ph D Cleveland Clinic, Euclid Ave and E 93rd St, Cleveland 6, Ohio *Research Staff* (1, 1946)
- Lewis, Robert C**, Ph D 4200 E 9th Ave, Denver, Colo *Prof of Biochemistry, Univ of Colorado School of Medicine* (2, 1931, 5, 1933)
- Ley, Herbert Leonard, Jr**, M D Army Medical Center, AMDR & GS, Dept Virus and Rickettsial Diseases, Washington 12, D C *Assistant* (6, 1949)
- Li, Choh Hao**, Ph D Univ of California, 4596 Life Sciences Bldg, Berkeley *Assoc Prof of Exper Biology* (2, 1944)
- Li, Richard C**, M D c/o Dr T S Yu, 392 MaDang Rd, Shanghai 25, China *Guest in Dept of Pharmacology, Natl Med College of Shanghai* (3, 1941)
- Libby, Raymond L**, Ph D Veterans Admin, Wilshire and Sawtelle Blvds, Los Angeles 25, Calif *Assoc Prof of Radio Biology, Univ of California Med School, Chief of Isotope Section, Veterans Hospital, Los Angeles* (6, 1938)
- Liberson, W T**, M D Inst of Living, Hartford, Conn *Neurophysiologist* (1, 1948)
- Libet, Benjamin**, Ph D Univ of California Med Center, Dept of Anatomy, San Francisco *Asst Prof of Physiology, Med School* (1, 1942)
- Licklider, J C R**, Ph D Harvard Univ, Psycho-Acoustic Lab, Cambridge, Mass *Lecturer in Psychology* (1, 1948)
- Liddell, Howard S**, Ph D Cornell Univ, Ithaca, N Y *Prof of Psychology* (1, 1925)
- Lieb, Charles C**, M D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City *Hosack Prof of Pharmacology* (1R, 1936, 3, 1915)
- Lieberman, Arnold L**, M D, Ph D 328 No Country Club Rd, Tucson, Ariz (1, 1934)
- Lieberman, Seymour**, Ph D Sloan-Kettering Inst, 444 E 68th St, New York City 21 *Associate* (2, 1948)
- Lifson, Nathan**, M D, Ph D 617 Kenwood Parkway, Minneapolis, Minn *Prof of Physiology, Univ of Minnesota Med School* (1, 1944)
- Lightbody, Howard D**, Ph D QM Food and Container Inst for the Armed Forces, 1849 W Pershing Rd, Chicago 9, Ill *Dir of Food Labs* (2, 1936)
- Ligon, Edgar Williams, Jr**, Ph D Production and Marketing Admin, Insecticide Div, Agriculture Bldg, Washington, D C (3, 1948)
- Lilienthal, Joseph L, Jr**, M D Johns Hopkins Hospital, Baltimore 5, Md *Assoc Prof of Medicine* (1, 1945)
- Lillie, Ralph Stayner**, Ph D Physiological Labs, Univ of Chicago, Chicago 37, Ill *Prof Emeritus of Physiology* (1R, 1905, 2, 1913)
- Lillie, R D**, M D Pathology Lab, Natl Insts

- of Health, Bethesda, Md *Chief Med Dir, USPHS* (4, 1941)
- Lim, Robert Kho-Seng, Ph D Academia Sinica, 320 Yo Yang Rd, Shanghai, China *Dir of Inst of Medicine* (1, 1923)
- Lindeman, V F, Ph D Syracuse Univ, 16 Lyman Hall, Syracuse, N Y *Prof of Zoology*, (1, 1949)
- Lindsay, Stuart, M D Univ of California Med School, Univ Hospital, San Francisco 22 *Pathologist* (4, 1949)
- Lindsley, Donald B, Ph D Northwestern Univ, Dept of Psychology, Evanston, Ill *Prof of Psychology* (1, 1937)
- Linegar, Charles R, Ph D E R Squibb and Sons, Research and Development Labs, New Brunswick, N J *Dir, Pharmacological Development Div*, (3, 1938)
- Lineweaver, Hans, Ph D U S Dept of Agriculture, Western Regional Research Lab, Albany 6, Calif *Head of Poultry Products Div* (2, 1941)
- Link, Karl Paul, Ph D Univ of Wisconsin, Biochemistry Bldg, Madison *Prof of Biochemistry* (2, 1931)
- Lintz, William, M D 36 Plaza St, Brooklyn, N Y *Late Prof of Immunology and Bacteriology and Clin Prof of Medicine, Long Island College of Medicine* (6, 1920)
- Lipman, Mrs Miriam O, A M Presbyterian Hospital, Dept of Medicine, 620 W 168th St, New York City *Research Asst, Edward Daniels Faulkner Arthritis Clinic* (6, 1931)
- Lipmann, Fritz, M D, Ph D Massachusetts General Hospital, Biochemical Research Lab, Boston 14 *Head, Prof of Biological Chemistry, Harvard Med School* (2, 1941)
- Lippincott, Stuart W, M D Univ of Washington School of Medicine, Seattle *Chairman, Dept of Pathology* (4, 1947)
- Lipton, Morris A, Ph D, M D Univ of Chicago, 59th St and Ellis Ave, Chicago 37, Ill *Resident, Dept of Psychiatry* (2, 1946)
- Lisco, Hermann, M D Argonne Natl Lab, Chicago 80, Ill *Dir, Med Div, Asst Prof of Pathology, Univ of Chicago* (4, 1947)
- Litchfield, John T, Jr, M D American Cyanamid Co, 1937 W Main St, Stamford, Conn *Dir of Pharmacology* (3, 1940)
- Little, James Maxwell, Ph D Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N C *Prof of Pharmacology, Assoc Prof of Physiology* (1, 1942, 3, 1947)
- Livingston, Alfred E, Ph D Temple Univ School of Medicine, Philadelphia, Pa *Prof of Pharmacology* (1, 1917, 3, 1920)
- Livingston, Robert B, M D 333 Cedar St, New Haven, Conn *Insti, Lab of Physiology* (1, 1949)
- Lloyd, David P C, Ph D Rockefeller Inst for Med Research, 66th St and York Ave, New York City 21 *Assoc Member* (1, 1939)
- Locke, Arthur P, Ph D Zonite Products Corp, New Brunswick, N J *Chief Research Chemist* (6, 1926)
- Lodholz, Edward, M D Univ of Pennsylvania, Med Labs, Philadelphia *Prof Emeritus of Physiology, Grad School of Medicine* (1, 1913)
- Loeb, Leo, M D Washington Univ Med School, St Louis, Mo *Prof Emeritus of Pathology* (1R, 1907, 4, 1913)
- Loefer, John B, Ph D Foundation of Applied Research, P O Box 2296, San Antonio 6, Tex *Chief Biologist* (1, 1949)
- Loew, Earl R, Ph D Boston Univ School of Medicine, 80 E Concord St, Boston 18, Mass (1, 1940, 3, 1946)
- Loewe, W S, M D Univ of Utah School of Medicine, Dept of Pharmacology, Salt Lake City 1 (3, 1936)
- Logan, Milan A, Ph D Univ of Cincinnati College of Medicine, Eden and Bethesda, Cincinnati 19, Ohio *Prof of Biological Chemistry* (2, 1936)
- Long, C N H, D Sc, M D Yale Univ, 333 Cedar St, New Haven 11, Conn *Dean, School of Medicine and Sterling Prof of Physiological Chemistry* (1, 1935, 2, 1927)
- Long, Esmond R, M D 7th and Lombard Sts, Philadelphia, Pa *Dir, Henry Phipps Inst, Prof of Pathology, Univ of Pennsylvania* (4, 1930)
- Long, Perrin Hamilton, M D Johns Hopkins Univ, 615 N Wolfe St, Baltimore, Md *Prof of Preventive Medicine* (3, 1940)
- Longcope, Warfield T, M D Cornhill Farm, Lee, Mass (3R, 1921, 4, 1913, 6R, 1923)
- Longenecker, Herbert Eugene, Ph D Univ of Pittsburgh, Pittsburgh 13, Pa *Dean, Grad School, Prof of Biochemistry* (2, 1940, 5, 1945)
- Longwell, Bernard B, Ph D Univ of Colorado School of Medicine, 4200 East 9th Ave, Denver 7, Colo *Prof of Biochemistry* (2, 1946)
- Loomis, Ted A, Ph D, M D Univ of Washington School of Medicine, Seattle *Asst Prof of Pharmacology* (3, 1948)
- Looney, Joseph M, M D 75 Park St, West Roxbury, Mass *Dir of Labs, Veterans Admin Hospital, West Roxbury, Mass* (2, 1922)
- Loosli, Clayton Garr, M D Univ of Chicago, Chicago, Ill *Assoc Prof of Medicine* (4, 1940)
- Loosli, J K, Ph D Cornell Univ, Animal Nutrition Lab, Ithaca, N Y *Assoc Prof of Animal Nutrition and Assoc Animal Nutritionist in Exper Station* (5, 1944)
- Lorber, Victor, M D, Ph D Western Reserve Univ School of Medicine, Dept of Biochemistry, Cleveland, Ohio *Assoc Prof* (1, 1944)

- Lorente de N6, Rafael**, M D Rockefeller Inst for Med Research, 66th St and York Ave, New York City *Member* (1, 1937)
- Lorenz, Egon**, Ph D Natl Cancer Inst, Bethesda, Md *Biophysicist* (4, 1942)
- Loring, H S**, Ph D Stanford Univ, Stanford Univ, Calif *Prof of Biochemistry* (2, 1938)
- Lotspeich, William D**, M D Oxford Univ, Dept of Biochemistry, Oxford, England *Research Fellow in Biochemistry* (1, 1948)
- Louis, Lawrence H**, D Sc Univ Hospital, Dept of Internal Medicine, Ann Arbor, Mich *Asst Prof of Biological Chemistry, Univ of Michigan* (2, 1949)
- Loveless, Mary H**, M D New York Hospital, 525 E 68th St, New York City *Research Assoc, Cornell Med School, Physician to Out-Patients, New York Hospital* (6, 1941)
- Lowell, Francis C**, M D 65 East Newton St, Boston, Mass *Assoc Prof of Medicine, Boston Univ School of Medicine* (6, 1942)
- Lowenbach, Hans**, M D Duke Univ Med School, Durham, N C *Assoc Prof of Neuropsychiatry and Physiology* (1, 1946)
- Lowry, Oliver H**, M D, Ph D Washington Univ School of Medicine, 4580 Scott Ave, St Louis 10, Mo *Prof and Head of Dept of Pharmacology* (2, 1942)
- Lu, Go**, M D Stanford Univ School of Medicine, Dept of Pharmacology and Therapeutics, 2398 Sacramento St, San Francisco 15, Calif *Research Assoc and Sterling-Winthrop Fellow* (3, 1949)
- Lubinski, Herbert**, M D Jewish General Hospital, 3755 St Catherine Rd, Montreal, Quebec, Canada *Bacteriologist and Serologist* (6, 1941)
- Lucas, Colin C**, Ph D Univ of Toronto, Banting and Best Dept of Med Research, Toronto, Ontario, Canada *Professor* (2, 1946)
- Lucas, George H W**, Ph D Univ of Toronto, Toronto, Ontario, Canada *Prof of Pharmacology* (2, 1925, 3, 1928)
- Luck, James Murray**, Ph D Stanford Univ, Stanford Univ, Calif *Prof of Biochemistry* (2, 1925)
- Lucké, Balduin**, M D 141 Montgomery Ave, Bala-Cynwyd, Pa *Prof of Pathology, Univ of Pennsylvania Med School, Philadelphia* (4, 1924)
- Luckhardt, Arno Benedict**, Ph D, M D Univ of Chicago, Chicago, Ill *The Dr Wm Beaumont Distinguished Service Prof of Physiology* (1, 1911)
- Ludewig, Stephan**, Ph D Univ of Virginia School of Medicine, Charlottesville *Assoc Prof of Biochemistry* (2, 1941)
- Luduena, Froilan P**, Ph D, M D Sterling-Winthrop Research Inst, Rensselaer, N Y (3, 1941)
- Lukens, Francis D W**, M D Univ of Pennsylvania 809 Maloney Clinic, 36th and Spruce Sts, Philadelphia *Assoc Prof of Medicine, Dir of George S Cox Med Research Inst* (1, 1938)
- Lund, E J**, Ph D Univ of Texas, Dept of Zoology and Physiology, Austin *Prof of General Physiology* (1, 1930)
- Lundberg, Walter O**, Ph D Hormel Inst, Austin, Minn *Director, Prof of Agricultural Biochemistry, Univ of Minn* (2, 1949)
- Lundgren, Harold P**, Ph D U S Dept of Agriculture, Western Regional Research Lab, Albany 6, Calif *Sr Chemist* (2, 1942)
- Lundy, John Silas**, M D Mayo Foundation, Rochester, Minn *Chief of Section on Anesthesia* (3, 1935)
- Lurie, Max B**, M D Henry Phipps Inst, 7th and Lombard Sts, Philadelphia, Pa *Assoc Prof of Exper Pathology* (4, 1934, 6, 1930)
- Lutz, Brenton R**, Ph D Boston Univ, 675 Commonwealth Ave, Boston, Mass *Prof of Biology* (1, 1925)
- Luyet, Basile J**, Sc D St Louis Univ School of Medicine, St Louis, Mo *Prof of Biology* (1, 1936)
- Lyall, Harold W**, Ph D New York State Dept of Health, Div of Labs and Research, Albany *Asst Dir in charge of Antitoxin, Serum, and Vaccine Labs* (6, 1937)
- Lyman, Carl M**, Ph D A and M College of Texas, College Station *Prof of Biochemistry and Nutrition* (2, 1940)
- Lyman, John F**, Ph D Ohio State Univ, Townsend Hall, Columbus *Prof of Agricultural Chemistry* (2, 1920, 5, 1933)
- Maaske, Clarence A**, Ph D Univ of Colorado School of Medicine, 4200 E 9th Ave, Denver *Assoc Prof of Physiology and Pharmacology* (1, 1945)
- Macallum, A Bruce**, M D, Ph D Univ of Western Ontario, Med School, London, Ontario, Canada *Research Prof of Biochemistry* (2, 1914)
- MacArthur, Edith H**, Ph D Skidmore College, Saratoga Springs, N Y *Prof and Dir of Home Economics* (5, 1933)
- MacCardle, Ross C**, Ph D Natl Cancer Inst, Bethesda, Md *Principal Cytologist* (4, 1948)
- MacCorquodale, D W**, Ph D Abbott Labs, North Chicago, Ill *Head of Biochemical Research* (2, 1934)
- MacFadyen, Douglas A**, M D Presbyterian Hospital, 1753 W Congress St, Chicago 12, Ill *Chairman of Dept of Biochemistry* (2, 1942)
- Macht, David Israel**, M D Sinai Hospital, Baltimore, Md *Consultant Pharmacologist, Research Pharmacologist, Sinai Hospital Labs* (1R, 1916, 3, 1915)
- Macht, Martin B**, Ph D The Jewish Hospital, Cincinnati 29, Ohio (1, 1948)
- MacKay, Eaton M**, M D Scripps Metabolic Clinic, La Jolla, Calif (1, 1930)

- MacKay, Ian F S**, Ph D Univ College of the West Indies, Jamaica, British West Indies *Prof of Physiology* (1, 1949)
- Mackenzie, Cosmo G**, Sc D Cornell Univ Med College, Dept of Biochemistry, 1300 York Ave, New York City 21 *Assoc Prof* (1, 1946, 2, 1946, 5, 1942)
- MacLeod, Colin M**, M D New York Univ College of Medicine, 477 First Ave, New York City *Prof of Bacteriology* (6, 1937)
- MacLeod, Florence L**, Ph D Univ of Tennessee, Knoxville *Prof of Nutrition* (2, 1927, 5, 1933)
- MacLeod, Grace**, Ph D 106 Morningside Drive, New York City 27 *Prof Emeritus of Nutrition, Teachers College, Columbia Univ* (2, 1924, 5, 1933)
- MacLeod, John**, Ph D Cornell Univ Med College, 1300 York Ave, New York City *Asst Prof of Anatomy* (1, 1942)
- MacNabb, Andrew L**, V S, B V Sc Guelph, Ontario, Canada *Principal, Ontario Veterinary College* (6, 1941)
- MacNider, William deB**, M D, Sc D Univ of North Carolina, Chapel Hill *Kenan Research Prof of Pharmacology* (1, 1912, 2, 1912, 3, 1909, 4, prior to 1920)
- MacPherson, Catherine F C**, Ph D 236 Brock Ave N, Montreal West, Quebec, Canada (2, 1947)
- MacVicar, Robert**, Ph D Oklahoma A & M College, Stillwater *Assoc Prof of Agricultural Chemistry Research* (2, 1949)
- Madden, Sidney C**, M D Brookhaven Natl Lab, Upton, L I, N Y *Head, Div of Pathology, Pathologist-in-Chief, Brookhaven Natl Lab Hospital* (4, 1939)
- Maddock, Stephen**, M D Boston City Hospital, Boston, Mass *Dir of Surgical Research Lab, Asst Prof of Surgery, Tufts Med School* (4, 1931)
- Madsen, Louis L**, Ph D Utah State Agricultural College, Dept of Animal Husbandry, Logan *Nutritionist* (5, 1940)
- Magath, Thomas B**, Ph D, M D Mayo Clinic, Rochester, Minn *Head of Div of Clinical Labs, Prof of Clinical Pathology and Parasitology, Univ of Minn, Mayo Foundation* (1, 1928)
- Magill, Thomas P**, M D Long Island College of Medicine, Brooklyn, N Y *Prof of Bacteriology* (6, 1937)
- Magoun, Horace W**, Ph D Northwestern Univ Med School, 303 E Chicago Ave, Chicago, Ill *Prof of Microscopic Anatomy* (1, 1937)
- Mahon, Eleanor Conway**, Ph D Iron River, Mich (4, 1940)
- Main, Rolland J**, Ph D Eaton Labs, Inc, Eaton Ave, Norwich, N Y *Med Editor and Consultant in Physiology* (1, 1936)
- Maison, George L**, M S, M D Boston Univ Med School, 80 E Concord St, Boston 18, Mass *Prof and Head of Pharmacology Dept* (1, 1939, 3, 1948)
- Major, Randolph T**, Ph D Merck and Co, Inc, Rahway, N J *Vice Pres and Scientific Dir* (2, 1942)
- Malkiel, Saul**, Northwestern Univ Med School, Evanston, Ill *Asst Prof of Medicine, Dir of Allergy Research Lab* (6, 1948)
- Mallory, G Kenneth**, M D Mallory Inst of Pathology, Boston City Hospital, Boston, Mass *Professor* (4, 1940)
- Mallory, Tracy B**, M D Massachusetts General Hospital, Boston *Dir of Pathology and Bacteriology, Prof of Pathology, Harvard Med School* (4, 1937)
- Maloney, Arnold H**, Ph D, M D Howard Univ School of Medicine, Washington, D C *Prof and Head of Dept of Pharmacology* (3, 1932)
- Maltaner, Frank**, Ph D Scarsdale Manor, Scarsdale, N Y (6, 1920)
- Maluf, N S Rustum**, Ph D 101 W Chestnut St, Louisville 2, Ky (1, 1942)
- Man, Evelyn B**, Ph D Yale Univ School of Medicine, 333 Cedar St, New Haven, Conn *Asst Prof in Biochemistry Lab, Dept of Medicine* (2, 1936)
- Manery, Jeanne Forest**, Ph D Univ of Toronto Med School, Toronto, Ontario, Canada *Demonstrator in Biochemistry* (1, 1937)
- Mangun, George H**, Ph D Henry Ford Hospital, 15713 Heyden St, Detroit 23, Mich *Sr Assoc in Clinical Chemistry* (2, 1947)
- Mann, Frank C**, M A, M D Mayo Clinic, Box 256, Rochester, Minn *Prof of Exper Medicine, Mayo Foundation* (1, 1916, 3, 1923, 4, 1924)
- Manning, G W**, M D 20 Woodington Ave, Toronto, Ontario Canada *Med Officer in Charge, No 2 R C A F Research Unit* (1, 1944)
- Manville, Ira Albert**, M D, Ph D 811 N W 19th Ave, Portland 9, Ore (1, 1933)
- Manwaring, Wilfred H**, M D Stanford Univ, Stanford University, Calif *Prof Emeritus of Bacteriology and Exper Pathology* (4, prior to 1920, 6, 1917)
- Marbarger, John P**, Ph D Univ of Illinois Physical Environment Unit, 1853 W Polk St, Chicago 12 *Research Dir, Aeromedical and Physical Environment Lab, Assoc Prof of Physiology* (1, 1949)
- Marcus, Stanley**, Ph D Univ of Utah, Dept of Bacteriology, Salt Lake City *Assoc Prof* (6, 1948)
- Marine, David**, M A, M D 18 Baltimore Ave, Rehoboth, Del (1R, 1910, 4, 1913)
- Markee, Joseph E**, Ph D Duke Univ School of Medicine, Durham, N C *Prof of Anatomy* (1, 1945)

- Markowitz, J, M D**, Ph D Univ of Toronto School of Medicine, Toronto, Ontario, Canada *Research Assoc in Physiology* (1, 1929)
- Marmont, George H**, Ph D Univ of Chicago, Inst of Radiobiology and Biophysics, Chicago 37, Ill *Asst Prof of Physiology* (1, 1941)
- Marmorston, Jessie** 416 N Bedford Drive, Beverly Hills, Calif *Asst Prof of Medicine, Univ of Southern California Staff, County and Cedars of Lebanon Hospitals* (6, 1932)
- Marrazzi, Amedeo S**, M D Med Div, Army Chemical Center, Md *Chief, Toxicology Section* (1, 1949, 3, 1938)
- Marsh, David F**, Ph D West Virginia Univ, Dept of Pharmacology, Morgantown *Head and Assoc Prof of Pharmacology* (3, 1946)
- Marsh, Gordon**, Ph D State Univ of Iowa, Iowa City *Assoc Prof of Zoology* (1, 1944)
- Marsh, M Elizabeth**, Ph D Kilian Research Labs, 49 W 45th St, New York City *Asst Dir* (1, 1929, 5, 1933)
- Marshak, Alfred Gordon**, Ph D New York Univ College of Medicine, New York City *Research Assoc* (1, 1940)
- Marshall, Eli Kennerly, Jr**, Ph D, M D Johns Hopkins Med School, Baltimore, Md *Prof of Pharmacology and Exper Therapeutics* (1, 1915, 2, 1913, 3, 1915)
- Marshall, Louise Hanson**, Ph D Natl Insts of Health, Lab of Physical Biology, Bethesda, Md *Physiologist* (1, 1946)
- Marshall, Wade H**, Ph D Natl Insts of Health, Bethesda, Md *Research Fellow, Lab of Physical Biology* (1, 1937)
- Martin, Arthur W, Jr**, Ph D Univ of Washington, Physiology Hall, Seattle *Assoc Prof of Physiology* (1, 1944)
- Martin, Donald S**, M D Duke Univ Hospital, Durham, N C *Assoc in Medicine* (6, 1943)
- Martin, Foster N, Jr**, Ph D, M D Tulane Univ Med School, Dept of Pharmacology, P O Station 20, New Orleans, La *Asst Prof of Pharmacology* (3, 1947)
- Martin, Stephens J**, Ph D St Francis Hospital, Hartford, Conn (1, 1933)
- Marx, Walter**, Ph D Univ of Southern California School of Medicine, Dept of Biochemistry, Los Angeles 7, Calif *Asst Prof of Biochemistry* (2, 1949)
- Mason, Edward C**, M D, Ph D Univ of Oklahoma School of Medicine, Oklahoma City *Prof of Physiology* (1, 1935)
- Mason, Eleanor Dewey**, Ph D Women's Christian College, Dept of Physiology and Nutrition, Cathedral P O, Madras, India *Prof of Physiology and Nutrition* (1, 1946)
- Mason, Herman C**, 3322 W Polk St, Chicago, Ill *Consulting Bacteriologist and Immunologist* (6, 1948)
- Mason, Harold L**, Ph D Mayo Clinic, Rochester, Minn *Prof of Physiological Chemistry, Mayo Foundation, Univ of Minnesota* (2, 1941)
- Mason, Howard S**, Ph D Natl Insts of Health, Bethesda 14, Md *Cancer Inst Fellow* (2, 1949)
- Mason, Karl Ernest**, Ph D Univ of Rochester School of Medicine and Dentistry, Rochester, N Y *Prof of Anatomy* (5, 1941)
- Mason, Morton F**, Ph D Parkland Hospital, Oak Lawn Ave, Dallas, Tex *Prof of Pathological Chemistry and Exper Medicine, Southwestern Med College* (2, 1938)
- Massengale, Oliver N**, Ph D Mead Johnson and Co, Research Lab, Evansville, Ind *Research Biochemist* (2, 1937)
- Masson, Georges M C**, Ph D Cleveland Clinic Foundation, Research Div, Cleveland, Ohio (1, 1944)
- Mathews, Albert P**, Ph D Marine Biological Lab, Woods Hole, Mass *Carnegie Prof Emeritus of Biochemistry, Univ of Cincinnati* (1R, 1898, 2, 1906)
- Mason, Gustave A**, Ph D Minneapolis Memorial Blood Bank, Inc, 1914 La Salle Ave, Minneapolis, Minn *Director* (6, 1946)
- Matthews, Samuel A**, Ph D Williams College, Williamstown, Mass *Prof of Biology* (1, 1948)
- Mattill, Henry A**, Ph D State Univ of Iowa, Iowa City *Prof and Head of Dept of Biochemistry* (1, 1913, 2, 1909, 5, 1933)
- Mattis, Paul A**, D Sc Atomic Energy Med Research Project, Western Reserve Univ, Cleveland, Ohio *Section Chief, Pharmacology* (3, 1946)
- Maurer, Frank W**, Ph D 301 Lake Ave, Newton Highlands 61, Mass (1, 1941)
- Mautz, Frederick R**, M D Western Reserve Univ School of Medicine, Cleveland 6, Ohio *Asst Prof of Surgery* (1, 1945)
- Maver, Mary E**, Ph D Natl Cancer Inst, Bethesda 14, Md *Sr Biochemist* (2, 1947)
- Mavor, James Watt**, Ph D 8 Gracewood Park, Cambridge, Mass (1, 1930)
- Maxfield, Mary E**, Ph D Woman's Med College of Pennsylvania, Dept of Pharmacology, Philadelphia *Assoc Prof* (1, 1947)
- Mayer, Manfred M**, Ph D 1739 Eutaw Place, Baltimore, Md *Assoc Prof of Bacteriology, Johns Hopkins Hospital, School of Hygiene* (6, 1946)
- Mayerson, Hymen S**, Ph D Tulane Univ School of Medicine, Station 20, New Orleans, La *Prof and Head of Dept of Physiology* (1, 1928)
- Maynard, L A**, Ph D Cornell Univ, Ithaca, N Y *Prof of Nutrition and Biochemistry, Dir of School of Nutrition* (2, 1930, 5, 1933)
- Mazur, Abraham**, Ph D City College of New York, 139th St and Convent Ave, New York City 31 *Asst Prof in Dept of Chemistry* (2, 1944)

- McCann, William S**, M D, D Sc Univ of Rochester School of Medicine, Rochester, N Y *The Charles A Dewey Prof of Medicine* (2, 1923, 5, 1933)
- McCarrell, Jane D** Hood College, Dept of Biology, Frederick, Md (1, 1942)
- McCarty, Maclyn, M D** 66th St and York Ave, New York City 21 Assoc, *Rockefeller Inst for Med Research* (6, 1947)
- McCawley, Elton Leeman**, Ph D Yale Med School, New Haven, Conn *Instr in Pharmacology* (3, 1944)
- McCay, Clive M**, Ph D Cornell Univ, Animal Nutrition Lab, Dairy Bldg, Ithaca, N Y *Prof of Nutrition* (2, 1929, 5, 1933)
- McChesney, Evan William**, Ph D Sterling-Winthrop Research Inst, Rensselaer, N Y *Member, Biological Div* (1, 1944)
- McClellan, Walter S**, M D Saratoga Spa, Saratoga Springs, N Y *Med Dir, Assoc Prof of Medicine, Albany Med College* (1, 1931)
- McClendon, J F**, Ph D Route 1, Box 383, Trooper Rd, Norristown, Pa *Research Prof of Physiology, Hahnemann Med College* (1, 1910, 2, 1914, 5, 1935)
- McClung, L S** Indiana Univ, Bloomington *Assoc Prof and Chairman of Dept of Bacteriology* (6, 1948)
- McCollum, Elmer Verner**, Ph D Johns Hopkins Univ, Baltimore 18, Md *Prof Emeritus of Biochemistry* (2, 1910, 5, 1933)
- McCollum, Ernestine Becker**, M A Johns Hopkins Univ School of Hygiene, Baltimore 5, Md *Asst Prof of Biochemistry* (5, 1938)
- McCouch, Grayson Prevost**, M D Univ of Pennsylvania School of Medicine, Philadelphia *Assoc Prof of Physiology* (1, 1925)
- McCouch, Margaret Sunwalt**, Ph D Rose Tree Rd, R F D 1, Media, Pa (1, 1934)
- McCoy, Richard H**, Ph D Univ of Pittsburgh, Pittsburgh, Pa *Assoc Research Prof of Chemistry* (5, 1948)
- McCrea, Forrest D**, Ph D Duke Univ School of Medicine, Durham, N C *Assoc Prof of Physiology and Pharmacology* (1, 1929, 3, 1937)
- McCrudden, Francis H**, M D 19 Stoneleigh Rd, West Newton 65, Mass, *Asst Med Dir, New England Mutual Life Insurance Co* (2, 1906)
- McCulloch, Warren Sturgis**, M A, M D Univ of Illinois College of Medicine, 912 S Wood St, Chicago *Assoc Prof of Psychiatry* (1, 1936)
- McCutcheon, Morton**, M D Univ of Pennsylvania Med School Philadelphia *Prof of Pathology* (4, 1925)
- McDonald, Francis Guy**, Ph D Mead Johnson and Co, Research Lab, 2404 Pennsylvania, Evansville, Ind *Biochemist* (2, 1936, 5, 1947)
- McDonald, Roger K**, M D Baltimore City Hospitals, Cardiovascular Diseases Section, Baltimore 24, Md *Sr Asst Surgeon, USPHS, Natl Heart Inst, Bethesda, Md* (1, 1949)
- McElroy, William Swindler**, M D Univ of Pittsburgh School of Medicine, Pittsburgh, Pa *Dean, Prof of Physiological Chemistry* (2, 1919)
- McElroy, L W** Ph D Univ of Alberta, Dept of Animal Science, Edmonton, Canada *Assoc Prof of Animal Husbandry* (5, 1944)
- McElroy, William D**, Ph D Johns Hopkins Univ, Dept of Biology, Baltimore, Md *Asst Prof of Biology* (1, 1945)
- McFarland, Ross A**, Ph D Harvard Univ School of Public Health, Boston, Mass *Assoc Prof of Industrial Hygiene* (1, 1943)
- McFarlane, William Douglas**, Ph D 496 Queen St, E, Toronto, Ontario, Canada *Dir of Research, Canadian Breweries, Ltd* (2, 1933)
- McGinty, Daniel A**, Ph D Parke, Davis and Co, Detroit, Mich *Research Physiologist* (1, 1925)
- McGugan, Hugh Alister**, Ph D, M D Univ of Illinois College of Medicine, 1853 W Polk St, Chicago *Prof Emeritus of Pharmacology* (1R, 1907, 2, 1906, 3R, 1913)
- McHargue, J S**, Ph D Univ of Kentucky, Dept of Chemistry, Kentucky Agricultural Experiment Station, Lexington *Member Emeritus* (2, 1927)
- McHenry, E W**, Ph D Univ of Toronto, School of Hygiene, Toronto, Ontario, Canada *Prof of Public Health Nutrition* (2, 1938, 5, 1935)
- McIntyre, A R**, Ph D, M D Univ of Nebraska College of Medicine, 42nd and Dewey Ave, Omaha *Prof of Physiology and Pharmacology* (1, 1933, 3, 1938)
- McKee, Albert P** R F D 7, Iowa City, Iowa (6, 1948)
- McKee, Clara M**, Squibb Inst for Med Research, New Brunswick, N J *Assoc in Microbiology* (6, 1941)
- McKee, Frank W**, M D Univ of Rochester School of Medicine and Dentistry, Rochester, N Y *Instr in Pathology, James E Gleason Fellow* (4, 1947)
- McKee, Ralph Wendell**, Ph D Harvard Med School, 25 Shattuck St, Boston 15, Mass *Asst Prof of Biological Chemistry* (2, 1946)
- McKennis, Herbert, Jr**, Ph D Med College of Virginia, Richmond *Assoc Prof of Biochemistry* (2, 1948)
- McKibbin, John M**, Ph D Syracuse Univ College of Medicine, Syracuse, N Y *Assoc Prof of Biochemistry* (2, 1948)
- McLain, Paul L**, M D Univ of Pittsburgh Med School Pittsburgh, Pa *Assoc Prof of Physiology and Pharmacology* (1, 1948, 3, 1940)
- McLean, Franklin C**, Ph D, M D Univ of Chicago, Chicago 37, Ill *Prof of Pathological Physiology* (1, 1914, 2, 1916, 3, 1916)

- McLean, I William, Jr, M D Parke, Davis Lab, Virus Research Div, Detroit, Mich *Research Virologist* (6, 1946)
- McLester, James S, M D Univ of Alabama, 930 S 20th St, Birmingham *Prof of Medicine* (5, 1933)
- McLimans, William F, Ph D Univ of Minnesota, 1834 Chelton Ave, St Paul *Asst Prof of Bacteriology and Immunology* (6, 1949)
- McManus, J F A, M D Med College of Alabama, Birmingham *Assoc Prof of Pathology* (4, 1948)
- McMaster, Philip D, M D Rockefeller Inst for Med Research, 66th St and York Ave, New York City (4, 1924)
- McMeekin, Thomas L, Ph D U S Dept of Agriculture, Eastern Regional Research Lab, Philadelphia, Pa *Head of Protein Div* (2, 1935)
- McNamara, Bernard P, Ph D Med Div, Army Chemical Center, Md *Pharmacologist* (3, 1947)
- McNaught, James Bernard, M D Univ of Colorado School of Medicine, Denver 7 *Prof and Head of Dept of Pathology* (4, 1936)
- McPhail, Murchie K, Ph D Natl Defense Board, Suffield Exper Station, Suffield, Alberta, Canada (3, 1941)
- McQuarrie, Irvine, Ph D, M D Univ of Minnesota, Minneapolis *Prof and Head of Dept of Pediatrics* (1, 1949, 4, 1927, 5, 1933)
- McShan, W H, Ph D Univ of Wisconsin, Biology Bldg, Madison 6 *Assoc Prof of Zoology* (2, 1947)
- Medes, Grace, Ph D Lankenau Hospital Research Inst, Philadelphia, Pa *Research Physiological Chemist* (2, 1930)
- Medlar, Edgar M, M D Veterans Admin Hosp, Sunmount, N Y *Chief Lab Pathologist* (4, 1927)
- Meek, Walter J, Ph D Univ of Wisconsin, Madison *Prof of Physiology, Assoc Dean of Med School* (1, 1908)
- Mehl, John W, Ph D Univ of Southern California, Dept of Biochemistry, 3551 Univ Ave, Los Angeles *Prof of Biochemistry* (2, 1946)
- Meier, Rolf, M D Basle Univ, Pelikanweg 7, Basle, Switzerland *Prof of Pathological Physiology* (3, 1949)
- Meiklejohn, Gordon, M D 678 Woodmont Ave, Berkeley, Calif *Instr in Medicine, Univ of California Med School* (6, 1948)
- Meites, Joseph Michigan State College, Dept of Physiology and Pharmacology, East Lansing *Asst Prof* (1, 1949)
- Mellon, Ralph R, M S, M D, D P H Western Pennsylvania Hospital, Inst of Pathology, Pittsburgh *Director* (6, 1918)
- Melnick, Daniel, Ph D The Best Foods, Inc, Research Labs, Bayonne, N J *Chief Technologist* (2, 1940, 5, 1942)
- Melnick, Joseph L, Ph D Yale Univ School of Medicine, 333 Cedar St, New Haven 11, Conn *Assoc Prof of Microbiology* (2, 1946, 6, 1948)
- Melville, Donald B, Ph D Cornell Univ Med College, 1300 York Ave, New York City 21 *Assoc Prof of Biochemistry* (2, 1947)
- Melville, Kenneth Ivan, M D McGill Univ, Montreal, Quebec, Canada *Asst Prof of Pharmacology* (3, 1931)
- Mendel, Bruno, M D Univ of Toronto, Banting and Best Dept of Med Research, 100 College St, Toronto 5, Ontario, Canada *Prof of Cellular Physiology* (2, 1947)
- Mendelson, E S, B A R F D 2, Telford, Pa *Physiologist, Head of Human Engineering Div, Naval Air Exper Station, Philadelphia, Pa* (1, 1949)
- Mendenhall, Walter L, M S, M D Milford, Iowa (1, 1915, 3, 1917)
- Mendez, Rafael, M D Natl Inst of Cardiology, Calzada de la Piedad 300, Mexico D F, Mexico *Head of Dept of Pharmacology* (3, 1944)
- Mendlowitz, Milton, M D 136 E 64th St, New York City 21 *Adjunct Physician in Mt Sinai Hospital of New York* (1, 1949)
- Meneely, George R, M D Thayer Veterans Admin Hospital, Nashville, Tenn *Asst Prof of Medicine* (4, 1946)
- Menkin, Valy, M A, M D Temple Univ School of Medicine, Philadelphia, Pa *Assoc Prof of Exper Pathology* (1, 1932, 4, 1932, 6, 1931)
- Menten, Maud L, M D, Ph D Univ of Pittsburgh, Pittsburgh, Pa *Prof of Pathology* (1, 1915, 4, 1927)
- Mettier, Stacy R, M D Univ of California Hospital, San Francisco *Prof of Medicine* (4, 1932)
- Mettler, Fred A, Ph D M D Columbia Univ College of Physicians and Surgeons, Dept of Neurology, New York City *Assoc Prof of Anatomy* (1, 1937)
- Meyer, Arthur E, Ph D Fellows Pharmaceutical Lab, New York City (1, 1948)
- Meyer, Curtis E, Ph D Upjohn Co, Kalamazoo, Mich *Sr Research Chemist* (2, 1942)
- Meyer, Frieda L, Ph D U S Dept of Agriculture, Bureau of Human Nutrition, Food and Nutrition Div, Washington 25, D C *Nutrition Specialist* (5, 1949)
- Meyer, Karl, M D, Ph D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 *Assoc Prof of Biochemistry, Dept of Medicine* (2, 1934)
- Meyer, Karl F, M D, Ph D George W Hooper Foundation, Univ of California Med Center, San Francisco *Director* (4, 1930, 6, 1922)
- Meyer, Roland K, Ph D Univ of Wisconsin, Dept of Zoology, Biology Bldg, Madison 6 *Prof of Zoology* (1, 1949)

- Meyerhof, Otto, M D Univ of Pennsylvania School of Medicine, Dept of Physiological Chemistry, Philadelphia *Research Prof of Biochemistry* (2, 1941)
- Michel, Harry O, Ph D Med Div, Biochemical Section, Army Chemical Center, Md *Biochemist* (2, 1949)
- Mickelsen, Olaf, Ph D Nutrition Branch USPHS, Washington 25, D C *Chief Chemist* (2, 1941)
- Mider, George Burroughs, M D Strong Memorial Hospital, Rochester 7 N Y *Prof of Cancer Research* (4, 1940)
- Miles, Walter R, Ph D Yale Univ, 333 Cedar St, New Haven, Conn *Prof of Psychology, School of Medicine and Inst of Human Relations* (1, 1919)
- Milhorat, Ade T, M D Cornell Univ Med College, New York City 21 *Assoc Prof of Medicine and Instr in Pharmacology, Research Fellow, Russell Sage Inst of Pathology* (1, 1934, 3, 1937, 5, 1935)
- Miller, Augustus Taylor, Jr, Ph D Univ of North Carolina Med School, Chapel Hill *Assoc Prof of Physiology* (1, 1944)
- Miller, Benjamin F, M D 71 Mt Vernon St, Boston 8, Mass *Assoc Physician, Massachusetts General Hospital, Clin Prof of Medicine (on leave), George Washington Med School* (2, 1938)
- Miller, Carey D, M S Univ of Hawaii, Honolulu *Prof of Food and Nutrition, Hawaii Agricultural Exper Station* (5, 1942)
- Miller, C Phillip, M S, M D Univ of Chicago, Chicago, Ill *Prof of Medicine* (4, 1925, 6, 1928)
- Miller, Edgar G, Jr, Ph D Columbia Univ, 630 W 168th St, New York City *Prof of Biological Chemistry* (2, 1930)
- Miller, Franklin R, M D Jefferson Med College and Hospital, Div of Hematology, Philadelphia, Pa *Assoc Prof of Medicine* (4, 1940)
- Miller, Frederick R, M A, M D Univ of Western Ontario, Faculty of Medicine London, Ontario, Canada *Research Prof of Neurophysiology* (1, 1908)
- Miller, Gail L, Ph D Inst for Cancer Research, Dept of General Biochemistry, Hasbrook and Hartel Aves, Philadelphia 11, Pa *Assoc Member* (2, 1949)
- Miller, G H, M D American College of Surgeons, 40 E Erie St, Chicago 11, Ill *Dir of Educational Activities* (3, 1925)
- Miller, George A, Ph D Harvard Univ, Psycho-Acoustic Lab, Cambridge, Mass *Research Fellow* (1, 1948)
- Miller, H R, M D Montefiore Hospital, 1020 Park Ave, New York City (1, 1947)
- Miller, James A, Ph D Univ of Wisconsin Med School, McArdle Mem Laboratory Research, Madison 6 *Assoc Prof of Oncology* (2, 1949)
- Miller, Leon L, Ph D, M D Univ of Rochester School of Medicine and Dentistry, P O Box 287, Crittenden Station, Rochester, N Y *Assoc Prof of Radiation Biology and Biochemistry* (2, 1947)
- Miller, Lila, Ph D Univ of Michigan, Dept of Biological Chemistry, Ann Arbor *Asst Prof of Biological Chemistry* (2, 1946)
- Miller, Lloyd C, Ph D Sterling-Winthrop Research Inst, 33 Riverside Ave, Rensselaer, N Y *Dir of Biology Div* (3, 1938)
- Miller, R C, Ph D Pennsylvania State College, State College *Asst Prof Agricultural and Biological Chemistry* (5, 1935)
- Miller, Zelma Baker, Ph D 71 Mt Vernon Boston, Mass (2, 1940)
- Mills, Clarence A, Ph D, M D Cincinnati General Hospital, Cincinnati 29, Ohio *Prof of Exper Medicine, Univ of Cincinnati* (1, 1921, 2, 1921)
- Minot, Annie Stone, Ph D Vanderbilt Univ Med School, Nashville, Tenn *Assoc Prof of Biochemistry* (1, 1923)
- Mirsky, Alfred E, Ph D Rockefeller Inst for Med Research, 66th St and York Ave, New York City 21 *Member* (2, 1941)
- Mirsky, I Arthur, M D The Jewish Hospital, Cincinnati, Ohio *Dir, May Inst for Med Research, Asst Prof of Biochemistry, Univ of Cincinnati* (1, 1936)
- Mitchell, Harold H, M D 120 Lasky Drive, Beverly Hills, Calif (6, 1943)
- Mitchell, Harold H, Ph D Univ of Illinois, 555 Davenport Hall, Urbana *Prof of Animal Nutrition* (2, 1919, 5, 1933)
- Mitchell, Helen S, Ph D Univ of Massachusetts, Amherst *Dean of School of Home Economics* (2, 1925, 5, 1933)
- Mitchell, Philip H, Ph D Brown Univ, Providence 12, R I *Robert P Brown Prof of Biology* (2, 1909)
- Modell, Walter, M D Cornell Univ Med College, 1300 York Ave, New York City *Instr in Pharmacology* (3, 1944)
- Moe, Gordon Kenneth, Ph D, M D Univ of Michigan, Ann Arbor *Assoc Prof of Pharmacology* (3, 1944)
- Mohn, James F, M D 24 High St, Buffalo, N Y *Asst Prof in Bacteriology and Immunology, Univ of Buffalo School of Medicine* (6, 1946)
- Molitor, Hans, M D Merck Inst for Therapeutic Research, Rahway, N J *Director* (1, 1933, 3, 1942)
- Molland, Jacob, M D, Ph D Univ of Oslo, Oslo, Norway *Prof of Pharmacology* (3, 1948)
- Molnar, George W, Ph D Med Dept, Field Research Lab, Fort Knox, Kv *Physiologist* (1, 1949)

- Molomut, Norman**, Ph D Biological Labs, 16 Clinton St, Brooklyn 2, N Y *Dir of research and of Labs* (6, 1942)
- Moon, Virgil H**, M Sc, M D Wake Forest College, Bowman Gray School of Medicine, Winston Salem, N C *Visiting Prof of Pathology* (4, 1934)
- Moore, Arthur R**, Ph D Univ of Portland, Dept of Biology, Portland, Ore (1, 1912)
- Moore, Carl Vernon**, M D Washington Univ School of Medicine, St Louis, Mo *Prof of Medicine* (4, 1938, 5, 1941)
- Moore, Dan H**, Ph D U S Navy No 100, c/o Fleet Post Office, New York City (1, 1948)
- Moore, Lane A**, Ph D U S Dept of Agriculture, Div of Nutrition and Physiology, Bureau of Dairy Industry, Beltsville, Md *Head, Section of Dairy Cattle Nutrition* (5, 1940)
- Moore, Robert A**, M D Washington Univ Med School, St Louis, Mo *Dean and Prof of Pathology* (4, 1929)
- Moore, Robert M**, M D Univ of Texas Med School, Galveston (1, 1932)
- Moore, Stanford**, Ph D Rockefeller Inst for Med Research, York Ave at 66th St, New York City 21 *Associate* (2, 1949)
- Moorhouse, Victor Henry K**, M B Univ of Manitoba, Winnipeg, Manitoba, Canada *Prof of Physiology* (1, 1912)
- More, Robert H**, M D Pathological Inst, 3775 University St, Montreal, Quebec, Canada *Assoc Prof of Pathology, McGill Univ* (4, 1949)
- Morehouse, Laurence E**, Ph D Univ of Southern California, Los Angeles 7 *Assoc Prof* (1, 1947)
- Morgan, Agnes Fay**, Ph D Univ of California, Berkeley 4 *Prof of Home Economics, Biochemist, Agric Exper Station* (2, 1929, 5, 1933)
- Morgan, Charles F**, Ph D Georgetown Univ School of Medicine, Washington, D C *Prof and Chairman of Dept of Physiology* (1, 1948, 3, 1947)
- Morgan, Clifford T**, Ph D Johns Hopkins Univ, Psychology Dept, Baltimore 18, Md (1, 1943)
- Morgulis, Sergius**, Ph D Univ of Nebraska College of Medicine, Omaha 5 *Prof and Chairman of Dept of Biochemistry* (1, 1914, 2, 1916)
- Morison, Robert S**, M D Rockefeller Foundation, 66th St and York Ave, New York City 21 *Asst Dir of Med Sciences* (1, 1938)
- Moritz, Alan R**, M D Western Reserve Univ, Cleveland, Ohio *Prof of Pathology and Dir of the Institute* (4, 1934)
- Morrell, Clarence Allison**, Ph D Lab of Hygiene, Dept of Pensions and Natl Health, Sussex and John Sts, Ottawa, Ontario, Canada *Sr Pharmacologist* (3, 1937)
- Morris, Harold P**, Ph D Natl Cancer Inst, Bethesda 14, Md *Principal Biochemist in Nutrition* (2, 1944, 5, 1943)
- Morris, Marion C**, Ph D Public Health Research Inst of City of New York, Foot of East 15th St, New York City *Assoc in Div of Infectious Diseases* (6, 1936)
- Morrison, Dempsey B**, Ph D Univ of Tennessee College of Medicine, Memphis *Assoc Prof of Chemistry* (2, 1936)
- Morrison, James L**, Ph D Emory Univ School of Medicine, Emory University, Ga *Assoc Prof of Pharmacology* (3, 1944)
- Morse, Minerva** Ph D 5525 Kimbark Ave, Chicago, Ill *Research Assoc, Dept of Pediatrics, Univ of Chicago* (2, 1934)
- Morse, Withrow**, Ph D 32 Manchester Rd, Eastchester, via Tuckahoe, N Y *Consultant* (2, 1914)
- Mortimer, Bernard**, Ph D, M D 25 N Ottawa St, Joliet, Ill, Cook County Hospital, Chicago (1, 1936)
- Morton, John J**, M D Univ of Rochester School of Medicine and Dentistry, Rochester, N Y *Prof of Surgery* (4, 1927)
- Moses, Campbell**, M D, Univ of Pittsburgh School of Medicine, Pittsburgh, Pa *Dir of Addison H Gibson Lab of Applied Physiology* (1, 1948)
- Motley, Hurley L**, Ph D, M D Jefferson Hospital, Philadelphia, Pa *Dir, Cardio-Respiratory Lab, Jefferson Med College and Assoc Prof of Medicine* (1, 1947)
- Mountain, Isabel M** Ph D 9 Coolidge Ave, White Plains, N Y (6, 1947)
- Mountcastle, Vernon B**, M D Johns Hopkins Univ Med School, Dept of Physiology, 710 N Washington St, Baltimore 5, Md *Asst Prof of Physiology* (1, 1949)
- Moxon, Alvin L**, Ph D College Station, Brookings, S D *Head, Chemistry Dept, South Dakota Agricultural Exper Station* (2, 1944)
- Moyer, Arden W**, Ph D 407 Mountain View Rd, Englewood, N J *Research Biochemist, Lederle Labs, Pearl River, N Y* (6, 1946)
- Moyer, Carl A**, Ph D 6417 Glenrose Ct, Dallas 1, Tex *Prof of Exper Surgery, Southwestern Med College* (1, 1943)
- Mudd, Stuart, M A**, M D Univ of Pennsylvania, Philadelphia *Prof of Bacteriology* (1, 1921, 4, 1927, 6, 1927)
- Muehlberger, Clarence W**, Ph D State Health Dept Labs Lansing, Mich *State Toxicologist* (3, 1928)
- Mueller, J Howard**, Ph D Harvard Med School, Boston, Mass *Charles Wilder Prof of Bacteriology and Immunology* (2, 1922, 4, 1927, 6, 1920)
- Mukherji, B**, D Sc All-India Inst of Hygiene and Public Health, Calcutta *Dir of Biochemical Standardization Lab* (3, 1938)
- Mulder, Arthur G**, Ph D Loyola Med School, 706 S Walcott St, Chicago, Ill (1, 1937)

- Mulford, Dwight J , Ph D 306 Riverway, Boston, Mass *Chief of Lab , Massachusetts Dept of Public Health, Assoc in Physical Chemistry, Harvard Univ* (2, 1948)
- Mulinos, M G , M D , Ph D New York Med College, Flower and Fifth Ave Hospitals, Fifth Ave and 105th St , New York City 29 *Assoc Prof of Pharmacology* (3, 1931)
- Mull, James W , Ph D 2020 State St , Quincy, Ill *Assoc , Quincy Specialties Co* (2, 1937)
- Müller, Otto H , R N Dr Syracuse Univ College of Medicine, Dept of Physiology, Syracuse 10, N Y (1, 1947)
- Mulligan, Richard M , M D Univ of Colorado School of Medicine, 4200 East 9th Ave , Denver *Prof of Pathology* (4, 1947)
- Mullin, F J , Ph D Univ of Chicago, Physiology Dept , Chicago 37, Ill *Asst Prof of Physiology* (1, 1937)
- Munro, F L , Ph D Jefferson Med College, 1025 Walnut St , Philadelphia 7, Pa *Research Chemist* (2, 1948)
- Munro, Muriel Platt, Ph D Jefferson Med College, 1025 Walnut St , Philadelphia 7, Pa *Research Chemist* (2, 1948)
- Munsell, Hazel E , Ph D Massachusetts Inst of Technology, Nutrition Biochemistry Labs , Dept of Food Technology, Cambridge *Research Assoc* (5, 1933)
- Muntwyler, Edward, Ph D Long Island College of Medicine, 350 Henry St , Brooklyn 2, N Y *Prof of Biochemistry* (2, 1931)
- Muntz, John A , Ph D 3356 Euclid Heights Blvd , Cleveland Heights, Ohio *Asst Prof , Western Reserve Univ* (2, 1948)
- Murlin, John R , Ph D Univ of Rochester School of Medicine and Dentistry, Rochester, N Y *Prof Emeritus of Physiology and Dir Emeritus of Dept of Vital Economics* (1R, 1906, 2, 1908, 5, 1933)
- Murphy, James B , M D Rockefeller Inst for Med Research, 66th St and York Ave , New York City *Member* (4, prior to 1920)
- Murphy, Quillian R , M D , Ph D Univ of Wisconsin Med School, Dept of Physiology, Madison 6 *Asst Prof* (1, 1949)
- Murray, Everitt G D , M A McGill Univ , Montreal, Quebec, Canada *Prof and Head of Dept of Bacteriology and Immunology, Bacteriologist-in-Chief to the Royal Victoria Children's Memorial and Alexandra Hospitals* (6, 1933)
- Mushett, Charles W , Ph D Merck Inst for Therapeutic Research, Rahway, N J *Head of Dept of Pathology* (4, 1948)
- Muus, Jytte, Mag Scient Mount Holyoke College, South Hadley, Mass *Assoc Prof* (2, 1946)
- Myers, Chester N , Ph D 34 Cedar Place, Yonkers 5, N Y *Chief, Div Chemotherapy, New York Skin and Cancer Hospital, Dir of Chemical and Clinical Research, H A Metz Labs , Inc* (2, 1922)
- Nachmansohn, David, M D Columbia Univ College of Physicians and Surgeons, Dept of Neurology, 630 W 168th St , New York City 32 *Research Assoc in Neurology* (1, 1940)
- Nadler, J Ernest, M D , Med D Sc 80-16 Lefferts Blvd , Kew Gardens 15, N Y (3, 1940)
- Nahum, Louis N , M D 1142 Chapel St , New Haven, Conn *Asst Prof of Physiology, Yale Univ* (1, 1934)
- Najjar, Victor A , M D Univ of Cambridge, Unit for Microbiology, Cambridge, England (2, 1946)
- Nash, Thomas P , Jr , Ph D 875 Monroe Ave , Memphis, Tenn *Prof of Chemistry, Univ of Tennessee College of Medicine, Dean of School of Biological Sciences* (2, 1923)
- Nasset, Edmund S , Ph D Univ of Rochester School of Medicine and Dentistry, Rochester, N Y *Prof of Physiology* (1, 1932, 5, 1940)
- Nastuk, William L , Ph D Columbia Univ College of Physicians and Surgeons, Dept of Physiology, 630 W 168th St , New York City 32 *Instr in Physiology* (1, 1949)
- Nathanson, Ira T , M S, M D Massachusetts General Hospital, Boston *Asst in Surgery, Instr in Surgery, Harvard Med School* (1, 1943)
- Nathanson, Morris D , M D 6333 Wilshire Blvd , Los Angeles, Calif *Assoc Clin Prof of Medicine, Univ of Southern California School of Medicine* (3, 1940)
- Necheles, Heinrich, M D , Ph D Michael Reese Hospital, Chicago, Ill *Dir of Dept of Gastrointestinal Physiology, Professorial Lecturer in Physiology, Univ of Chicago* (1, 1929)
- Neill, James M , Ph D Cornell Univ Med College, 1300 York Ave , New York City *Prof. of Bacteriology and Immunology* (6, 1930)
- Neilson, Charles Hugh, Ph D , M D Humboldt Bldg , St Louis, Mo *Assoc Dean and Prof of Medicine, St Louis Univ Med School* (2, 1906)
- Nelson, Arthur A , M D , Ph D Food and Drug Admin Div of Pharmacology, Washington 25, D C *Med Officer (Pathology)* (4, 1942)
- Nelson, Carl Ferdinand, M D , Ph D Univ of Kansas, Dept of Biochemistry, Lawrence *Prof of Physiological Chemistry* (2, 1914)
- Nelson, Carl T , M A , M D Harkness Pav Presbyterian Hosp , 180 Ft Washington Ave , New York City 32 (6, 1943)
- Nelson, Erwin E , Ph D , M D Food and Drug Admin , Drug Div , Washington 25, D C *Chief, New Drug Section, Adjunct Clin Prof of Pharmacology, George Washington Univ School of Medicine* (3, 1924)
- Nelson, E M , Ph D Food and Drug Admin , Washington, 18, D C *Chief, Vitamin Div* (2, 1927, 5, 1933)

- Nelson, John B**, Ph.D Rockefeller Inst for Med Research, Princeton, N J *Assoc Member* (4, 1934)
- Nelson, John M**, Ph D Columbia Univ, New York City 27 *Prof Emeritus of Organic Chemistry* (2, 1923)
- Nelson, Norton**, Ph D New York Univ College of Medicine, 477 First Ave, New York City 16 *Assoc Prof of Industrial Medicine* (2, 1946)
- Nelson, P Mabel**, Ph D Iowa State College, Ames *Dean, Div of Home Economics* (5, 1934)
- Nelson, Tell, M A**, M D 1415 Kalakaua Ave, Honolulu 19, T H *Practicing Physician* (6, 1938)
- Nelson, Victor E**, M S 559 Putnam St, Eau Claire, Wis *Prof of Physiological Chemistry* (2, 1924)
- Nelson, Warren O**, Ph D Univ of Iowa School of Medicine, Dept of Anatomy, Iowa City *Prof of Anatomy* (1, 1937)
- Neter, Erwin**, M D Children's Hospital, 219 Bryant St, Buffalo, N Y *Attending Bacteriologist* (6, 1937)
- Nettleship, Anderson**, M D Univ of Arkansas School of Medicine, Little Rock *Prof and Head of Dept of Pathology* (4, 1942)
- Neuberg, Carl**, Ph D, M D Med, Chem D 536 W 113th St, New York City 25 *Research Prof, New York Univ* (2, 1944)
- Neuman, William F**, Ph D, Univ of Rochester School of Medicine and Dentistry, 20 Alpine St, Rochester 7, N Y *Asst Prof of Biochemistry and Pharmacology* (2, 1949)
- Neumann, Charles**, M D 525 E 68th St, New York City 21 *Resident Surgeon, New York Hospital, Instr in Surgery, New York Univ Post-Grad Med School* (1, 1944)
- Neurath, Hans**, Ph D Duke Univ School of Medicine, Durham, N C *Prof of Physical Biochemistry* (2, 1940, 6, 1944)
- Neuwelt, Frank**, M D 504 Broadway, Gary, Ind *Research Assoc, Dept of Gastrointestinal Research, Michael Reese Hospital* (1, 1940)
- Neuwirth, Isaac**, Ph D 209 E 23rd St, New York City 10 *Prof of Pharmacology, New York Univ College of Dentistry* (2, 1924, 3, 1931)
- Newman, Elliot V**, M D, Johns Hopkins Hospital, Baltimore, Md *Asst Prof of Medicine* (1, 1948)
- Newman, Henry W**, M D Stanford Med School, Clay and Webster Sts, San Francisco 15, Calif *Assoc Prof of Medicine* (3, 1949)
- Nice, Leonard B**, Ph D Chicago Med School, 710 S Wolcott Ave, Chicago, Ill *Prof of Physiology and Pharmacology* (1, 1921)
- Nicholas, John S**, Ph D Yale Univ, Osborn Zoological Lab, New Haven, Conn *Bronson Prof of Comparative Anatomy* (1, 1927)
- Nicholson, Hayden C**, M S, M D Natl Research Council, Div of Med Sciences, 2101 Constitution Ave, Washington 25, D C (1, 1932)
- Nickerson, John L**, Ph D Columbia Univ, 630 W 168th St, New York City 32 *Assoc Prof of Physiology* (1, 1945)
- Nickerson, Mark**, Ph D Univ of Utah School of Medicine, Salt Lake City *Asst Prof of Pharmacology* (3, 1947)
- Nicoll, Paul A**, Ph D Indiana Univ, Bloomington *Asst Prof of Physiology* (1, 1945)
- Niemann, Carl G**, Ph D California Inst of Technology, Pasadena 4 *Prof of Organic Chemistry* (2, 1940)
- Nigg, Clara**, Ph D E R Squibb and Sons, New Brunswick, N J *Head of Depts of Bacteriology and Virology, and of Microbiological Development* (6, 1929)
- Nims, Leslie F**, Ph D Brookhaven Natl Lab, Biology Dept, Upton, Long Island, N Y *Acting Head of Dept* (1, 1940)
- Noble, Robert Laing**, M D, Ph D Univ of Western Ontario, Dept of Med Research, London, Ontario, Canada (1, 1941)
- Nord, F F**, Ph D Fordham Univ, Dept of Organic Chemistry, New York City *Prof of Organic Chemistry and Enzymology* (2, 1940)
- Norris, Earl R**, Ph D Univ of Washington, Seattle *Prof of Biochemistry and Exec Officer* (2, 1938)
- Norris, L C**, Ph D Cornell Univ, Rice Hall, Ithaca, N Y *Prof of Nutrition* (2, 1939, 5, 1934)
- Northrop, J H**, Ph D Univ of California, Berkeley 4 *Member of Rockefeller Inst* (2, 1938)
- Northrup, David W**, Ph D West Virginia Univ Med School, Morgantown *Prof of Physiology* (1, 1936)
- Novy, F G**, M D, Sc D 721 Forest Ave, Ann Arbor, Mich *Dean Emeritus of Med School and Prof Emeritus of Bacteriology, Univ of Michigan* (2, 1906, 4R, prior to 1919)
- Nowinski, W W**, Ph D Univ of Texas School of Medicine, Galveston *Research Assoc* (1, 1948)
- Nungester, Walter James**, Ph D, M D Univ of Michigan, Dept of Bacteriology, Ann Arbor *Professor* (6, 1949)
- Oberst, Fred W**, Ph D U S School of Aviation Medicine, Randolph Air Force Base, Randolph Field, Tex *Pharmacologist-Biochemist* (2, 1936, 3, 1949)
- Ochoa, Severo**, M D New York Univ College of Medicine, 477 First Ave, New York City 16 *Prof of Pharmacology* (2, 1942)
- Ogden, Eric** Ohio State Univ College of Medicine, Dept of Physiology, Columbus 10 *Prof of Physiology* (1, 1941)

- O'Hare, James P**, M D 520 Commonwealth Ave, Boston, Mass *Physician, Peter Bent Brigham Hospital, Lecturer in Medicine, Harvard Med School* (4, 1927)
- Ohlson, Margaret A**, Ph D Michigan State College, Dept of Foods and Nutrition, East Lansing *Prof and Head of Dept of Foods and Nutrition* (5, 1945)
- Okey, Ruth**, Ph D Univ of California, 1583 Life Sciences Bldg, Berkeley 4 *Prof of Home Economics and Biochemist, State Exper Station* (2, 1922, 5, 1933)
- Olcott, Harold S**, Ph D U S Dept of Agriculture, Western Regional Research Lab, Albany 6, Calif *Head of Vegetable Processing Div* (2, 1935)
- Oldham, Helen**, Ph D Univ of Chicago, Chicago, Ill *Asst Prof, Dept of Home Economics* (5, 1946)
- Olitsky, Peter K**, M D Rockefeller Inst for Med Research, 66th St and York Ave, New York City *Member* (6, 1917)
- Oliver, Jean Redman**, M D Hoagland Lab, 335 Henry St, Brooklyn, N Y *Prof of Pathology, Long Island College of Medicine* (1, 1924, 4, 1924)
- Oliver, Wade W**, M D Rockefeller Foundation, New York City *Assoc Dir of Div of Med Sciences* (4, 1925)
- Olmsted, J M D**, Ph D Univ of California, Berkeley *Prof of Physiology* (1, 1920)
- Olsen, Norman S**, Ph D Washington Univ Med School, 4580 Scott Ave, St Louis 10, Mo *Asst Prof of Biological Chemistry* (1, 1948, 2, 1949)
- Olson, Byron J**, Ph D, M D National Insts of Health, Bethesda, Md *Surgeon, Div of Infectious Diseases* (6, 1948)
- Olson, Carl, Jr**, D V M, Ph D Univ of Nebraska, Lincoln *Chairman of Dept of Animal Pathology and Hygiene* (4, 1937)
- Opdyke, David F**, Ph D Western Reserve Univ Med School, Cleveland 6, Ohio *Assoc Prof of Physiology* (1, 1945)
- Opie, Eugene L**, M D, Sc D Rockefeller Inst for Med Research, 66th St and York Ave, New York City 21 *Member* (1R, 1906, 4, 1913, 6R, 1923)
- Oppenheimer, Enid Tribe** 124 E 61st St, New York City *Instr in Physiology, Columbia Univ* (1, 1932)
- Oppenheimer, Ernst**, M D Ciba Pharmaceutical Products, Inc, Lafayette Park, Summit, N J *Vice-Pres in charge of Med Research* (3, 1944)
- Oppenheimer, Morton Joseph**, Ed M, M D 3400 N Broad St, Philadelphia, Pa *Prof of Physiology, Temple Univ School of Medicine* (1, 1942)
- Orent-Keiles, Elsa**, D Sc U S Dept of Agriculture, Bureau of Human Nutrition and Home Economics, Beltsville, Md. *In charge of Nutrition Investigations, Asst Chief, Foods and Nutrition Div* (2, 1935, 5, 1935)
- Ort, John M**, Ph D 401 Codwise Ave, New Brunswick, N J *Dir of Research, Carroll Dunham Smith Pharmacal Co* (2, 1932)
- Orten, Aline Underhill**, Ph D Wayne Univ College of Medicine, Detroit 26, Mich *Research Assoc, Dept of Physiological Chemistry* (5, 1946)
- Orten, James M**, Ph D Wayne Univ College of Medicine, Detroit 26, Mich *Assoc Prof of Physiological Chemistry* (2, 1936, 5, 1937)
- Orth, O Sidney**, Ph D, M D Univ of Wisconsin, S M I, Madison *Prof of Pharmacology* (1, 1942, 3, 1944)
- Osborne, Stafford L**, Ph D Northwestern Univ Med School, 303 E Chicago Ave, Chicago, Ill *Prof of Physical Medicine* (1, 1941)
- Oser, Bernard L**, Ph D Food Research Labs, Inc, 48-14 33rd St, Long Island City 1, N Y *Director* (5, 1945)
- Osler, Abraham G**, Ph D Johns Hopkins Hospital, Monument and Wolfe Sts, Baltimore, Md *Dir of Serologic Lab* (6, 1949)
- Oster, Robert H**, Ph D Univ of Maryland Med School, Greene and Lombard Sts, Baltimore *Asst Prof of Physiology* (1, 1938)
- Osterberg, Arnold E**, Ph D Abbott Labs, Med Dept, N Chicago, Ill *Assoc in Med Dept* (2, 1933)
- Osterhout, Marian I** Rockefeller Inst for Med Research, 66th St and York Ave, New York City 21 *Assoc, Div of General Physiology* (1, 1927)
- Osterhout, W J V**, Ph D Rockefeller Inst for Med Research, 66th St and York Ave, New York City 21 *Member Emeritus* (1, 1910)
- Otis, Arthur B**, Ph D Univ of Rochester, Dept of Physiology, Rochester 7, N Y *Asst Prof of Physiology* (1, 1946)
- Ott, Walther H**, Ph D Merck Inst for Therapeutic Research, Rahway, N J *Head of Dept of Biological Control and Poultry Nutrition* (5, 1949)
- Overman, Richard R**, Ph D Univ of Tennessee College of Medicine, Division of Physiology, Memphis *Assoc Prof* (1, 1946)
- Owen, Seward E**, Ph D 418 S 20th Ave, Maywood, Ill (1, 1938)
- Pace, Donald M**, Ph D Univ of Nebraska College of Pharmacy, Dept of Physiology and Pharmacology, Lincoln *Assoc Prof of Physiology* (1, 1944)
- Pace, Nello**, Ph D Univ of California, Div of Physiology, Berkeley 4 *Asst Prof of Physiology* (1, 1947)
- Pack, George T**, M D 139 E 36th St, New York City 16 *Fellow in Cancer Research, Memorial Hospital* (1, 1924)

- Packchanian, Ardzoony**, Ph D Univ of Texas School of Medicine, Galveston *Assoc Prof of Bacteriology and Tropical Medicine, Dir of Lab of Microbiology* (6, 1943)
- Page, Edouard**, Ph D Laval Univ Med School, Dept of Biochemistry, Quebec, Canada (1, 1947)
- Page, Ernest W**, M D Univ of California Hospital, Dept of Obstetrics and Gynecology, San Francisco 22 *Asst Prof* (1, 1947)
- Page, Irvine H**, M D Cleveland Clinic Foundation, 2040 E 93rd St, Cleveland 6, Ohio *Dir of Research* (1, 1937, 2, 1932)
- Painter, Elizabeth E**, Ph D Univ of Illinois School of Medicine, 1853 W Polk St, Chicago *Asst Prof of Physiology* (1, 1941)
- Palmer, John W**, Ph D E R Squibb and Sons, Georges Rd, New Brunswick, N J *Head of Dept of Protein Fractionation* (6, 1949)
- P'An, S Y**, M D Columbia Univ College of Physicians and Surgeons, New York City (3, 1941)
- Pangborn, Mary C**, Ph D New Scotland Ave, Albany, N Y *Sr Biochemist, New York State Dept of Health, Div of Labs and Research* (2, 1941)
- Pappenheimer, A M, Jr**, Ph D New York Univ College of Medicine, 477 First Ave, New York City 16 *Assoc Prof of Microbiology* (2, 1941, 6, 1938)
- Pappenheimer, Alwin M**, M D 45 Holden St, Cambridge, Mass *Prof Emeritus of Pathology, Columbia Univ* (4, 1922)
- Pappenheimer, John R**, Ph D Harvard Med School, Boston, Mass *Assoc in Physiology* (1, 1946)
- Papper, E M**, M D Columbia Presbyterian Med Center, 630 W 168th St, New York City *Prof of Anesthesiology* (3, 1949)
- Park, Edwards A**, M D Johns Hopkins Hospital, Baltimore, Md *Prof Emeritus of Pediatrics, Johns Hopkins Univ* (4, 1923)
- Parker, Robert F**, M D Univ Hosps, 2065 Adelbert Rd, Cleveland, Ohio *Assoc Prof of Microbiology and Medicine* (4, 1942, 6, 1935)
- Parkins, William M**, Ph D Route 2, Chariton, Iowa (1, 1939)
- Parpart, Arthur K**, Ph D Princeton Univ, Guyot Hall, Princeton, N J *Prof of Physiology* (1, 1937)
- Parr, Leland W**, Ph D George Washington Univ School of Medicine, 1335 H St NW, Washington, D C *Prof of Bacteriology* (4, 1940)
- Parrack, Horace O**, Ph D Wright-Patterson Air Force Base Aero-Med Lab, Dayton, Ohio *Research Physiologist* (1, 1948)
- Parsons, Helen T**, M D, Ph D Univ of Wisconsin, Madison *Prof of Home Economics, in charge of Purnell Research in Nutrition* (2, 1929, 5, 1933)
- Parsons, Robert J**, M D Highland Alameda County Hospital, 2701 14th Ave, Oakland, Calif *Pathologist, Dir of Labs* (4, 1939)
- Paschkis, Karl E**, M D Jefferson Med College, 1025 Walnut St, Philadelphia 7, Pa *Asst Prof of Medicine and Assoc in Physiology, Chief of Endocrine Clinic, Jefferson Hospital* (1, 1942)
- Patt, Harvey M**, Ph D Argonne National Lab, Chicago, Ill *Physiologist* (1, 1948)
- Patterson, Thos L**, Ph D Wayne Univ College of Medicine, 1512 St Antoine St, Detroit, Mich *Prof of Oro-Physiology and Dir of Dental Research, Univ of Detroit College of Dentistry* (1, 1920)
- Patterson, Wilbur I**, Ph D Food and Drug Admin, Washington 25, D C *Chief, Organic Analytical Methods* (2, 1948)
- Patton, H D**, Ph D, M D Univ of Washington School of Medicine, Dept of Physiology and Biophysics, Seattle 5 *Asst Prof* (1, 1947)
- Paul, John R**, M A, M D Yale Univ Med School, 333 Cedar St, New Haven, Conn *Prof of Preventive Medicine* (4, 1927, 6, 1937)
- Pearce, John Musser**, M D 525 E 68th St, New York City 21 *Prof of Pathology, Cornell Univ Med College, Attending Pathologist, New York Hospital* (4, 1942)
- Pearce, Louise**, M D Rockefeller Inst for Med Research, Princeton, N J *Assoc Member in Pathology and Bacteriology* (3, 1915, 4, 1925)
- Pearcy, Frank**, Ph D, M D 2606 Oak Lawn Ave, Dallas, Tex (1, 1928)
- Pearlman, William H**, Ph D Jefferson Med College, 1025 Walnut St, Philadelphia 7, Pa *Assoc Prof, Biochemistry Dept* (2, 1946)
- Pearse, Herman E**, M D Univ of Rochester School of Medicine and Dentistry, Rochester, N Y *Prof of Surgery* (4, 1932)
- Pearson, Harold E**, M D, M P H Univ of Southern California Med School, Los Angeles *Assoc Prof of Bacteriology, Med Microbiologist, Los Angeles County Hospital* (6, 1948)
- Pearson, P B**, Ph D Atomic Energy Commission, Div of Biology and Medicine, Washington 25, D C *Chief of Biology Branch* (2, 1944, 5, 1940)
- Pease, Marshall C, Jr**, M D Branchville Rd, R F D 4, Ridgefield, Conn *Historian of Am Acad of Pediatrics* (6, 1920)
- Peck, Robert L**, Ph D 939 Madison Ave, Plainfield, N J *Sr Chemist, Merck and Co, Inc* (2, 1947)
- Pemberton, Ralph**, M S, M D Univ of Pennsylvania, Philadelphia *Prof of Medicine, Graduate School of Medicine* (5, 1933)
- Penfield, Wilder G**, M D, D Sc McGill Univ, Montreal, Quebec, Canada *Prof of Neurology and Neurosurgery* (1, 1932)
- Pennington, Mary Engle**, Ph D 233 Broadway, New York City 7 *Consultant in Connection with*

- the Handling, Transportation and Storage of Perishables* (2, 1908)
- Penrod, Kenneth E**, Ph D Boston Univ School of Medicine, Dept of Physiology, 80 E Concord St, Boston, Mass *Asst Prof of Physiology* (1, 1946)
- Peoples, S Anderson**, M D Univ of California, Dept of Veterinary Science, Davis *Prof of Pharmacology* (3, 1937)
- Perlman, Ely**, M D 77-14 113th St, Forest Hills, L I, N Y (6, 1944)
- Perlzweig, William A**, Ph D Box 3711, Duke Univ School of Medicine, Durham, N C *Prof and Chairman of Dept of Biochemistry and Nutrition, Biochemist, Duke Hospital* (2, 1924, 5, 1944)
- Permar, Howard H**, M D Pathologic Labs, Mercy Hospital, Pittsburgh, Pa *Dir of Labs* (4, 1925)
- Petermann, Mary L**, Ph D Sloan-Kettering Inst for Cancer Research, 444 E 68th St, New York City 21 *Associate* (2, 1947)
- Peters, John P**, M D 123 Marvel Rd, New Haven 15, Conn *John Slade Ely Prof of Medicine, Yale Univ School of Medicine* (2, 1922)
- Peters, Lawrence**, Ph D Western Reserve Univ Med School, Dept of Pharmacology, 2109 Adelbert Rd, Cleveland 6, Ohio *Sr Instr in Pharmacology* (3, 1946)
- Petersen, William E**, Ph D Univ of Minnesota, Div of Dairy Husbandry, St Paul 1 *Professor* (1, 1947)
- Petersen, William F**, M D 1322 Astor St, Chicago, Ill *Prof of Pathology, Univ of Illinois* (3, 1923, 4R, 1923)
- Peterson, William H**, Ph D Univ of Wisconsin, Biochemistry Bldg, Madison *Prof of Biochemistry* (2, 1919, 5, 1936)
- Pett, L B**, M D, Ph D Dept of Natl Health and Welfare, Ottawa, Ontario, Canada *Dir of Nutrition* (2, 1937, 5, 1945)
- Pfeiffer, Carl C**, Ph D, M D Univ of Illinois, Dept of Pharmacology, 1853 W Polk St, Chicago 12 *Prof and Chairman of Dept of Pharmacology* (3, 1938)
- Pfiffner, Joseph J**, Ph D Parke, Davis & Co, Research Labs, Detroit 32, Mich *Research Chemist* (1, 1931, 2, 1931, 5, 1946)
- Phatak, Nilkanth M**, Ph D North Pacific College of Oregon, School of Dentistry, Portland *Assoc Prof of Physiology, Pharmacology, and Research, Instr, Dept of Pharmacology, Univ of Oregon Med School* (3, 1941)
- Philips, Frederick S**, Ph D Sloan-Kettering Inst for Cancer Research, 444 E 68th Street, New York City 21 *Chief, Pharmacology Dept* (3, 1947)
- Phillips, Paul H**, Ph D Univ of Wisconsin, Madison *Prof of Biochemistry* (2, 1940, 5, 1938)
- Phillips, Robert Allan**, M D U S Naval Med Research Unit No 3, American Embassy at Cairo, Egypt, %Navy Pouch Section, Navy Dept, Washington 25, D C (1, 1938)
- Pick, Ernst Peter**, M D 19 E 98th St, New York City *Assoc Pharmacologist, Mt Sinai Hospital, Clin Prof of Pharmacology, Columbia Univ* (3, 1940)
- Pierce, Harold B**, Ph D Univ of Vermont College of Medicine, Burlington *Prof and Chairman of Dept of Biochemistry* (2, 1929, 5, 1933)
- Pierce, Harold Fisher**, Ph D, M D State Veterans Hospital, Rocky Hill Conn (1, 1928)
- Pierce, Ira H**, Ph D Univ of Iowa, Iowa City *Assoc Prof of Pharmacology* (3, 1933)
- Pike, Frank H**, Ph D 437 W 59th St, New York City 19 *Assoc Prof of Physiology, Columbia Univ* (1, 1907)
- Pillemer, Louis**, Ph D Western Reserve Univ, Inst of Pathology, Cleveland, Ohio *Assoc Prof of Immunochemistry* (6, 1942)
- Pincus, Gregory**, Sc D Worcester Foundation for Exper Biology, 222 Maple Ave, Shrewsbury, Mass (1, 1935)
- Pincus, I J**, M D Jefferson Med College, Philadelphia, Pa *Assoc in Physiology* (1, 1948)
- Pinkerton, Henry**, M D St Louis Univ School of Medicine, St Louis, Mo *Prof of Pathology* (4, 1931)
- Pinkston, James O**, Ph D American Univ of Beirut, Beirut, Lebanon *Pharmacologist* (1, 1936, 3, 1939)
- Pinson, Ernest A**, Ph D 139 N Walnut St, Yellow Springs, Ohio (1, 1943)
- Pittman, Martha S**, Ph D Manhattan, Kan (5, 1933)
- Pitts, Robert F**, Ph D, M D Syracuse Univ College of Medicine, Syracuse, N Y *Prof of Physiology* (1, 1934)
- Pollack, Herbert**, Ph D, M D 45 E 66th St, New York City 21 *Assoc Physician and Chief of Metabolism Clinics, Mt Sinai Hospital* (1, 1933, 5, 1935)
- Pomerat, Charles Marc**, Ph D Univ of Texas Med School, Galveston *Prof of Anatomy* (1, 1944)
- Pommerenke, W T**, Ph D, M D Univ of Rochester School of Medicine and Dentistry, Rochester 7, N Y *Assoc Prof of Obstetrics and Gynecology* (1, 1947)
- Pond, Samuel E**, Ph D 400 S Main St, East Hartford, Conn *Consulting Engineer, P and W A Div, United Aircraft Corp* (1, 1924)
- Ponder, Eric**, M D, Sc D Nassau Hospital, Mineola, Long Island, N Y (1, 1931)
- Poppen, John R**, M D Aero Med Acceleration Lab, Naval Air Development Center, Johnsville, Pa *Director* (1, 1948)
- Popper, Hans**, Ph D, M D Cook County Hospital, 1825 W Harrison St, Chicago 12, Ill

- Dir of Dept of Pathology and Scientific Div of Hektoen Inst for Med Research, Assoc Prof of Pathology, Northwestern Univ Med School* (4, 1942)
- Porter, Eugene L**, Ph D Univ of Texas Med Branch, Galveston *Prof of Physiology* (1, 1913)
- Porter, Thelma**, Ph D Univ of Chicago, Chicago, Ill *Prof and Chairman of Dept of Home Economics* (5, 1944)
- Poth, Edgar J**, M D, Ph D Univ of Texas Med School, Galveston *Prof of Surgery* (1, 1946)
- Potter, Truman S**, M D 82 N Prospect St, Amherst, Mass *Independent Research Worker* (6, 1939)
- Potter, Van R**, Ph D Univ of Wisconsin Med School, McArdle Memorial Lab, Madison *Prof of Oncology* (2, 1941)
- Powell, Horace M**, Sc D 5565 Washington Blvd, Indianapolis, Ind *Head Bacteriologist, Eli Lilly & Co* (6, 1934)
- Power, Marschelle H**, Ph D Mayo Clinic, Rochester, Minn *Prof of Physiological Chemistry, Mayo Foundation, Univ of Minnesota* (2, 1932)
- Pratt, Joseph H**, M D, Sc D New England Med Center, 25 Bennet St, Boston, Mass *Physician-in-Chief, Boston Dispensary and Joseph H Pratt Diagnostic Clinic, Prof Emeritus of Clin Medicine, Tufts Med School* (1, 1910, 3, 1910, 4, 1927)
- Preisler, Paul W**, Ph D 4580 Scott Ave, St Louis 10, Mo *Asst Prof of Biochemistry, Washington Univ School of Medicine* (2, 1931)
- Pressman, David**, Ph D Sloan-Kettering Inst for Cancer Research, 444 East 68 St, New York City 21 *Sr Fellow in Cancer Research* (6, 1949)
- Price, Clifford W**, Ph D U S Food and Drug Admin, Washington, D C *Bacteriologist, Antibiotics Analyst, Div of Penicillin Control and Immunology* (6, 1946)
- Prinzmetal, Myron, M.A.**, M D 300 S Beverly Drive, Beverly Hills, Calif *Sr Attending Physician, Cedars of Lebanon Hospital, Los Angeles* (3, 1941)
- Prosser, C Ladd**, Ph D Univ of Illinois, Natural History Bldg, Urbana (1, 1935)
- Puestow, Charles B**, M D, Ph D Univ of Illinois College of Medicine, 1853 W Polk St, Chicago *Asst Prof of Surgery* (1, 1934)
- Pugsley, L I**, Ph D Dept of Natl Health and Welfare Food and Drug Lab, Ottawa, Ontario, Canada *Chief of Lab Service* (2, 1937)
- Putnam, Frank W**, Ph D Univ of Chicago, 947 E 58th St, Chicago 37, Ill *Asst Prof of Biochemistry* (2, 1947)
- Quackenbush, Forrest W**, Ph D R-2, Brookston, Ind *Prof and Head of Dept of Agricultural Chemistry, Purdue Univ* (2, 1946)
- Quaife, Mary L**, Ph D Distillation Products, Inc, 755 Ridge Rd W, Rochester 13, N Y *Sr Research Chemist, Dept of Biochemistry* (2, 1948)
- Quastel, J H**, D Sc, Ph D McGill Univ, Montreal, Quebec, Canada *Prof of Biochemistry* (2, 1948)
- Queen, Frank B**, M D Univ of Oregon School of Medicine, 3181 S W Marquam Hill Rd, Portland *Prof of Pathology* (4, 1941)
- Quick, Armand J**, M D, Ph D 561 N 15th St, Milwaukee 3, Wis *Prof and Dir of Dept of Biochemistry, Marquette Med School* (2, 1932, 3, 1937)
- Quigley, J P**, Ph D Univ of Tennessee, Dept of Pharmacology, Memphis 3 *Prof and Chief of Div of Pharmacology* (1, 1929, 3, 1945)
- Quinn, Edmond John**, Ph D 106 N Lee Ave, Rockville Center, Long Island, N Y *Medicinal Sales Div, Merck & Co, Inc, Rahway, N J* (2, 1927, 5, 1933)
- Raab, Wilhelm**, M D Univ of Vermont College of Medicine, Div of Exper Medicine, Burlington *Prof, Head of Division and of Cardiovascular Clin Research Unit* (1, 1949)
- Rabinowitch, I M**, D Sc, M D C M 1020 Med Arts Bldg, Sherbrooke and Guy Sts, Montreal, Quebec, Canada *Assoc Prof of Medicine and Lecturer in Med Jurisprudence and Toxicology, McGill Univ, Dir, Inst for Research, Montreal General Hospital* (2, 1928, 5, 1933)
- Rachele, Julian R**, Ph D Cornell Univ Med College, 1300 York Ave, New York City 21 *Assoc Prof of Biochemistry* (2, 1948)
- Rackemann, Francis M**, M D 263 Beacon St, Boston, Mass *Physician, Massachusetts General Hospital, Lecturer in Medicine, Harvard Med School* (6, 1923)
- Racker, Efraim**, M D New York Univ College of Medicine, New York City 16 *Asst Prof of Microbiology* (2, 1948)
- Raffel, Sidney**, Sc D, M D Stanford Univ, Dept of Bacteriology and Exper Pathology, Stanford Univ, Calif *Asst Prof* (6, 1938)
- Rahn, Hermann**, Ph D Univ of Rochester School of Medicine and Dentistry, Rochester, N Y *Asst Prof of Physiology* (1, 1944)
- Rake, Geoffrey W**, M B Squibb Inst for Med Research, Div of Microbiology, New Brunswick, N J *Head of Div* (6, 1939)
- Rakestraw, Norris W**, Ph D Univ of California, Scripps Inst of Oceanography, LaJolla *Prof of Chemistry* (2, 1925)
- Rakieten, Nathan**, Ph D Bristol Labs, Inc, P O Box 657, Syracuse 2, N Y *Pharmacologist and Toxicologist* (1, 1941)
- Ralli, Elaine P**, M D 477 First Ave, New York City *Assoc Prof of Medicine, New York Univ College of Medicine* (1, 1934, 5, 1933)
- Ralston, H J**, Ph D College of Physicians and Surgeons, Dept of Physiology, 344 14th St,

- San Francisco 3, Calif *Assoc Prof of Physiology* (1, 1947)
- Rammelkamp, Charles H , Jr** , M D Western Reserve Univ , Dept of Preventive Medicine, Cleveland 6, Ohio *Assoc Prof of Medicine* (6, 1943)
- Ramsey, Robert Weberg**, Ph D Med College of Virginia, Richmond *Assoc Prof of Physiology and Pharmacology* (1, 1939)
- Randall, Lowell O** , Ph D Hoffmann-LaRoche, Inc , Nutley 10, N J *Pharmacologist* (2, 1939)
- Randall, Walter C** , Ph D St Louis Univ School of Medicine, 1402 S Grand Blvd , St Louis, Mo *Instr in Physiology* (1, 1943)
- Randall, William A** , Ph D Food and Drug Admin , Washington 25, D C *Bacteriologist, Div of Penicillin Control and Immunology* (6, 1946)
- Rane, Leo**, Ph D Lederle Labs , Inc , Pearl River, N Y *Dept Head, Normal Blood Plasma* (6, 1942)
- Rantz, Lowell A** , M D Stanford Univ Hospital, San Francisco 15, Calif *Asst Prof of Medicine* (3, 1946)
- Rapoport, Samuel**, M D , Ph D Children's Hospital Research Foundation, Elland and Bethesda, Cincinnati, Ohio *Assoc Prof of Pediatrics and Asst Prof of Biochemistry, Univ of Cincinnati* (2, 1941)
- Rapport, David**, M D 416 Huntington Ave , Boston, Mass *Prof of Physiology, Tufts College Med School* (1, 1922)
- Raska, Sigwin**, Ph D Univ of North Dakota, Dept of Physiology and Pharmacology, Grand Fork *Prof of Pharmacology* (1, 1947)
- Rasmussen, A F** , Ph D , M D Univ of Wisconsin Med School, Dept of Med Microbiology , Madison *Assoc Prof* (6, 1949)
- Rasmussen, Andrew Theodore**, Ph D Univ of Minnesota Med School, Minneapolis *Prof of Neurology* (1, 1919)
- Ratner, Bret**, M D 50 E 78th St , New York City *Prof of Pediatrics, New York Univ College of Medicine* (4, 1940, 6, 1928)
- Ratner, Sarah**, Ph D New York Univ College of Medicine, Dept of Pharmacology, 477 First Ave , New York City 16 *Asst Prof of Pharmacology* (2, 1944)
- Raulston, B O** , M D 200 S Hudson Ave , Los Angeles, Calif *Prof of Medicine and Dir of Clinical Teaching, Univ of Southern California School of Medicine* (3, 1942)
- Ravdin, I S** , M D Univ of Pennsylvania School of Medicine, Philadelphia *John Rhea Barton Prof of Surgery, Chief Surgeon, Hospital of the Univ of Pennsylvania* (1, 1930, 4, 1930)
- Rawson, Rulon W** , M D Memorial Hospital, New York City *Attending Physician, Chief, Dept of Clinical Investigation, Sloan-Kettering Inst* (1, 1947)
- Raymond, Albert L** , Ph D G D Searle and Co , P O Box 5110, Chicago 80, Ill *Vice Pres in charge of Research and Development* (2, 1932)
- Redfield, Alfred C** , Ph D Woods Hole, Mass *Prof of Physiology, Harvard Univ* (1, 1919)
- Reed, Carlos I** , Ph D Univ of Illinois College of Medicine, 1853 W Polk St , Chicago *Prof of Physiology* (1, 1923)
- Reed, Emerson A** , Ph D Hahnemann Med College, Philadelphia, Pa *Asst Prof of Physiology* (1, 1948)
- Reed, Howard S** , Ph D 3048 Life Sciences Bldg , Univ of California, Berkeley *Prof of Plant Physiology* (2, 1909)
- Reese, John D** , M D North Carolina Sanatorium, McCain *Pathologist and Lab Dir* (4, 1949)
- Rehm, Warren S , Jr** , Ph D , M D Univ of Louisville School of Medicine, Louisville, Ky *Prof of Physiology* (1, 1945)
- Reid, Marion Adelaide**, Ph D New Jersey College for Women, New Brunswick *Asst Prof , Dept of Zoology* (1, 1941)
- Reid, Mary E** , Ph D Natl Insts of Health, Bethesda 14, Md *Cytologist* (5, 1947)
- Reimann, Hobart A** , M D Jefferson Hospital, Philadelphia, Pa *Prof of Medicine, Jefferson Med College* (4, 1933)
- Reimann, Stanley P** , M D , Sc D 703 W Phil-Ellena St , Mount Airy, Philadelphia, Pa *Dir of Research Inst of Lankenau Hospital, Prof of Oncology, Hahnemann Med College and Hospital, Philadelphia* (1, 1921, 4, 1924)
- Reinecke, Roger M** , Ph D , M D Univ of Minnesota, Dept of Physiology, Minneapolis 14 *Asst Prof* (1, 1947)
- Reiner, John M** , Ph D Tufts College Med School, Dept of Physiology, Boston, Mass (1, 1947)
- Reiner, Laszlo**, M D , Ph D Wallace and Tiernan Products, Inc , 165 Franklin St , Bloomfield, N J *Dir , Pharmaceutical Research* (2, 1942, 6, 1933)
- Reinhold, John G** , Ph D Univ of Pennsylvania Hospital, 702 Maloney Bldg , Philadelphia, Pa *Asst Prof of Physiological Chemistry and Medicine* (2, 1936)
- Remington, John W** , Ph D Univ of Georgia School of Medicine, Augusta *Asst Prof of Physiology* (1, 1943)
- Remington, Roe E** , Ph D , P O Box 1252, Hendersonville, N C *Consultant* (2, 1930, 5R, 1934)
- Renfrew, Alice G** , Ph D Mellon Inst of Industrial Research, Univ of Pittsburgh, Pittsburgh, Pa *Sr Fellow* (2, 1939)
- Reynolds, Chapman**, M D Louisiana State Univ School of Medicine, New Orleans *Asst Prof of Pharmacology* (3, 1937)

- Reynolds, Orr E**, Ph D Office of Naval Research, Med Sciences Div, Washington, D C *Head of Physiology Branch* (1, 1948)
- Reynolds, Samuel R M**, Ph D 4028 Deepwood Rd, Baltimore 18, Md *Staff Member and Physiologist, Carnegie Inst of Washington, Dept of Embryology* (1, 1932)
- Reznikoff, Paul**, M D New York Hospital, 525 E 68th St New York City *Assoc Prof of Clin Medicine, Cornell Univ Med College* (1, 1927)
- Rhoads, Cornelius Packard**, M D Memorial Hospital, 444 E 68th St, New York City *Dir, Prof of Pathology, Cornell Univ Med College* (4, 1930)
- Rhoads, Jonathan Evans**, M D, D Sc 4023 Pine St, Philadelphia 4, Pa *Asst Prof of Surgical Research* (1, 1946)
- Rice, Christine E**, Ph D Animal Diseases Research Inst, Canadian Dept of Agriculture, Hull, Quebec, Canada *Agricultural Scientist* (6, 1938)
- Rice, Harold V**, Ph D Univ of Alberta, Edmonton, Alberta, Canada *Prof of Physiology* (1, 1948)
- Rice, James C**, Ph D Univ of Mississippi, P O Box 475, University *Prof of Pharmacology* (3, 1941)
- Rich, Arnold Rice**, M D Johns Hopkins Hospital, Baltimore, Md *Baxley Prof of Pathology, Johns Hopkins Univ* (4, 1924)
- Richards, Alfred N**, Ph D, M D, Univ of Pennsylvania School of Medicine, Philadelphia 4 *Prof Emeritus of Pharmacology* (1R, 1900, 2, 1906, 3R, 1909)
- Richards, Oscar W**, Ph D American Optical Co, Scientific Instrument Div, Box A, Buffalo 15, N Y *Chief Biologist* (1, 1934)
- Richards, Richard Kohn**, M D Abbott Labs, North Chicago, Ill *Dir of Pharmacologic Research, Lecturer in Pharmacology, Northwestern Univ Med School* (1, 1938, 3, 1947)
- Richardson, Arthur P**, M D Emory Univ School of Medicine, Dept of Pharmacology, Emory University, Ga *Prof of Pharmacology* (3, 1939)
- Richardson, Luther R**, Ph D P O Box 102, College Station, Tex (5, 1942)
- Richter, Curt P**, Ph D Phipps Psychiatric Clinic, Johns Hopkins Hospital, Baltimore, Md *Assoc Prof of Psycho-biology, Johns Hopkins Univ* (1, 1924)
- Richter, Maurice N**, M D 303 E 20th St, New York City *Prof of Pathology, Bellevue Med Center, Post-Grad Med School Director, Univ Hosp* (4, 1931)
- Ricketts, Henry T**, M D Univ of Chicago, Dept of Medicine, Chicago, Ill *Assoc Prof of Medicine* (1, 1940)
- Riegel, Byron**, Ph D Northwestern Univ, Dept of Chemistry, Evanston, Ill *Professor* (2, 1942)
- Riegel, Cecilia**, Ph D Univ of Pennsylvania Hospital, Philadelphia 4, Pa *Assoc, Dept of Research Surgery, Univ of Pennsylvania School of Medicine* (2, 1938)
- Rigdon, R H**, M D Univ of Texas Med Branch, Galveston *Prof of Exper Pathology* (4, 1941)
- Riggs, Douglas S**, M D Harvard Med School, Boston, Mass *Instr in Pharmacology* (3, 1948)
- Riggs, Lloyd K**, Ph D Natl Dairy Research Labs, Oakdale, L I, N Y *Dir of Nutritional Research* (2, 1929)
- Riker, Walter F, Jr**, M D Cornell Univ Med College, 1300 York Ave, New York City 21 *Asst Prof of Pharmacology* (3, 1947)
- Riley, Richard L**, M D New York Univ Post-Grad Med School, Inst of Industrial Medicine, 477 First Ave, New York City *Assoc in Medicine* (1, 1948)
- Rinehart, James F**, M D Univ of California Med School, Parnassus and Third Aves, San Francisco *Prof of Pathology* (4, 1933)
- Ring, Gordon C**, Ph D Temple Univ Med School, Broad St, Philadelphia, Pa (1, 1933)
- Rioch, David McKenzie**, M D Chestnut Lodge Sanitarium, 500 W Montgomery Ave, Rockville, Md *Dir of Research* (1, 1931)
- Rittenberg, David**, Ph D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 *Assoc Prof* (2, 1939)
- Ritzman, E G**, A M Univ of New Hampshire, Durham *Research Prof* (5, 1933)
- Rivers, T M**, M D, Sc D Hospital of Rockefeller Inst for Med Research, 66th St and York Ave, New York City *Director* (4, 1925, 6, 1921)
- Robb, Jane Sands**, Sc D, M D Syracuse Univ College of Medicine, 761 Irving Ave, Syracuse, N Y *Assoc Prof of Pharmacology* (1, 1924)
- Robbie, W A**, Ph D State Univ of Iowa College of Medicine, Dept of Ophthalmology, Iowa City *Research Assoc, Prof of Ophthalmology and Physiology* (1, 1947)
- Robbins, Benjamin Howard**, M S, M D Vanderbilt Univ School of Medicine, Nashville, Tenn *Assoc Prof of Pharmacology* (3, 1936)
- Robbins, Mary L**, Ph D George Washington Univ School of Medicine, 1335 H St, N W, Washington 5, D C *Asst Prof of Bacteriology* (6, 1946)
- Roberts, Edward F**, M D, Ph D Wyeth, Inc, 1600 Arch St, Philadelphia 3, Pa *Dir of Clinical Investigation* (6, 1932)
- Roberts, Joseph T**, M D, Ph D Veterans Admin Hospital, Batavia, N Y *Chief of Med Service* (1, 1947)
- Roberts, Lydia J**, Ph D Univ of Puerto Rico, Rio Piedras, P R *Prof and Chairman of Dept of Home Economics* (5, 1933)

- Roberts, Sidney, Ph D Univ of California Med School, Los Angeles *Asst Clinical Prof of Physiological Chemistry* (1, 1946)
- Robertson, Elizabeth Chant, M D , Ph D Univ of Toronto, Toronto, Ontario, Canada *Research Fellow in Pediatrics* (5, 1939)
- Robinson, Charles Summers, Ph D Vanderbilt Univ Med School, Nashville, Tenn *Prof of Biochemistry* (2, 1925)
- Robinson, Elliott S , M D , Ph D R F D 4, Lacomia, N H *Dir of Div of Biologic Labs , Mass Dept of Health (Leave of Absence)* (6, 1935)
- Robinson, G Canby, M D , 4712 Keswick Rd , Baltimore, Md *Excc Sec , Maryland Tuberculosis Assoc* (1R, 1912, 3, 1921)
- Robinson, Harry J , Ph D Merck Inst for Therapeutic Research, Rahway, N J *Asst Dir* (3, 1946)
- Robinson, Herbert E , Ph D Swift and Co , Research Labs , Union Stock Yards, Chicago 9, Ill *Asst Dir of Research* (5, 1947)
- Robinson, Howard W , Ph D Broad and Ontario Sts , Philadelphia, Pa *Prof of Physiological Chemistry, Temple Univ School of Medicine* (2, 1929)
- Robinson, Sid, Ph D Indiana Univ Med School, Bloomington *Prof of Physiology* (1, 1941)
- Robinson, True W , Ph D Wright-Patterson Air Force Base, Aero-Med Lab , Dayton, Ohio *Chief of Metabolism Unit* (1, 1948)
- Roblin, Richard O , Jr , Ph D 1937 W Main St , Stamford, Conn *Dir of Chemotherapy Div , American Cyanamid Co* (2, 1946, 6, 1947)
- Robschert-Robbins, F S , Ph D Univ of Rochester School of Medicine and Dentistry, Rochester, N Y *Assoc in Pathology* (1, 1925, 4, 1930)
- Rodbard, Simon, Ph D Michael Reese Hospital, Cardiovascular Dept , 29th and Ellis Aves , Chicago, Ill (1, 1942)
- Roe, Joseph Hiram, Ph D George Washington Univ School of Medicine, Washington, D C *Prof of Biochemistry* (2, 1927, 5, 1933)
- Roeder, Kenneth D , M A Tufts College, Medford, Mass *Assoc Prof of Biology* (1, 1942)
- Roepke, Martin Henry, Ph D Univ Farm, St Paul, Minn *Prof of Veterinary Medicine* (3, 1937)
- Rogers, Fred T , Ph D , M D Dallas Med and Surgical Clinic, 4105 Live Oak St , Dallas 1, Tex (1, 1917)
- Rogoff, Julius M , M D , Sc D Univ of Pittsburgh School of Medicine, Pittsburgh, Pa *Prof of Endocrinology* (1, 1916, 3, 1916)
- Ronzoni, Ethel, Ph D Washington Univ Med School, St Louis 4, Mo *Asst Prof of Biological Chemistry* (2, 1923)
- Roos, Albert, M D Washington Univ School of Medicine, Dept of Physiology, Euclid Ave and Kingshighway, St Louis 10, Mo *Instr in Physiology and Surgery* (1, 1949)
- Root, Howard F , M D 44 Dwight St , Brookline, Mass *Assoc in Medicine, Harvard Med School* (5, 1933)
- Root, Raymond W , Ph D College of the City of New York, Dept of Biology, 139th St and Convent Ave , New York City *Assoc Prof of Biology, Head of Div of Physiology* (1, 1949)
- Root, Walter S , Ph D Columbia Univ College of Physicians and Surgeons, 630 W 168th St , New York City 32 *Prof of Physiology* (1, 1932)
- Rosahn, Paul D , M D 92 Grand St , New Britain, Conn *Pathologist, New Britain General Hospital, Assoc Clinical Prof of Pathology, Yale Univ School of Medicine* (4, 1934)
- Rose, Harry M , M D Columbia Univ College of Physicians and Surgeons, 630 W 168th St , New York City 32 *Assoc Prof of Bacteriology* (6, 1949)
- Rose, Jerzy E , M D Johns Hopkins Univ Med School, Dept of Physiology, 710 N Washington St , Baltimore 5, Md *Asst Prof of Physiology and Psychiatry* (1, 1949)
- Rose, William C , Ph D Univ of Illinois, Urbana *Prof of Biochemistry* (2, 1912, 5, 1933)
- Rosenblueth, Arturo, M D Instituto Nacional de Cardiologia, Calzada de la Piedad 300, Mexico D F , Mexico (1, 1932)
- Rosenfeld, Morris, M D Johns Hopkins School of Medicine, Baltimore, Md *Assoc Prof of Pharmacology and Exper Therapeutics* (3, 1934)
- Rosenow, Edward C , M D Longview State Hospital, Research Dept , Cincinnati 16, Ohio (4, 1913, 6, 1915)
- Rosenthal, Otto, M D Univ of Pennsylvania School of Medicine, Philadelphia, Pa *Asst Prof of Cancer Research, Harrison Dept of Surgical Research* (2, 1946)
- Rosenthal, Sanford M , M D Natl Insts of Health, Bethesda, Md *Sr Pharmacologist USPHS* (3, 1925)
- Rosenthal, S R , M D , Ph D Univ of Illinois College of Medicine, Chicago *Assoc Prof of Preventive Medicine and Public Health, Dir , Institution for Tuberculosis Research of Univ of Illinois* (1, 1948, 4, 1941)
- Ross, Joseph F , M D Robert Dawson Evans Memorial, 65 E Newton St , Boston, Mass *Physician, Massachusetts Memorial Hospital, Assoc Prof of Medicine, Boston Univ School of Medicine* (4, 1941)
- Rossiter, R J , Ph D Univ of Western Ontario, London, Ontario, Canada *Prof of Biochemistry* (2, 1948)
- Rostorfer, Howard Hayes, Ph D Indiana Univ , Dept of Physiology, Bloomington *Asst Prof of Physiology* (1, 1946)

- Roth, Grace M , Ph D Mayo Clinic, Rochester, Minn *Assoc in Clin Physiology* (1, 1939)
- Roth, L W , Ph D Abbott Research Labs , Dept of Pharmacology, North Chicago, Ill *Research Pharmacologist* (1, 1947)
- Rothchild, Irving, Ph D Ohio State Univ , Dept of Obstetrics and Gynecology, Univ Hospital, Columbus, Ohio (1, 1949)
- Rothmund, Paul W K , Dr -Ing Antioch College, Yellow Springs, Ohio *Prof of Chemistry, Research Chemist, C F Kettering Foundation* (2, 1940)
- Rothstein, A , Ph D Univ of Rochester School of Medicine and Dentistry, Rochester 7, N Y *Asst Prof in Pharmacology, Chief of Physiological Section, Pharmacological Div , Atomic Energy Project* (1, 1949)
- Rous, Peyton, M D , Sc D Rockefeller Inst for Med Research, York Ave at 66th St , New York City *Member Emeritus* (4, 1913)
- Routh, Joseph I , Ph D State Univ of Iowa, Iowa City *Assoc Prof of Biochemistry* (2, 1942)
- Rovenstone, Emery Andrew, M D 477 First Ave , New York City *Prof of Anesthesia, New York Univ , Dir of Div of Anesthesia, Bellevue Hospital* (3, 1944)
- Rowntree, Jennie I , Ph D Univ of Washington, Seattle *Prof of Home Economics* (5, 1933)
- Rowntree, L G , M D , Sc D DuPont Bldg , E Flagler St at 2nd Ave , Miami, Fla (1, 1911, 2, 1910, 3, 1908, 4, prior to 1920, 5, 1933)
- Rubenstein, Boris B , Ph D , M D Michael Reese Hospital, Dept of Metabolic and Endocrine Research, E 59th St and Ellis Ave , Chicago, Ill (1, 1934)
- Rubin, Saul H , Ph D Hoffmann-LaRoche, Inc , Nutley 10, N J *Dir of Nutrition Labs* (2, 1947, 5, 1947)
- Ruch, Theodore C , Ph D Univ of Washington, 204B Physiology Hall, Seattle 5 *Prof of Physiology and Biophysics* (1, 1933)
- Ruckman, Isaac, Ph D Cincinnati General Hospital, Cincinnati, Ohio *Asst Prof of Bacteriology* (6, 1948)
- Rusch, Harold Paul, M D Univ of Wisconsin Med School, McArdle Memorial Lab , Madison 6 *Prof of Oncology, Dir of Lab* (4, 1940)
- Rushmer, Robert F , M D Univ of Washington School of Medicine, Dept of Physiology and Biophysics, Seattle 5 *Asst Prof* (1, 1949)
- Rusoff, Louis L , Ph D Louisiana State Univ , Baton Rouge *Assoc Dairy Nutritionist in Exper Station and Assoc Prof of Nutrition* (5, 1948)
- Russell, Jane A , Ph D Yale Univ School of Medicine, 333 Cedar St , New Haven, Conn *Instr in Physiological Chemistry* (1, 1939)
- Russell, Walter C , Ph D New Jersey Agricultural Exper Station, Rutgers Univ , New Brunswick *Research Specialist and Prof of Agricultural Biochemistry* (2, 1932, 5, 1933)
- Ryan, Andrew Howard, M D Chicago Med School, 710 S Wolcott Ave , Chicago, Ill *Assoc Prof of Physiology and Pharmacology* (1, 1912)
- Rytand, David A , M D Stanford Univ Hospital, San Francisco 15, Calif *Assoc Prof of Medicine, Stanford Univ School of Medicine* (3, 1946)
- Sabin, Albert, M D Children's Hospital Research Foundation, Cincinnati, Ohio *Prof of Research Pediatrics, Univ of Cincinnati* (6, 1946)
- Sacks, Jacob, Ph D , M D Brookhaven Natl Lab , Biology Dept , Upton, L I , N Y *Scientist* (1, 1948, 3, 1933)
- Sah, Peter P T , Ph D Univ of Calif , Div of Pharmacology and Exper Therapeutics, San Francisco *Lecturer in Pharmacology* (3, 1941)
- Sahyun, Melville, Ph D Sahyun Research Lab , 1425 Bath St , Santa Barbara, Calif (2, 1932)
- Sakami, Warwick, Ph D Western Reserve Univ School of Medicine, 2109 Adelbert Rd , Cleveland 6, Ohio *Asst Prof of Biochemistry* (2, 1949)
- Salk, Jonas E , M D Univ of Pittsburgh School of Medicine, Pittsburgh, Pa *Assoc Research Prof of Bacteriology* (6, 1947)
- Salmon, W D , A.M Alabama Polytechnic Inst , Auburn *Animal Nutritionist* (2, 1929, 5, 1933)
- Salter, William T , M D Yale Univ School of Medicine, 333 Cedar St , New Haven, Conn *Prof of Pharmacology* (1, 1933, 3, 1942, 5, 1934)
- Sammis, Florence E , M D 133 E 58th St , New York City *Physician, Allergy, OPD, New York Hospital* (6, 1943)
- Sampson, John J , M D 2211 Post St , San Francisco, Calif (1, 1932)
- Sampson, Myra M , Ph D Smith College, Northampton, Mass *Prof of Zoology* (5, 1935)
- Samuels, Leo T , Ph D Univ of Utah Med School, Salt Lake City *Prof and Head of Dept of Biological Chemistry* (2, 1941, 3, 1937, 5, 1949)
- Sandels, Margaret R , Ph D Florida State Univ , Tallahassee *Dean of School of Home Economics, Prof of Nutrition* (5, 1933)
- Sandiford, Irene, Ph.D Billings Hospital, Univ of Chicago, Chicago, Ill *Asst Prof of Medicine* (2, 1925, 5, 1933)
- Sandow, Alexander, Ph D Washington Square College of Arts and Sciences, New York Univ , New York City *Assoc Prof of Biology* (1, 1945)
- Sandweiss, David J , M D 9739 Dexter Ave , Detroit, Mich *Instr in Clin Medicine, Wayne Univ College of Medicine, Physician, Harper Hospital (OPD)* (1, 1944)
- Sanford, Arthur H , M A , M D Clin Labs , Mayo Clinic, Rochester, Minn *Head of Div of Clin Labs* (6, 1920)

- Santos, Francisco O , Ph D Univ of the Philippines, Los Banos, Laguna *Prof and Head of Dept of Agricultural Chemistry, College of Agriculture* (5, 1936)
- Saphir, Otto, M D Michael Reese Hospital, 29th St and Ellis Ave , Chicago 16, Ill *Pathologist, Clin Prof of Pathology, Univ of Illinois Med School* (4, 1927)
- Saphra, Ivan, M D Beth Israel Hospital, New York City *Assoc Bacteriologist* (6, 1946)
- Sarett, Herbert P , Ph D Tulane Med School, 1430 Tulane Ave , New Orleans 13, La *Asst Prof of Biochemistry* (2, 1946, 5, 1947)
- Sarnoff, Stanley J , M D Harvard School of Public Health, Dept of Physiology, 55 Shattuck St , Boston 15, Mass *Asst Prof of Physiology* (1, 1949)
- Saslow, George, Ph D , M D Washington Univ Med School, Dept of Neuropsychiatry, 640 S Kingshighway, St Louis, Mo *Asst Prof of Psychiatry, Assoc Physician to the Student Health Service* (1, 1936)
- Satterfield, G Howard, A M Univ of North Carolina, State College of Agriculture and Engineering, Raleigh *Prof of Biochemistry* (2, 1944, 5, 1941)
- Saul, Leon Joseph, M A , M D Room 1907, 255 S 17th St , Philadelphia 3, Pa (1, 1933)
- Sawyer, Charles H , Ph D Duke Univ School of Medicine, Dept of Anatomy, Durham, N C *Asst Prof of Anatomy* (1, 1949)
- Sawyer, Margaret E MacKay, Ph D 142 Lower Albert St , Kingston, Ontario, Canada (1, 1935)
- Sawyer, Wilbur A , M D 770 Hilldale Ave , Berkeley 8, Calif (4, 1930)
- Saxton, John A , Jr , M D Snodgrass Lab of Pathology and Bacteriology, 1430 Carroll St , St Louis, Mo *Asst Prof of Pathology, Washington Univ School of Medicine, Lab Dir , Hospital Div , City of St Louis* (4, 1944)
- Sayers, George, Ph D Univ of Utah, Dept of Pharmacology, Salt Lake City 1 *Assoc Prof of Pharmacology* (1, 1948 3, 1947)
- Scantlebury, Ronald E , Ph D Natl Insts of Health, Div of Research Grants and Fellowships, Bethesda 14, Md (1, 1948)
- Schales, Otto, D Sc Ochsner Clinic, Prytania and Aline Sts , New Orleans, La *Dir of Chemical Research, Ochsner Foundation, Asst Prof of Biochemistry, Tulane Univ School of Medicine* (2, 1944)
- Scharles, Frederick H , M D 911 Alma Ave , Oakland, Calif (5, 1935)
- Schattenberg, Herbert John, M S , M D Lab of Clin Pathology, 220-222 Med Arts Bldg , San Antonio, Tex *Director* (4, 1940)
- Schenken, John R , M D Univ of Nebraska College of Medicine, Omaha *Prof of Pathology and Bacteriology* (4, 1942)
- Scherago, Morris, D V M Univ of Kentucky, Lexington *Prof and Head of Dept of Bacteriology* (6, 1948)
- Scherp, Henry W , Ph D Univ of Rochester School of Medicine and Dentistry, Rochester 7, N Y *Assoc Prof of Bacteriology and Immunology* (6, 1940)
- Schick, Bela, M D 17 E 84th St , New York City *Pediatrician, Mt Sinai and Sea View Hospitals* (6, 1924)
- Schiffman, Milton J , Ph D Hoffmann-LaRoche, Inc , Chicago, Ill *Research Consultant* (1, 1943)
- Schiller, Alfred A , M D Univ of Illinois College of Medicine, Chicago *Asst Prof of Physiology* (1, 1948)
- Schiller, Joseph, M D , Ph D 55 West 11th St , New York City 11 *Assoc Prof of Physiology, Long Island Univ* (1, 1949)
- Schlenk, Fritz, Ph D Science Hall, Iowa State College, Ames *Prof of Bacteriology* (2, 1942)
- Schlesinger, M J , Ph D , M D Beth Israel Hospital, 330 Brookline Ave , Boston, Mass *Dir of Pathology, Asst Prof of Pathology, Harvard Med School* (4, 1942, 6, 1921)
- Schlomovitz, Benjamin H , M D 1210 Majestic Bldg , 231 W Wisconsin Ave , Milwaukee, Wis (1, 1919)
- Schlumberger, Hans G , M D Ohio State Univ School of Medicine, Columbus *Assoc Prof in Pathology* (4, 1945)
- Schmeisser, Harry C , M D Univ of Tennessee, Memphis *Prof of Pathology* (4, 1937)
- Schmidt, Carl F , M D Univ of Pennsylvania Med School, Philadelphia *Prof of Pharmacology* (1, 1929, 3, 1924)
- Schmidt, C Robert, Ph D , M D Hertzler Clinic, Halstead, Kan *Resident Surgeon* (1, 1940)
- Schmidt, Gerhard, M D Boston Dispensary, 25 Bennett St , Boston, Mass *Sr Research Fellow, Tufts College Med School* (2, 1939)
- Schmidt, L H , Ph D Christ Hospital Inst of Med Research, Cincinnati, Ohio *Dir of Research, Assoc Research Prof of Biochemistry, Univ of Cincinnati College of Medicine* (2, 1936, 3, 1946)
- Schmidt-Nielsen, Bodil, D D S Kettering Lab , Univ of Cincinnati, Cincinnati, Ohio *Research Assoc* (1, 1949)
- Schmidt-Nielsen, Knut, Ph D Kettering Lab , Univ of Cincinnati, Cincinnati, Ohio *Asst Prof* (1, 1949)
- Schmitt, Francis Otto, Ph D Dept of Biology, Massachusetts Inst of Technology, Cambridge *Prof of Biology* (1, 1930)
- Schmitt, Otto H , Ph D Univ of Minnesota, Dept of Physics, Minneapolis 14 *Assoc Prof of Zoology and Physics* (1, 1947)

- Schneider, Charles L , M D , Ph D Herman Kiefer Hospital, Detroit 2, Mich *Resident in Obstetrics, College of Medicine, Wayne Univ* (1, 1949)
- Schneider, Edward C , Ph D 25 Gordon Place, Middletown, Conn *Prof Emeritus of Biology, Wesleyan Univ* (1R, 1912, 2, 1912)
- Schneider, Howard A , Ph D Rockefeller Inst for Med Research, 66th Street and York Avenue, New York City 21 *Associate* (5, 1947)
- Schneerson, S Stanley, M D Mount Sinai Hospital, 2 East 100th St , New York City 29 *Assoc Bacteriologist* (6, 1946)
- Schoenbach, Emanuel B , M D Johns Hopkins School of Hygiene, 615 N Wolfe St , Baltimore, Md *Assoc Prof of Preventive Medicine* (6, 1941)
- Schoepfle, Gordon M , Ph D Washington Univ School of Medicine, St Louis, Mo *Asst Prof of Physiology* (1, 1943)
- Scholander, P F , M D , Ph D Swarthmore College, Dept of Zoology, Swarthmore, Pa *Research Biologist* (1, 1947)
- Schradieck, Constant E , M D General Delivery, Germantown, Philadelphia, Pa (6, 1921)
- Schreiner, Oswald, Ph D U S Dept of Agriculture, Bureau of Plant Industry, Washington 25, D C *Chief of Div of Soil Fertility Investigations* (2, 1908)
- Schroeder, E F , Ph D G D Searle & Co , P O Box 5110, Chicago 80, Ill *Research Biochemist* (2, 1938)
- Schroeder, Henry A , M D Dept of Internal Medicine, Hypertension Div , Washington Univ , 640 S Kingshighway, St Louis 10, Mo (1, 1947)
- Schubert, Maxwell, Ph D Dept of Therapeutics, New York Univ College of Medicine, 477 First Ave , New York City 10 *Research Assoc in Therapeutics* (3, 1947)
- Schuck, Cecelia, Ph D Purdue Univ , Dept of Home Economics, Lafayette, Ind *Prof of Nutrition* (5, 1941)
- Schueler, Fred W , Ph D State Univ of Iowa College of Medicine, Dept of Pharmacology, Iowa City *Instructor* (3, 1949)
- Schultz, Edwin William, M D Stanford Univ , 743 Cooksey Lane, Calif *Prof of Bacteriology and Exper Pathology* (4, 1927, 6, 1928)
- Schultz, Fred H , Jr , Ph D Commercial Solvents Corp , Terre Haute, Ind *Research Pharmacologist* (3, 1948)
- Schultze, Max O , Ph D Univ of Minnesota, Div of Agricultural Biochemistry, St Paul 8 *Professor* (2, 1938)
- Schweigert, B S , Ph D Univ of Chicago, American Meat Inst Foundation, 939 E 57th St , Chicago 37, Ill *Asst Prof of Biochemistry, Biochemist, American Meat Inst Foundation* (2, 1949)
- Schweizer, Malvina, Ph D New York Univ , Washington Square College of Arts and Sciences, New York City *Instr in Biology* (1, 1944)
- Schwerma, Henry, Ph D Los Angeles County General Hospital, 1200 N State St , Los Angeles 33, Calif (1, 1948)
- Schwert, George W , Ph D Duke Univ School of Medicine, Dept of Biochemistry, Durham, N C *Asst Prof* (2, 1949)
- Schwimmer, Sigmund, Ph D Enzyme Research Lab , 800 Buchanan St , Albany 6, Calif *Chemist, U S Bureau of Agricultural and Industrial Chemistry* (2, 1947)
- Scott, Charles Covert, Ph D , M D Inlow Clinic, Shelbyville, Ind (3, 1945)
- Scott, David Alymer, Ph D Connaught Labs , Univ of Toronto, Toronto 5, Ontario, Canada *Research Member* (2, 1935)
- Scott, Ernest L , Ph D 64 South St , Bogota, N J *Assoc Prof Emeritus of Physiology, Columbia Univ* (1R, 1914, 2, 1915)
- Scott, Frederick Hughes, Ph D , M B Univ of Minnesota, Minneapolis *Prof Emeritus of Physiology* (1R, 1908, 2, 1909)
- Scott, James K , M D Univ of Rochester School of Medicine and Dentistry, Atomic Energy Project, Rochester 7, N Y *Chief, Pathology Section, AEC, Assoc in Pathology* (4, 1949)
- Scott, John C , Ph D Hahnemann Med College, Philadelphia Pa *Prof and Head of Dept of Physiology* (1, 1936)
- Scott, R W , M A , M D City Hospital, Cleveland, Ohio *Physician in Chief, Prof of Clin Medicine, Western Reserve Univ* (1, 1917, 3, 1917)
- Scott, V Brown, Ph D , M D Inlow Clinic, Shelbyville, Ind *Internist, Div of Medicine* (1, 1941)
- Scott, W W , Ph D , M D Brady Urological Inst , Johns Hopkins Hospital, Baltimore 5, Md (1, 1943)
- Scrimshaw, Nevin S , Ph D , M D Instituto de Nutricion de Centro America y Panama Guatemala City, Guatemala, Central America *Dir of Div of Nutrition, Pan American Sanitary Bureau* (5, 1949)
- Scudi, John V , Ph D Pyridium Corp , Nepera Park, Yonkers 2, N Y *Dir of Research* (2, 1942, 5, 1945)
- Seager, Lloyd D , M S , M D Woman's Med College of Pennsylvania, East Falls, Philadelphia *Prof of Pharmacology and Toxicology* (3, 1939)
- Sealock, Robert R , Ph D Iowa State College, Ames *Assoc Prof of Chemistry* (2, 1940, 5, 1941)
- Seastone, C V , Jr , M D Univ of Wisconsin Med School, Madison *Prof and Chairman of Med Bacteriology* (6, 1939)

- Sebrell, W H , Jr , M D Natl Insts of Health, Bethesda 14, Md *Dir , Exper Biology and Medicine Inst , Professorial Lecturer on Nutrition, George Washington Univ* (2, 1938, 5, 1937)
- Seecof, David P , M D 1970 Daly Ave , Bronx, New York City (4, 1927)
- Seegal, Beatrice Carrie, M D Columbia Univ College of Physicians and Surgeons, 630 W 168th St , New York City 32 *Assoc Prof of Bacteriology* (6, 1949)
- Seegal, David, M D Maimonides Hospital, Brooklyn, N Y *Dir of Med Services* (6, 1930)
- Seegers, Walter H , Ph D Wayne Univ College of Medicine, 1512 St Antoine St , Detroit 26, Mich *Prof of Physiology* (1, 1947, 2, 1941)
- SeEVERS, Maurice Harrison, Ph.D , M D Univ of Michigan, Ann Arbor *Prof of Pharmacology* (1, 1933, 3, 1930)
- Segaloff, Albert, M D Alton Ochsner Med Foundation, 3503 Prytania St , New Orleans, La *Dir of Endocrine Research* (4, 1946)
- Seibert, Florence B , Ph D Univ of Pennsylvania, Henry Phipps Inst , 7th and Lombard Sts , Philadelphia *Assoc Prof of Biochemistry* 2, 1925)
- Seidell, Atherton, Ph D 2301 Connecticut Ave , Washington D C *Special Expert, Natl Insts of Health* (2, 1924)
- Seifter, Joseph, M D Wyeth Inst of Applied Biochemistry, Philadelphia, Pa *Dir of Research* (3, 1940)
- Seifter, Sam, Ph D Long Island College of Medicine, 350 Henry St , Brooklyn 2, N Y *Assoc Prof of Biochemistry* (2, 1946)
- Selkurt, Ewald E , Ph D Western Reserve Univ School of Medicine, Cleveland 6, Ohio *Assoc Prof of Physiology* (1, 1945)
- Selle, Wilber Arthur, Ph D Univ of Texas Med School, Galveston *Prof of Physiology* (1, 1938)
- Sellers, E A , M D , Ph D Univ of Toronto, Dept of Physiology, Toronto, Ontario, Canada *Assoc Prof of Physiology, Assoc Prof in Banting and Best Dept of Med Research* (1, 1947)
- Selye, Hans, M D , Ph D Inst of Exper Medicine and Surgery , Univ of Montreal, Montreal, Quebec, Canada *Prof and Dir* (1, 1934)
- Sendroy, Julius, Jr , Ph D Naval Med Research Inst , Natl Naval Med Center, Bethesda 14, Md *Chief Chemist* (2, 1928)
- Sevag, M G , Ph D Univ of Pennsylvania School of Medicine, Dept of Bacteriology, Philadelphia *Assoc Prof Biochemistry in Bacteriology* (2, 1949, 6, 1941)
- Sevringhaus, Elmer L , M A , M D Hoffmann La Roche, Inc, Nutley 10, N J *Dir of Clin Research, Clin Prof of Medicine, New York Med College* (2, 1923, 5, 1939)
- Shaffer, Morris F , Ph D Tulane Univ School of Medicine, Dept of Bacteriology, New Orleans, La *Professor* (4, 1939, 6, 1937)
- Shaffer, Philip A , Ph D Washington Univ Med School, St Louis 10, Mo *Distinguished Service Prof of Biological Chemistry* (2, 1906, 5, 1935)
- Shanes, Abraham M , Ph D Georgetown Univ School of Medicine, Dept of Physiology and Biophysics, Washington 7, D C (1, 1946)
- Shank, Robert E , M D Washington Univ School of Medicine, St Louis 10, Mo *Prof of Preventive Medicine* (2, 1947)
- Shannon, James A , Ph D , M D Squibb Inst for Med Research, New Brunswick, N J *Director* (1, 1933, 3, 1945)
- Shapiro, Herbert, Ph D Henry Phipps Inst , 7th and Lombard Sts , Philadelphia 47, Pa *Research Assoc* (1, 1937)
- Sharpless, George R , Sc D Lederle Labs , Pearl River, N Y *Research Biochemist* (5, 1942)
- Shaw, J C , Ph D Univ of Maryland, Dept of Dairy Husbandry, College Park, Md *Professor* (1, 1947)
- Shaw, James H , Ph D Harvard School of Dental Medicine, Boston, Mass *Assoc in Nutrition* (5, 1948)
- Shaw, Myrtle, Ph D 11 S Lake Ave Albany, N Y *Sr Bacteriologist, Div of Labs and Research, N Y State Dept of Health* (6, 1937)
- Shay, Harry, M D Temple Univ School of Medicine, Philadelphia, Pa *Dir of Fels Research Inst and Clin Prof of Medicine* (1, 1944)
- Shear, M J , Ph D Natl Cancer Inst , Bethesda, 14, Md *Chief of Chemotherapy Section* (2, 1930)
- Sheard, Charles, Ph D Mayo Foundation, Rochester, Minn *Chief of Div of Physics and Biophysical Research, Prof of Physiological Optics and Biophysics, Univ of Minnesota* (1, 1925)
- Sheehan, Donal, M D , D Sc New York Univ , Bellevue Med Center, 477 First Ave , New York City (1, 1938)
- Shelesnyak, M C , Ph D Johns Hopkins Univ , Arctic Inst of North America, Baltimore, Md (1, 1948)
- Shellev, Walter B , Ph D , M D Hitchcock Clinic, Hanover, N H (1, 1946)
- Shemin, David, Ph D Columbia Univ College of Physicians and Surgeons, 630 W 168th St , New York City 32 *Assoc Prof of Biochemistry* (2, 1944)
- Sheppard, Fay, M S Univ of Oklahoma Med School, Oklahoma City *Instr in Biochemistry* (2, 1936)
- Sherman, Henry C , Ph D Columbia Univ , New York City *Mitchell Prof Emeritus of Chemistry* (1R, 1923, 2, 1906 5, 1933)
- Sherman, William C , Ph D Ralston Purina Co , St Louis, Mo *Head of Biological Labs* (5, 1949)
- Sherwin, Carl Paxson, Sc D , M D , Dr P H 6 Carstensen Rd Scarsdale, N Y *Dir of Metabolic Service, St Vincent's Hospital, Assoc Physician, French Hospital* (2, 1917)

- Sherwood, Noble P , Ph D , M D Univ of Kansas, 517 Snow Hall, Lawrence *Prof of Bacteriology* (6, 1928)
- Sherwood, Thomas Cecil, Ph D , M D 1824 Robert St , New Orleans, La Southern Baptist Hospital *Staff Member, Internal Medicine* (1, 1938)
- Shettles, Landrum B , Ph D , M D Columbia Univ , College of Physicians and Surgeons, Box 330, 622 W 168th St , New York City (1, 1946)
- Shideman, Frederick E , Ph D Univ of Michigan, Dept of Pharmacology, Ann Arbor *Instr in Pharmacology* (3, 1944)
- Shimkin, Michael Boris, M D Univ of California Med School, San Francisco *Dir , Lab of Exper Oncology* (4, 1940)
- Shunowara, George Y , Ph D Ohio State Univ College of Medicine, Dept of Pathology, Columbus *Assoc Prof , Dept of Pathology, Chem Pathologist, Univ Hospital* (2, 1949)
- Shipley, Reginald A , M D Western Reserve Univ School of Medicine, Cleveland 6, Ohio *Asst Prof of Medicine* (1, 1945)
- Shupley, Robert E , M D Lilly Lab for Clin Research, Indianapolis City Hospital, Indianapolis, Ind (1, 1945)
- Shive, William, Ph D Univ of Texas, Austin *Assoc Prof of Chemistry* (2, 1948)
- Shlaer, Simon, Ph D Box 1663, Los Alamos, N M (1, 1938)
- Shock, Nathan W , Ph D Baltimore City Hospitals, Baltimore 24, Md *Chief, Section on Gerontology, USPHS, Natl Insts of Health, Bethesda, Md* (1, 1942)
- Shoemaker, Harold A , Ph D Univ of Oklahoma School of Medicine, 801 E 13th St , Oklahoma City *Asst Dean, Prof of Pharmacology* (3, 1941)
- Shope, Richard E , M D Ridge Road, Kingston N J *Merck Inst for Therapeutic Research* (4, 1934)
- Shorr, Ephraim, M D New York Hospital, 525 E 68th St , New York City *Asst Attending Physician, Asst Prof of Medicine, Cornell Univ Med College* (1, 1931, 3, 1942)
- Shrigley, E W , Ph D , M D Indiana Univ Med Center, Dept of Microbiology, 1040-1232 W Michigan St , Indianapolis 7 (6, 1946)
- Shwartzman, Gregory, M D 230 E 50th St , New York City *Head of Dept of Bacteriology, Mount Sinai Hospital, Prof of Bacteriology, Columbia Univ* (4, 1929, 6, 1930)
- Sichel, F J M , Ph D Univ of Vermont College of Medicine, Burlington *Prof and Chairman of Dept of Physiology and Biophysics* (1, 1939)
- Sickles, Grace M , B A 2201 Twelfth St , Troy, N Y *Assoc Bacteriologist, Div of Labs and Research, New York State Dept of Health* (6, 1932)
- Sickles, Gretchen R , A B New York State Dept of Health, Div of Labs and Research, Albany, N Y *Asst Bacteriologist* (6, 1937)
- Siebenmann, Charles O , D Eng Connaught Med Research Labs , Univ of Toronto, Toronto 5, Ontario, Canada *Research Assoc* (3, 1946)
- Siebert, Walter J , M D Lutheran Hospital, St Louis, Mo *Dir of Labs , Pathologist* (4, 1932)
- Silber, Robert H , Ph D Merck Inst , Rahway, N J *Head, Dept of Biochemistry* (2, 1948)
- Silberberg, Martin, M D Snodgras Lab of Pathology, City Hospital, 1430 Carroll St , St Louis 4, Mo *Instr in Pathology, Washington Univ School of Medicine* (4, 1944)
- Silberberg, Ruth, M D Snodgras Lab of Pathology, City Hospital, 1430 Carroll St , St Louis 4, Mo *Instr in Pathology, Washington Univ School of Medicine* (4, 1944)
- Silvette, Herbert, Ph D Meharry Med College, Dept of Pharmacology, Nashville, Tenn *Visiting Prof of Pharmacology* (1, 1943, 3, 1940)
- Simmonds, Sofia, Ph D Yale Univ , Osborn Botanical Lab , New Haven 11, Conn *Asst Prof of Microbiology* (2, 1948)
- Simonds, James P , Ph D , M D 2033 W Morse Ave , Chicago 45, Ill *Prof Emeritus of Pathology, Northwestern Univ Med School* (4, prior to 1920)
- Simonsen, Daisy G , Ph D Los Angeles County Hospital, 1200 N State St , Los Angeles 33, Calif *Chemist and Toxicologist, Instr in Medicine, Univ of Southern California Med School* (2, 1949)
- Simonson, Ernst, M D Univ of Minnesota, Lab of Physiological Hygiene, Stadium South Tower, Minneapolis 14 *Assoc Prof of Physiology* (1, 1941)
- Simpson, Miriam E , Ph D , M D Univ of California, Div of Anatomy, Berkeley *Prof of Anatomy* (1, 1946)
- Singal, Sam A , Ph D Univ of Georgia School of Medicine, Augusta *Assoc Prof of Biochemistry* (2, 1948)
- Singer, Thomas P , Ph D Western Reserve Univ , Cleveland, Ohio *Asst Prof of Biochemistry* (2, 1948)
- Sizer, Irwin W , Ph D Massachusetts Inst of Technology, Cambridge *Assoc Prof of Physiology* (1, 1944)
- Skinner, John Taylor, Ph D The Grapette Co , Camden, Ark *Chief Chemist* (2, 1946)
- Slaughter, Donald, M D Univ of South Dakota School of Med Sciences, Vermillion *Dean* (3, 1938)
- Smadel, Joseph Edwin, M D Army Med Dept , Dept of Virus and Rickettsial Diseases, Re-

- search and Grad School, Washington 12, D C *Scientific Dir* (4, 1940, 6, 1937)
- Small, James C, M D 101 S 39th St, Philadelphia, Pa *Assoc in Medicine, Univ of Pennsylvania Grad School of Medicine* (4, 1927)
- Smetana, Hans F, M D Armed Forces Inst of Pathology, 7th St and Independence Ave, Washington 25, D C (4, 1934)
- Smith, Arthur H, Ph D Wayne Univ College of Medicine, Detroit 26, Mich *Prof of Physiological Chemistry* (1, 1923, 2, 1921, 5, 1933)
- Smith, Austin Edward, M D American Med Assoc, 535 N Dearborn St, Chicago, Ill *Acting Sec of Council on Pharmacy and Chemistry, Research Assoc, Univ of Chicago, Dept of Pharmacology* (3, 1942)
- Smith, Clarence A, Ph D Standard Brands, Inc, 595 Madison Ave, New York City *Technical Dir, Agricultural Dept* (1, 1921)
- Smith, David T, M D Duke Hospital, Durham, N C *Prof of Bacteriology and Assoc Prof of Medicine* (5, 1943, 6, 1949)
- Smith, Dietrich Conrad, Ph D Univ of Maryland School of Medicine, Lombard and Greene Sts, Baltimore *Assoc Prof of Physiology* (1, 1937)
- Smith, Douglas E, Ph D Argonne Natl Lab, P O Box 5207, Chicago 80, Ill (1, 1947)
- Smith, Edwin L, Ph D Univ of Texas Dental School, Houston *Prof of Physiology* (1, 1948)
- Smith, Elnor Van Dorn, Ph D 5 Middle St, Hadley, Mass *Assoc Prof of Bacteriology, Smith College* (6, 1940)
- Smith, Elizabeth R B, Ph D % Dr, Paul K Smith, 1335 H St, N W, Washington 5, D C (2, 1938)
- Smith, Emul L, Ph D Univ of Utah School of Medicine, Salt Lake City 1 *Assoc Prof of Biochemistry* (2, 1946)
- Smith, Erma A, Ph D, M D 1600 W Maypole St, Chicago 12, Ill (1, 1928)
- Smith, George H, Ph D Yale Univ School of Medicine, New Haven, Conn *Prof of Immunology and Asst Dean, Chairman of Dept of Bacteriology, Yale Univ* (6, 1918)
- Smith, H P, M S, M D Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City 32 *Delafield Prof of Pathology* (1, 1937, 4, 1925)
- Smith, Homer W, Sc D New York Univ, Bellevue Med Center, 477 First Ave, New York City *Prof of Physiology* (1, 1923, 2, 1930)
- Smith, Janice M, Ph D Univ of Illinois, Dept of Home Economics, Urbana *Prof and Chief of Nutrition* (5, 1947)
- Smith, John R, A M, M D Washington Univ School of Medicine, St Louis 10, Mo *Asst Prof of Medicine* (1, 1947)
- Smith, Lawrence Weld, M D 119 E 26th St, New York City 10 (4, 1927)
- Smith, Lee Irvin, Ph D Univ of Minnesota School of Chemistry, Minneapolis 14 *Prof and Chief of Div of Organic Chemistry* (2, 1942)
- Smith, Margaret Cammack, Ph D El Encanto Estates, Tuscon, Ariz (5, 1933)
- Smith, Maurice I, M D Natl Insts of Health, Bethesda 14, Md *Principal Pharmacologist, USPHS* (1, 1920, 3, 1916)
- Smith, Paul K, Ph D George Washington Univ School of Medicine, Dept of Pharmacology, 1335 H St, N W, Washington 5, D C *Prof of Pharmacology and Exec Officer of Dept* (2, 1937, 3, 1937)
- Smith, Paul W, Ph D Univ of Oklahoma School of Medicine, 801 E 13th St, Oklahoma City *Assoc Prof of Pharmacology* (1, 1933)
- Smith, Philip Edward, Ph D Columbia Univ, 630 W 168th St, New York City 32 *Prof of Anatomy* (1, 1923)
- Smith, Ralph G, M D, Ph D Tulane Univ, Station 20, New Orleans, La *Prof of Pharmacology* (3, 1929)
- Smith, R Blackwell, Jr, Ph D Med College of Virginia, Richmond 19 *Lecturer in Pharmacology* (3, 1944)
- Smith, Sedgwick E, Ph D Cornell Univ, Dept of Animal Husbandry, Ithaca, N Y *Animal Physiologist* (5, 1945)
- Smith, Susan Gower, M A Duke Univ, Durham, N C *Assoc in Dept of Medicine and Nutrition, School of Medicine* (5, 1939)
- Smith, Wilbur Kenneth, M D Univ of Rochester School of Medicine and Dentistry, Rochester, N Y *Assoc. Prof of Anatomy* (1, 1939)
- Smith, Willie W, Ph D Natl Insts of Health, Bethesda, Md *Physiologist* (1, 1941)
- Smithburn, Kenneth C, M D Rockefeller Foundation, 49 W 49th St, New York City *Staff Member, International Health Div* (6, 1937)
- Smolens, Joseph, B S Wyeth Research Inst, 900 N Broad St, Philadelphia, Pa *Head of Dept of Immunology* (6, 1943)
- Smythe, C V, Ph D 5000 Richmond St, Philadelphia, Pa *Head of Biochemistry, Rohm & Haas Co* (2, 1934)
- Snape, William J, M D Jefferson Med College, Philadelphia, Pa *Assoc in Physiology* (1, 1948)
- Snell, Albert M, M D Mayo Clinic, Rochester, Minn *Head of Section on Medicine, Prof in Medicine, Mayo Foundation Grad School, Univ of Minnesota* (4, 1930)
- Snell, Esmond E, Ph D Univ of Wisconsin, Madison 6 *Prof of Biochemistry* (2, 1942, 5, 1946)
- Snider, Ray S, Ph D Northwestern Univ School of Medicine, 303 E Chicago Ave, Chicago 11, Ill *Assoc Prof of Anatomy* (1, 1949)
- Snyder, Franklin Faust, M D Boston Lying-In Hospital, Boston, Mass (1, 1936)

- Sobel, Albert E**, Ph D Jewish Hospital of Brooklyn, Prospect Place and Classon Ave, Brooklyn, N Y *Head of Dept of Chemistry, Adjunct Prof of Chemistry, Polytechnic Inst of Brooklyn* (2, 1939)
- Sobin, Sidney S**, M D, Ph D 405 N Bedford Drive, Beverly Hills, Calif *Research Assoc in Physiology, Univ of Southern California* (1, 1949)
- Sobotka, Harry H**, Ph D Mount Sinai Hospital, Fifth Ave and 100th St, New York City 29 *Head of Dept of Chemistry* (2, 1932, 5, 1933)
- Solandt, Donald Young**, M D, Ph D Univ of Toronto, Toronto, Ontario, Canada *Prof of Physiology in charge of Biophysics, Faculty of Medicine, Prof and Head of Dept of Hygiene, School of Hygiene* (1, 1937)
- Sollmann, Torald**, M D 14327 Superior Rd, Cleveland, Ohio *Dean and Prof Emeritus of Pharmacology, Western Reserve Univ* (1R, 1902, 2, 1906, 3, 1908)
- Solotorovsky, Morris**, Ph D 203 W 5th St, Plainfield, N J *Merck Inst for Therapeutic Research, Rahway, N J, Research Assoc in Chemotherapy* (6, 1946)
- Somogyi, Michael**, Ph D 216 S Kingshighway, St Louis, Mo *Biochemist, Jewish Hospital of St Louis* (2, 1927)
- Soskin, Samuel**, M D, Ph D Michael Reese Hospital, 29th St and Ellis Ave, Chicago 16, Ill *Med Dir and Dir of Research Inst, Dean, Postgrad School* (1, 1930, 5, 1933)
- Soule, Malcolm H**, Sc D Univ of Michigan, Ann Arbor *Prof of Bacteriology, and Chairman of Dept of Bacteriology* (4, 1927, 6, 1925)
- Spain, Will C**, M D 116 E 53rd St, New York City *Clin Prof of Medicine, Post-Grad Med School, Columbia Univ* (6, 1923)
- Spealman, C R**, Ph D Dept of Commerce, Civil Aeronautics Admin, Washington 25, D C (1, 1940)
- Specht, Heinz**, Ph D Natl Insts of Health, Bethesda, Md *Scientist, USPHS* (1, 1941)
- Speirs, Mary**, Ph D Georgia Agricultural Experiment Station, Experiment *Head of Dept of Home Economics* (5, 1949)
- Sperry, Roger W**, Ph D Univ of Chicago, Dept of Anatomy, Chicago 37, Ill (1, 1945)
- Sperry, Warren M**, Ph D New York State Psychiatric Inst, 722 W 168th St, New York City 32 *Principal Research Biochemist, Assoc Prof of Biological Chemistry, Columbia Univ* (2, 1929, 5, 1933)
- Spiegel, Ernest A**, M D Temple Univ School of Medicine, Broad and Ontario Sts, Philadelphia, Pa *Prof of Exper Neurology* (1, 1938)
- Spiegel-Adolf, Mona**, M D Temple Univ School of Medicine, Broad and Ontario Sts, Philadelphia, Pa *Prof and Head of Dept of Colloid Chemistry* (2, 1933)
- Spiegelman, Sol**, Ph D Univ of Minnesota Med School, Dept of Physiology, Minneapolis 14 (1, 1946)
- Spies, Tom D**, M D Hillman Hospital, Birmingham, Ala *Dir of Nutrition Clinic, Prof of Nutrition and Metabolism, Northwestern Univ Med School* (3, 1941, 4, 1940, 5, 1938)
- Spink, Wesley W**, M D Univ of Minnesota Hospital, Minneapolis *Assoc Prof of Medicine, Univ of Minnesota Med School* (6, 1940)
- Spohn, Adelaide**, Ph D Elizabeth McCormick Memorial Fund, 848 N Dearborn St, Chicago, Ill *Nutritionist* (5, 1933)
- Spoor, Herbert J**, Ph D, M D Bristol Myers Co, New York City *Asst Dir of Med Div* (1, 1945)
- Sprent, J F A**, Ph D Univ of Toronto, 43 Queens Park, Toronto 5, Ontario, Canada *Asst Prof, Sr Research Fellow, Ontario Research Foundation* (6, 1949)
- Sprinson, David B**, Ph D Columbia Univ College of Physicians and Surgeons, Dept of Biochemistry, 630 W 168th St, New York City 32 *Research Assoc* (2, 1949)
- Sproul, Edith E**, M D Columbia Univ College of Physicians and Surgeons, Dept of Pathology, 630 W 168th St, New York City 32 (4, 1941)
- Sprunt, Douglas H**, M D, M S Univ of Tennessee, Memphis *Prof of Pathology, Head of Dept of Pathology and Bacteriology* (4, 1934, 6, 1936)
- Stacy, Ralph W**, Ph D Ohio State Univ, Dept of Physiology, Hamilton Hall, Columbus 10 *Asst Prof* (1, 1949)
- Stadie, William C**, M D 821 Maloney Clinic, 36th and Spruce Sts, Philadelphia 4, Pa *John Herr Musser Prof of Research Medicine, Univ of Pennsylvania* (2, 1922)
- Stainsby, Wendell J**, M D Geisinger Memorial Hospital, Danville, Pa *Chief Physician* (6, 1930)
- Stanley, Wendell M**, Ph D Univ of California, Berkeley 4 *Prof and Chairman of Div of Biochemistry, Dir of Virus Lab* (2, 1936)
- Stannard, James Newell**, Ph D Univ of Rochester Med School, Atomic Energy Project, Rochester 7, N Y *Assoc Prof of Radiation Biology* (1, 1938)
- Stare, Fredrick J**, Ph D, M D 695 Huntington Ave, Boston 15, Mass *Prof of Nutrition, Harvard Univ* (2, 1937, 5, 1942)
- Starr, Isaac**, M D Hospital of the Univ of Pennsylvania, Philadelphia *Hartzell Prof of Research Therapeutics* (1, 1929, 3, 1942)

- Stavraky, George W , M D Univ of Western Ontario, Med School, London, Ontario, Canada *Assoc Prof of Physiology* (1, 1937, 3, 1944)
- Stead, Eugene A , Jr , M D Duke Univ , Dept of Medicine, Durham, N C (1, 1945)
- Stearns, Genevieve, Ph D State Univ of Iowa, College of Medicine, Iowa City *Research Prof of Pediatrics* (2, 1932, 5, 1937)
- Steel, Matthew, Ph D Long Island College of Medicine, 350 Henry St , Brooklyn, N Y *Prof of Biological Chemistry* (2, 1909)
- Steele, J Murray, M D Thurd (N Y U) Research Div , Goldwater Memorial Hospital, Welfare Island, New York City *Assoc Prof of Medicine, New York Univ , Dir of 3rd Med Div , Welfare Hospital* (1, 1936)
- Steenbock, Harry, Ph D Univ of Wisconsin, Madison *Prof of Biochemistry* (2, 1912, 5, 1933)
- Steggerda, F R , Ph D Univ of Illinois, 416 Natural History Bldg , Urbana *Assoc Prof of Physiology* (1, 1934)
- Stehle, Raymond Louis, Ph D McGill Univ , Faculty of Medicine, Montreal Quebec, Canada *Prof of Pharmacology* (2, 1920, 3, 1922)
- Steigman, Alex J , M S , M D Natl Foundation for Infantile Paralysis, 120 Broadway, New York City 5 (6, 1949)
- Steigmann, Frederick, M S , M D 348 S Hamlin Ave , Chicago, Ill *Assoc in Medicine, Univ of Illinois, College of Medicine, Assoc Attending Physician, Cook County Hospital* (3, 1942)
- Steiman, S E , Ph D , M D 23 Broad St , Lynn, Mass *Asst Physician, Metropolitan State Hospital, Waltham, Mass* (1, 1939)
- Stein, George J , Ph D 406th Med General Lab APO 500 %P M , San Francisco, Calif (6, 1947)
- Stein, William Howard, Ph D Rockefeller Inst for Med Research, 66th and York Ave , New York City 21 *Assoc in Chemistry* (2, 1946)
- Steinbach, H Burr, Ph D Univ of Minnesota, Dept of Zoology, Minneapolis (1, 1934)
- Steinberg, Bernhard, M D Toledo Hospital Inst of Med Research, Toledo, Ohio *Dir of Toledo Hospital Inst , Chief Pathologist and Dir of Labs , Toledo Hospital* (4, 1928, 6, 1946)
- Steiner, Paul E , M D Univ of Chicago, Chicago, Ill *Prof of Pathology* (4, 1939)
- Steinhardt, Jacinto, Ph D 1548 East-West Highway, Silver Spring, Md *Dir , Operations Evaluation Group, Mass Inst of Technology* (2, 1939)
- Steinhaus, Arthur H , Ph D 5315 Drexel Ave , Chicago, Ill *Prof of Physiology, George Williams College, Hyde Park* (1, 1928)
- Stekol, Jakob A , D Sc Lankenau Hospital Research Inst , Philadelphia 30, Pa *Assoc Member* (2, 1936)
- Stern, Kurt G , Ph D Polytechnic Inst of Brooklyn, 99 Livingston St , Brooklyn 2, N Y *Adjunct Prof of Biochemistry* (2, 1938)
- Stetten, DeWitt, Jr , M D , Ph D Harvard Med School, 25 Shattuck St , Boston 15, Mass *Chief, Div of Nutrition and Physiology* (2, 1944)
- Stetten, Marjorie R , Ph D Harvard Med School, 25 Shattuck St , Boston 15, Mass *Assoc , Div of Nutrition and Physiology* (2, 1947)
- Stevens, S Smith, Ph D Harvard Univ , Memorial Hall, Cambridge, Mass *Prof of Psychology, Div of Psychological Labs* (1, 1937)
- Stewart, Dorothy R , Ph D Rockford College, Rockford, Ill (1, 1947)
- Stewart, Fred W , M D Memorial Hospital, 444 E 68th St , New York City *Pathologist, Prof of Pathology, Cornell Med School* (4, 1928)
- Stewart, Harold L , M D Natl Cancer Inst , Bethesda, Md *Chief of Pathology Section* (4, 1936)
- Stewart, Winifred Bayard, M D , M A 1930 Spruce St , Philadelphia, Pa *Prof of Neurology, Woman's Med College of Pennsylvania* (1, 1941)
- Stickney, J Clifford, Ph D West Virginia Univ School of Medicine Morgantown *Assoc Prof of Physiology* (1, 1944)
- Stiebeling, Hazel K , Ph D U S Dept of Agriculture Washington, D C *Chief, Bureau of Human Nutrition and Home Economics* (5, 1933)
- Stier, Theodore J B , Ph D Indiana Univ Med School, Bloomington *Assoc Prof of Physiology* (1, 1938)
- Still, Eugene U , Ph D 820 So Orange, Sarasota, Fla (1, 1929)
- Stillman, Ernest G , M D 45 E 75th St , New York City (6, 1930)
- Stimmel, Benjamin F , Ph D Rees-Stealy Med Research Fund, Ltd , 2001 Fourth Ave , San Diego, Calif *Research Biochemist* (2, 1947)
- Stock, Aaron H , M D Univ of Pittsburgh School of Medicine, Pittsburgh, Pa *Asst Prof of Bacteriology and Immunology* (6, 1947)
- Stockton, Andrew Benton, M D 655 Sutter St , San Francisco, Calif (3, 1931)
- Stoerk, Herbert C , M D Merck Inst for Therapeutic Research, Rahway, N J *Head of Dept of Cancer Research* (4, 1948)
- Stoesser, Albert V , M D , Ph D Univ of Minnesota Med School, 1409 Willow St , Loring Park, Minneapolis *Clin Assoc Prof of Pediatrics* (6, 1949)
- Stohlman, Edward F , LL B Natl Insts of Health, Bethesda, Md *Assoc Pharmacologist* (3, 1948)
- Stokinger, Herbert B , Ph D 250 Meigs St , Rochester, N Y *Assoc Prof of Pharmacology and Toxicology* (6, 1947)
- Stokstad, E L Robert, Ph D Lederle Labs , Pearl River, N Y *Chemist* (2, 1947, 5, 1942)
- Stoland, O O , Ph D 1845 Learnard Ave , Lawrence, Kan *Prof of Physiology and Pharmacology, Univ of Kansas* (1, 1913)

- Stone, William E, Ph D Univ of Wisconsin, Dept of Physiology, Madison Asst Prof of Physiology (1, 1945)
- Stormont, Robert T, Ph D, M D Food and Drug Admin, Washington, D C Med Dir, Professional Lecturer in Pharmacology, Georgetown Univ Med School (3, 1941)
- Storvick, Clara A, Ph D Oregon State College, School of Home Economics, Corvallis, Ore Assoc Prof of Foods and Nutrition (5, 1947)
- Stotz, Elmer, H, Ph D Univ of Rochester School of Medicine and Dentistry, Rochester 7, N Y Prof of Biochemistry (2, 1939)
- Stoughton, Roger W, Ph D Mallinckrodt Chemical Works, 3600 N Second St, St Louis, Mo Research Chemist (3, 1939)
- Strong, Frank M, Ph D Univ of Wisconsin, Dept of Biochemistry, Madison 6 Prof of Biochemistry (2, 1941)
- Struck, Harold Carl, Ph D Creighton Univ School of Medicine, 302 N 14th St, Omaha 2, Nebr (1, 1940)
- Stuart, Charles A, Ph D 372 Lloyd Ave, Providence, R I Assoc Prof of Biology, Brown Univ (6, 1935)
- Sturgis, Cyrus Cressey, M D Simpson Memorial Inst, Ann Arbor, Mich Dir of Thomas Henry Simpson Memorial Inst for Med Research, Chairman, Dept of Medicine, Univ of Michigan Hospital (4, 1927)
- Sturkie, Paul D, Ph D Rutgers Univ, New Brunswick, N J Assoc Research Specialist in Poultry Husbandry, Assoc Prof of Poultry Husbandry (1, 1948)
- Stutzman, Jacob W, Ph D, M D Boston Univ School of Medicine, Boston, Mass Assoc Prof of Pharmacology (1, 1946, 3, 1948)
- Sugg, John Y, Ph D Cornell Univ Med College, 1300 York Ave, New York City Assoc Prof of Bacteriology and Immunology (6, 1938)
- Sulkin, S Edward, Ph D Southwestern Med College, Dallas, Tex Prof and Chairman of Dept of Bacteriology and Immunology (6, 1944)
- Sullivan, Michael Xavier, Ph D Chemo-Medical Research Inst, Georgetown Univ, 37th & O Sts, N W, Washington, D C Dir and Research Prof of Chemistry (2, 1909)
- Sulzberger, Marion B, M D 999 5th Ave, New York City Assoc Clin Prof of Dermatology and Syphilology, N Y Post-Grad Med School of Columbia Univ, Assoc Dir of Skin and Cancer Unit of N Y Post-Grad Hospital (6, 1936)
- Summerson, William H, Ph D Med Div, Army Chemical Center, Md Chief, Biochemistry Section (2, 1942)
- Sumner, J B, Ph D Cornell Univ, Ithaca, N Y Dir, Enzyme Chemistry Lab (2, 1919)
- Sunderman, F William, M D, Ph D Univ of Texas, M D Anderson Hospital, 2310 Baldwin St, Houston 6 Prof of Exper Medicine and Clin Pathology, Dir of Clin Research (2, 1931)
- Sundstroem, Edward S, M D Univ of California, Berkeley 4 Prof Emeritus of Biochemistry (2, 1919)
- Sure, Barnett, Ph D Univ of Arkansas, Fayetteville Head of Dept and Prof of Agricultural Chemistry (2, 1923, 5, 1933)
- Sutherland, George F, M D Duke Univ School of Medicine, Durham, N C (1, 1939)
- Sutton, T Scott, Ph D Ohio State Univ, Columbus Prof, Dir of Inst of Nutrition and Food Technology (5, 1936)
- Svirbely, Joseph L, Ph D Mound Lab, Monsanto Chemical Co, Miamisburg, Ohio Pharmacologist (3, 1945)
- Swain, Robert E, Ph D 634 Mirada Ave, Stanford Univ, Calif Prof Emeritus of Chemistry (2, 1909)
- Swann, Howard G, Ph D Univ of Texas Med School, Dept of Physiology, Galveston Asst Prof of Physiology (1, 1940)
- Swanson, Pearl P, Ph D Iowa State College, Ames Prof of Foods and Nutrition, Dept of Foods and Nutrition (5, 1933)
- Swanson, William W, M S, M D 2376 E 71st St, Chicago, Ill Asst Prof of Pediatrics, Northwestern Univ (2, 1938)
- Sweeney, H Morrow, Ph D Aero-Med Lab, Wright Field, Dayton, Ohio (1, 1939)
- Swift, Homer, M D, D Sc 888 Park Ave, New York City Member, Rockefeller Inst for Med Research, Physician to Hospital of Rockefeller Inst for Med Research (6, 1920)
- Swift, Raymond W, Ph D Pennsylvania State College, State College Prof of Dept of Animal Nutrition (5, 1934)
- Swingle, Wilbur Willis, Ph D Princeton Univ, Princeton, N J Prof of Biology (1, 1924)
- Swinyard, Ewart A, Ph D Univ of Utah, Salt Lake City Prof of Pharmacy, School of Pharmacy, Lecturer in Pharmacology, School of Medicine (3, 1948)
- Sydenstricker, V P, M D Univ of Georgia School of Medicine, Augusta Prof of Medicine (5, 1944)
- Sykes, Joseph F, Ph D U S Dept of Agriculture, Bureau of Dairy Industry, Beltsville, Md Physiologist (1, 1942)
- Syvertson, Jerome T, M D Univ of Minnesota, Minneapolis, Minn Prof and Head, Dept of Bacteriology and Immunology (4, 1940, 6, 1947)
- Szego, Clara M, Ph D Univ of California, Med School, Los Angeles Asst Clin Prof of Biophysics (1, 1946)
- Szent-Gyorgyi, Albert, Ph D, M D Natl Insts of Health, Bethesda 14, Md (2, 1949)

- Szepsenwol, Josel, M D Emory Univ School of Medicine, Emory University, Ga *Asst Prof of Anatomy* (1, 1948)
- Tabor, Herbert, M D Natl Insts of Health, Pharmacology Section, Bethesda 14, Md *Sr Asst Surgeon, USPHS* (3, 1947)
- Tager, Morris Western Reserve Univ, Cleveland, Ohio *Dept of Microbiology* (6, 1948)
- Tainter, M L, M A, M D Sterling-Winthrop Research Inst, 33 Riverside Ave, Rensselaer, N Y *Director* (1, 1929, 3, 1927)
- Talbot, Samuel Armstrong, Ph D Johns Hopkins Hospital Baltimore, Md *Instr in Physiological Optics, Johns Hopkins Univ* (1, 1940)
- Tahaferro, William H, Ph D Univ of Chicago, Dept of Bacteriology, Chicago, Ill *Ehakim H Moore Distinguished Service Prof of Parasitology and Dean of Div of Biological Sciences* (6, 1930)
- Tannenbaum, Albert, M D Michael Reese Hospital, 29th St & Ellis Ave, Chicago, Ill *Dir of Dept of Cancer Research* (4, 1942)
- Tarver, Harold, Ph D Univ of California, Div of Biochemistry, Berkeley 4 *Asst Prof of Biochemistry* (2, 1947)
- Tashiro, Shiro, Ph D, M D Univ of Cincinnati College of Medicine, Cincinnati, Ohio *Prof of Biochemistry* (1, 1913, 2, 1913)
- Tatum, Arthur L, Ph D, M D Service Memorial Inst, Univ of Wisconsin, Madison *Prof of Pharmacology* (1, 1913, 3, 1919)
- Tatum, Edward L, Ph D Stanford Univ, School of Biological Sciences, Stanford University, Calif *Prof of Biology* (2, 1947)
- Tauber, Henry, Ph D V D Research Lab, U S Marine Hospital, Staten Island, N Y *Biochemist, USPHS* (2, 1933)
- Taylor, A N, Ph D Univ of Oklahoma School of Medicine, Oklahoma City *Asst Prof and Chairman of Dept of Physiology* (1, 1948)
- Taylor, Alton R, Ph D Parke, Davis & Co, Detroit 32, Mich *Sr Researcher, Research Div* (2, 1947, 6, 1943)
- Taylor, Craig L, Ph D Univ of California, Dept of Engineering, Los Angeles *Assoc Prof of Engineering* (1, 1945)
- Taylor, Fred A, Ph D 320 E North Ave, N S, Pittsburgh, Pa *Biochemist, Singer Memorial Lab* (2, 1933)
- Taylor, Haywood M, Ph D Duke Univ School of Medicine, Durham, N C *Assoc Prof of Biochemistry and Toxicology, Biochemist and Toxicologist to Duke Hospital* (4, 1942)
- Taylor, Henry Longstreet, Ph D Univ of Minnesota, School of Public Health, Minneapolis *Asst Prof of Physiological Hygiene* (1, 1944)
- Taylor, John Fuller, Ph D Washington Univ School of Medicine, Euclid and Kingshighway St Louis, Mo *Asst Prof of Biological Chemistry* (2, 1944)
- Taylor, M Wight, Ph D New Jersey Agricultural Exper Station, New Brunswick *Assoc Biochemist in Nutrition and Assoc Prof of Agricultural Biochemistry, Rutgers Univ* (5, 1944)
- Taylor, Norman Burke, M D Univ of Toronto, Toronto 5, Ontario, Canada *Prof of Physiology* (1, 1922)
- Taylor, Richard M, Rockefeller Foundation, 66th St and York Ave, New York City (6, 1949)
- Taylor, Robert D, M D Clinical Research Div, Cleveland Foundation, Cleveland 6, Ohio *Member* (1, 1945)
- Teague, Robert S, Ph D, M D Medical College of Alabama, Dept of Pharmacology and Physiology, Birmingham 5 *Assoc Prof of Physiology and Pharmacology* (3, 1942)
- Templeton, Roy D, B S 5630 South Flores, San Antonio, Tex (1, 1935)
- Ten Broeck, Carl, M D Rockefeller Inst for Med Research, Dept of Animal and Plant Pathology, Princeton, N J *Director* (4R, 1932, 6, 1924)
- Tepperman, Jay, M D Syracuse Univ School of Medicine, Dept of Pharmacology, Syracuse, N Y *Assoc Prof of Pharmacology* (1, 1944)
- Terplan, Kornel L, M D Univ of Buffalo School of Medicine, Buffalo, N Y *Prof of Pathology* (4, 1935)
- Terry, Roger, M D Univ of Rochester School of Medicine and Dentistry, Dept of Pathology, Rochester 7, N Y *Instr in Pathology* (4, 1949)
- Thannhauser, S J, M D, Ph D Pratt Diagnostic Hospital, 30 Bennet St, Boston, Mass *Prof of Clin Medicine, Tufts Med School, Assoc Chief of Pratt Diagnostic Hospital* (2, 1937)
- Thatcher, Jonathan S, Ph D Ohio State Univ, Dept of Physiology, Hamilton Hall, Columbus 10 *Instructor* (1, 1949)
- Thauer, Rudolf, M D 3226 Fuller St, Philadelphia 36, Pa *Physiologist, Research Worl in N A M C U S Navy* (1, 1949)
- Thayer, Sidney Allen, Ph D 1402 S Grand Blvd, St Louis 4, Mo *Assoc Prof of Biochemistry, St Louis Univ School of Medicine* (2, 1933)
- Thienes, Clinton H, M D, Ph D Univ of Southern California School of Medicine, Los Angeles *Prof of Pharmacology* (3, 1928)
- Thomas, Arthur W, Ph D Columbia Univ, New York City 27 *Prof of Chemistry* (2, 1924)
- Thomas, Byron H, Ph D Iowa State College, Ames *Prof and Head of Animal Chemistry and Nutrition, Iowa Agric Exper Station* (5, 1933)
- Thomas, Caroline Bedell, M D Johns Hopkins Hospital, Baltimore, Md *Assoc Prof of Medicine, Johns Hopkins Univ School of Medicine* (1, 1939)

- Thomas, J Earl**, M D Jefferson Med College, Philadelphia, Pa *Prof of Physiology* (1, 1922, 3, 1924)
- Thompson, Marvin R**, Ph D 67 Greenwich Ave, Stamford, Conn (3, 1944)
- Thompson, Randall L**, Sc D, M D Indiana Univ, Med Center, Indianapolis 7 *Prof of Bacteriology* (6, 1937)
- Thompson, William R**, Ph D 1 Darrock Rd, Delmar, N Y *Sr Biochemist, Div of Labs and Research, New York State Dept of Health* (2, 1934)
- Thomson, David Landsborough**, Ph D McGill Univ, Montreal, Quebec, Canada *Prof of Biochemistry and Dean of Faculty of Grad Studies and Research* (2, 1929)
- Thorn, George Widmer**, M D Peter Bent Brigham Hospital, Boston, Mass *Prof of Medicine, Harvard Univ* (1, 1939)
- Thorp, W T S**, D V M Natl Insts of Health, Bethesda, Md *Veterinary Pathologist* (4, 1948)
- Tidwell, Herbert C**, Ph D Southwestern Med College, 2211 Oak Lawn Ave, Dallas, Tex *Prof and Chairman of Dept of Biochemistry* (2, 1948)
- Tillett, William S**, M D New York Univ College of Medicine, Dept of Bacteriology, 477 First Ave, New York City *Prof of Medicine* (6, 1927)
- Tilt, Jennie**, Ph D Florida State Univ, Tallahassee *Prof of Physiological Chemistry and Nutrition* (5, 1937)
- Tipson, R Stuart**, Ph D Mellon Inst of Industrial Research, Univ of Pittsburgh, Pittsburgh, Pa *Sr Fellow, Dept of Research in Pure Chemistry* (2, 1937)
- Tipton, Samuel R**, Ph D Univ of Tennessee, Dept of Zoology, Knoxville *Prof of Zoology* (1, 1940)
- Tislow, Richard**, M D Schering Corp, Bloomfield, N J *Dir of Biological Labs* (1, 1944)
- Titus, Harry W**, Ph D Lime Crest Research Lab, R F D 1, Newton, N J *Technical Counsellor and Dir of Nutritional Research* (2, 1929, 5, 1933)
- Tobias, Julian M**, M D Univ of Chicago, Chicago, Ill *Instr in Physiology* (1, 1944)
- Tocantins, Leandro Maués**, M D Jefferson Med College, Philadelphia, Pa *Assoc Prof of Medicine* (1, 1939)
- Todd, Wilbert R**, Ph D Univ of Oregon Med School, Portland, Ore *Assoc Prof of Biochemistry* (2, 1948, 5, 1948)
- Todhunter, Elizabeth Neige**, Ph D Univ of Alabama, University *Prof of Nutrition* (5, 1939)
- Toennies, Gerrit**, Ph D Lankenau Hospital, Philadelphia 30, Pa *Sr Member, Inst for Cancer Research* (2, 1934)
- Tolle, Chester D**, Ph D Federal Security Agency, Food and Drug Admin, Washington, D C *Sr Biochemist* (5, 1942)
- Toman, James E P**, Ph D Univ of Utah School of Medicine, Dept of Pharmacology and Physiology, Salt Lake City (1, 1945)
- Tomlinson, Wray Joseph**, M D Fort Logan Veterans Hospital, Denver, Colo *Chief of Labs, Asst Prof of Pathology, Univ of Colorado School of Medicine* (4, 1945)
- Tompkins, Edna H**, M D Yale Univ, Lab of Applied Physiology, 52 Hillhouse Ave, New Haven, Conn *Research Assoc, Assoc Prof* (4, 1941)
- Toomey, John A**, LL B City Hospital, Div of Contagious Diseases, 3395 Scranton Rd, Cleveland 9, Ohio *Prof of Pediatrics, Western Reserve Univ School of Medicine* (6, 1943)
- Torda, Clara**, Ph D, M D Cornell Univ Med College, New York City *Research Fellow in Pharmacology* (1, 1943, 3, 1944)
- Toth, Louis A**, Ph D Louisiana State Univ School of Medicine, Dept of Physiology, New Orleans 13 *Assoc Prof of Physiology* (1, 1940)
- Totter, John R**, Ph D Univ of Arkansas School of Medicine, Little Rock *Assoc Prof, Dept of Biochemistry* (2, 1946)
- Tourtellotte, Dee**, D Sc Charles B Knox Gelatin Co, 4th and Erie Sts, Camden, N J *Head of Nutrition Lab* (5, 1935)
- Tower, Sarah Sheldon**, M D, Ph D Johns Hopkins Univ, Baltimore, Md *Instr in Psychiatry* (1, 1932)
- Trager, William**, M D, Ph D Rockefeller Inst for Med Research, Dept of Animal and Plant Pathology, Princeton, N J *Associate* (4, 1947)
- Traub, Frederick B**, M D 205 E 82nd St, New York City 28 *Assoc Bacteriologist, Jewish Hospital of Brooklyn* (6, 1946)
- Travell, Janet**, M D Cornell Univ Med College, New York City *Asst Prof of Clin Pharmacology* (3, 1933)
- Travis, Lee Edward**, Ph D Univ of Southern California, Los Angeles *Prof of Psychology and Dir of Psychological Center, Major, YAAF (Yuma, Ariz)* (1, 1929)
- Treadwell, Carleton R**, Ph D George Washington Univ School of Medicine, Dept of Biochemistry, 1335 H St, N W, Washington, D C *Assoc Prof of Biochemistry* (2, 1944, 5, 1949)
- Treffers, Henry P**, Ph D Yale Med School, Dept of Immunology, New Haven, Conn *Assoc Prof of Immuno-chemistry* (6, 1942)
- Trimble, Harry C**, Ph D Harvard Med School, 25 Shattuck St, Boston, Mass *Asst Prof of Biological Chemistry* (2, 1929, 5, 1936)
- Tuft, Louis H**, M D 1530 Locust St, Philadelphia, Pa *Asst Prof of Medicine, Temple Univ Med School, Chief of Clinic of Allergy and Applied Immunology, Temple Univ Hospital* (6, 1928)
- tum Suden, Caroline**, Ph D % J L Wolff, 38 Crane Rd, Scarsdale, N Y (1, 1936)

- Tunturi, Archie Robert, Ph D Univ of Oregon Med School, Portland Asst Prof of Anatomy (1, 1946)
- Tuohy, Edward B, M S, M D Mayo Clinic, Rochester, Minn Asst Prof of Anesthesiology, Mayo Foundation (3, 1941)
- Turner, Abby H, Ph D Mount Holyoke College, South Hadley, Mass Prof of Physiology (1, 1928)
- Tuttle, Waid Wright, Ph D State Univ of Iowa, Iowa City Prof of Physiology (1, 1925)
- Tweedy, Wilbur R, Ph D Veterans Admin Hospital, Radioisotope Unit, Hines, Ill Assoc Director (2, 1931)
- Tyler, Albert, Ph D California Inst of Technology, Pasadena, Calif Assoc Prof of Embryology (6, 1946)
- Tyler, David B, Ph D Carnegie Inst of Washington, Dept of Embryology, Wolfe and Madison Sts, Baltimore 5, Md Member of Staff (1, 1943)
- Umbreit, Wayne W, Ph D Merck Inst for Therapeutic Research, Rahway, N J Head of Dept of Enzyme Chemistry (2, 1947)
- Unna, Klaus R W, M D Univ of Illinois College of Medicine, 1853 W Polk St, Chicago 12, Ill Assoc Prof, Dept of Pharmacology (1, 1941, 3, 1944, 5, 1942)
- Upton, Morgan, Ph D Rutgers Univ, Dept of Psychology, New Brunswick, N J (1, 1934)
- Urban, Frank, Ph D, M D 302 Northern Bldg, Green Bay, Wis (2, 1932)
- Utter, Merton F, Ph D Western Reserve Univ, Dept of Biochemistry, Cleveland, Ohio Assoc Prof of Physiological Chemistry (2, 1946)
- Vahlteich, Ella McCollum, Ph D 310 Walnut St, Englewood, N J (5, 1933)
- Valle, J R, M D Escola Paulista de Medicina, Caixa Postal 144-A, Sao Paulo, Brazil Prof of Pharmacology (3, 1947)
- Vanderscheer, James, Ch E 136 Linwood Ave, Ridgewood, N J Research Chemist, Lederle Labs (6, 1946)
- Van Dyke, H B, Ph D, M D Columbia Univ College of Physicians and Surgeons, 630 W 168th St New York City 32 Hosack Prof of Pharmacology (1, 1925, 3, 1927)
- van Harreveld, Anthonie, M A, M D California Inst of Technology, Pasadena Assoc Prof of Physiology (1, 1941)
- Van Liere, Edward J, M D, Ph D West Virginia Univ School of Medicine, Morgantown Prof of Physiology and Dean (1, 1927)
- Van Middlesworth, Lester, Ph D Univ of Tennessee, Memphis Instr in Physiology (1, 1948)
- Van Slyke, Donald D, Ph D, M D Brookhaven Natl Lab, Upton, L I, N Y Asst Dir in Charge of Depts of Biology and Med Sciences, Member Emeritus, Rockefeller Inst (2, 1908)
- van Wagenen, Gertrude, Ph D Yale Univ School of Medicine, New Haven, Conn Assoc Prof (1, 1932)
- van Wagtendonk, Willem J, Ph D Indiana Univ Dept of Zoology, Bloomington Assoc Prof (2, 1946)
- Van Winkle, Walton, Jr, M D American Med Assoc, 535 N Dearborn St, Chicago 10, Ill (3, 1939)
- Varney, Philip L Washington Univ School of Medicine, St Louis, Mo Asst Prof of Bacteriology (6, 1948)
- Vars, Harry M, Ph D Univ of Pennsylvania Med School, Harrison Dept of Surgical Research, Philadelphia Assoc Prof of Physiological Chemistry (2, 1935, 5, 1935)
- Velick, Sidney Frederick Washington Univ School of Medicine, Dept of Biochemistry, Euclid Ave and Kingshighway, St Louis 10, Mo Asst Prof of Biochemistry (2, 1946)
- Vennesland, Birgit, Ph D Univ of Chicago, Dept of Biochemistry, Chicago, Ill Assoc Prof (2, 1944)
- Venning, Eleanor H, Ph D Univ Clinic, Royal Victoria Hospital, Pine Ave, Montreal, Quebec, Canada Asst Prof of Medicine, McGill Univ (2, 1938)
- Vestling, Carl Swensson, Ph D Univ of Illinois, Noyes Lab, Urbana, Ill Asst Prof of Biochemistry (2, 1946)
- Vickery, Hubert B, Ph D Connecticut Agric Exper Station, New Haven Biochemist in Charge, Dept of Biochemistry, Lecturer in Physiological Chemistry, Yale Univ (2, 1923)
- Victor, Joseph, M D Camp Detrick, Frederick, Md Chief, Pathology Branch (4, 1935)
- Villee, Claude A, Jr, Ph D Harvard Med School, 25 Shattuck St, Boston 15, Mass Assoc in Biological Chemistry (2, 1948)
- Virtue, Robert W, Ph.D, M D Denver General Hospital, Denver 4, Colo Anesthesiologist (2, 1939)
- Visscher, Frank E, Ph D Upjohn Co, Kalamazoo 99, Mich Research Scientist, Dept of Pharmacology and Endocrinology (1, 1947)
- Visscher, Maurice B, Ph D, M D Univ of Minnesota, Minneapolis Prof and Head of Dept of Physiology (1, 1927)
- Voegtlin, Carl, Ph D Univ of Rochester Med School, Rochester, N Y Lecturer in Pharmacology (1R, 1908, 2, 1908, 3, 1908)
- von Haam, Emmerich, M D Ohio State Univ, Columbus Prof of Pathology (4, 1938)
- Von Oettingen, W F, M D, Ph D Natl Insts of Health, Lab of Physical Biology, Exper Biology and Medicine Inst, Bethesda, Md Chief Industrial Toxicologist (3, 1925)
- Voris, LeRoy, Ph D Natl Research Council, 2101 Constitution Ave, Washington 25, D C Exec Sec, Food and Nutrition Board (5, 1949)

- Vorwald, Arthur J , Ph D , M.D Saranac Lake, N Y *Dir , Edward L Trudeau Foundation and Saranac Lab* (4, 1937)
- Vos, Bert J , Ph D , M D Food and Drug Admin , Div of Pharmacology, Washington, D C *Assoc Pharmacologist* (3, 1941)
- Wachstein, Max, M D St Catherine's Hospital, Brooklyn, N Y *Dir of Lab , Research Asst , Mt Sinai Hospital* (4, 1947)
- Waddell, James, Ph D E I duPont de Nemours & Co , New Brunswick, N J *Dir of Biological Lab* (2, 1930, 5, 1935)
- Wadsworth, Augustus B , M D Manchester, Vt (4, 1935, 6, 1920)
- Waelsch, Heinrich, M D , Ph D 722 West 168th St , New York City 32 *Assoc Research Biochemist, N Y State Psychiatric Inst and Hospital, Assoc Prof of Biochemistry, Columbia Univ* (2, 1941)
- Wagman, Irving H , Ph D Jefferson Med College, Dept of Physiology, Philadelphia 7, Pa *Asst Prof of Physiology* (1, 1946)
- Waisman, Harry A , M D , Ph D Univ of Illinois Med School, 1819 W Polk St , Chicago 12 *Resident Fellow in Pediatrics* (2, 1944)
- Wakeman, Alfred J , Ph D Hatfield Hill Rd , Bethany, Conn *Retired* (2, 1906)
- Wakerlin, George E , Ph D , M D Univ of Illinois Med School, 1853 W Polk St , Chicago *Prof of Physiology* (1, 1933, 3, 1934)
- Wakim, Khalil G , M D , Ph D Mayo Clinic, Rochester, Minn *Consultant, Prof of Physiology, Mayo Foundation* (1, 1942)
- Walcott, William W , Ph D Columbia Univ College of Physicians and Surgeons, Dept of Physiology, New York City 32 *Instructor* (1, 1947)
- Wald, George, Ph D Harvard Univ , Biological Labs , Cambridge, Mass (1, 1934)
- Walker, Arthur M , M.D Univ of Pennsylvania, Philadelphia 4 *Assoc Prof of Pharmacology, Major (M C) , U S A* (1, 1932, 3, 1939)
- Walker, Burnham S , Ph D , M D Boston Univ School of Medicine, 80 E Concord St , Boston, Mass *Prof of Biochemistry* (2, 1940)
- Walker, Harry A , Ph D Emory Univ School of Medicine, Emory University, Ga *Asst Prof of Pharmacology* (3, 1948)
- Walker, Sheppard M , Ph D Washington Univ School of Medicine, St Louis, Mo *Asst Prof of Physiology* (1, 1946)
- Wallen-Lawrence, Zonja, Ph D 4534 W Pine Blvd , St Louis 8, Mo (2, 1937)
- Walter, Annabel W 29 Perry St , New York City 14 *Bacteriologist, New York City Dept of Health, Bureau of Labs* (6, 1946)
- Walter, Carl W , M D Harvard Med School, 25 Shattuck Street, Boston, Mass *Asst Clin Prof of Surgery, Sr Assoc in Surgery, Peter Bent Brigham Hosp* (4, 1942)
- Walters, Orville S , Ph D , M D McPherson, Kan *Physician* (1, 1936)
- Walton, Robert P , Ph.D , M D Med College of the State of South Carolina, Charleston *Prof of Pharmacology* (3, 1933)
- Walton, Seth T , V.M D , Ph D Lab , Veterans Hospital, Oteen, N C *Dir of Labs and Research* (6, 1936)
- Walzer, Matthew, M D 20 Plaza St , Brooklyn, N Y *Attending in Allergy, Jewish Hospital of Brooklyn* (6, 1924)
- Wang, Chu Che, Ph D U S Veterans Admin Hospital, Hines, Ill *In charge of Biochemical Research* (2, 1922, 5, 1933)
- Wang, Shih-Chun, M D , Ph D Columbia Univ College of Physicians and Surgeons, 630 W 168th St , New York City 32 *Assoc Prof of Physiology* (1, 1943)
- Wangeman, Clayton P , M D Broadway Med Center, Associated Anesthesiologists, Seattle, Washington (3, 1946)
- Wangensteen, Owen Harding, M D Univ Hospital, Minneapolis 14, Minn *Prof of Surgery, Univ of Minnesota* (1, 1947, 4, 1931)
- Ward, Arthur A , Jr , M D 8009 W 31st Ave , N E , Seattle, Wash *Asst Prof of Surgery, Head of Div of Neurosurgery, Univ of Washington School of Medicine* (1, 1949)
- Ward, Walter E , Ph D , M D Cutter Labs Fourth and Parker Sts , Berkeley 1, Calif *Assoc Med Dir* (6, 1947)
- Ware, Arnold G , Ph D Wayne Univ College of Medicine, Dept of Physiology, 1512 Antoine St , Detroit 26, Mich *Research Assoc* (2, 1949)
- Warner, Emory D , M D Med Labs Bldg , Iowa City, Ia *Prof of Pathology* (4, 1937)
- Warner, Robert C , Ph D New York Univ College of Medicine, 477 First Ave , New York City 16 *Asst Prof of Chemistry* (2, 1946)
- Warren, Charles O , Ph D , M D The Commonwealth Fund, 41 E 57th St , New York City 22 (1, 1941)
- Warren, James V , M D Emory Univ School of Medicine, Atlanta, Ga *Prof of Physiology, Assoc Prof of Medicine* (1, 1947)
- Warren, Joel 8900 Flower Ave , Silver Spring, Md (6, 1949)
- Warren, Marshall R , Ph D , M D Univ of Tennessee College of Medicine, Memphis *Asst Prof of Pharmacology* (3, 1948)
- Warren, Shields, M D 195 Pilgrim Rd , Boston, Mass *Prof of Pathology, Harvard Med School; Dir , Div of Biology and Medicine, USAEC* (4, 1929)
- Wariman, William Beckman, M D Northwestern Univ , 303 East Chicago Ave , Chicago 11, Ill *Morrison Prof and Chairman of Dept of Pathology* (4, 1940)

- Wasteneys, Hardolph, Ph D Univ of Toronto, Toronto, Ontario, Canada *Prof and Head of Dept of Biochemistry* (2, 1915)
- Waterman, Robert E, B S Schering Corp, 86 Orange St, Bloomfield, N J *Vice-Pres* (2, 1940)
- Waters, Ralph Milton, M D 1300 University Ave, Madison, Wis *Prof of Anesthesia, Univ of Wisconsin* (3, 1937)
- Watson, Cecil J, M D, Ph D University Hospital, Dept of Medicine, Minneapolis, Minn *Prof and Head of Dept of Medicine* (4, 1941)
- Watson, Dennis W Univ of Minnesota Med School, Dept of Bacteriology and Immunology, Minneapolis *Assoc Prof* (6, 1949)
- Watson, John B, Ph D Box 526, Westport, Conn (1, 1907)
- Waud, Russell A, M.D, Ph D Univ of Western Ontario Med School, London, Ontario, Canada *Prof of Pharmacology* (3, 1931)
- Waugh, David F, Ph D Massachusetts Inst of Technology, Dept of Biology and Biological Engineering, Cambridge *Asst Prof of Physical Biology* (1, 1943)
- Way, E Leong, Ph D Univ of California Med Center, San Francisco, Calif *Asst Prof of Pharmacology* (3, 1947)
- Wearn, Joseph T, M D Lakeside Hospital, Cleveland, Ohio *Dir of Medicine, Prof of Medicine, Western Reserve Univ* (1, 1921)
- Weatherby, J H, Ph D Med College of Virginia, Dept of Physiology and Pharmacology, Richmond, Va *Assoc Prof of Pharmacology* (3, 1941)
- Weber, Clarence J, M D, Ph D *Chief, Lab Service* (2, 1931)
- Webster, Bruce, M D, C M Cornell Univ Med College, 1300 York Ave, New York City *Asst Prof of Medicine, Assoc Attending Physician, New York Hospital* (5, 1935)
- Wégria, René, M D Presbyterian Hospital, Dept of Medicine, 622 W 168th St, New York City *Asst Prof of Medicine* (1, 1941)
- Weichert, Charles K, Ph D Univ of Cincinnati, Cincinnati, Ohio *Prof of Zoology* (1, 1935)
- Weil, Alfred J, M D The Bronx Hospital, New York City *Dir of Dept of Bacteriology* (6, 1940)
- Weil, Arthur, M D 952 5th Ave, New York City (4, 1940)
- Weil, Leopold, Ph D U S Dept of Agriculture, Eastern Regional Research Lab, Chestnut Hill Station, Philadelphia 18, Pa *Chemist* (2, 1942)
- Weinhouse, Sidney, Ph D Temple Univ, Research Inst, 3223 N Broad St, Philadelphia 40, Pa *Biochemical Dir* (2, 1948)
- Weir, Everett G, Ph D 231 S 13th Ave, Maywood, Ill (1, 1941)
- Weiser, Russell S, Ph D Univ of Washington School of Medicine, Seattle 5, Washington *Assoc Prof of Microbiology* (6, 1948)
- Weiss, Charles, Ph D, M D Jewish Hospital, York and Tabor Rds, Philadelphia, Pa *Dir of Labs* (4, 1934, 6, 1920)
- Weiss, Emil, M D, Ph D 5036 Bernard St, Chicago, Ill *Pathologist, People's Hospital* (6, 1927)
- Weiss, Paul, Ph D Univ of Chicago, Chicago, Ill *Prof of Zoology* (1, 1936)
- Welch, Arnold D, Ph D, M D Western Reserve Univ School of Medicine, Cleveland, Ohio *Prof of Pharmacology* (3, 1942, 5, 1944)
- Welch, Henry, Ph D U S Food and Drug Admin, Rm 6171 S Agriculture Bldg, Washington, D C, *Chief of Div of Penicillin Control and Immunology* (6, 1932)
- Weld, Charles Beecher, M A, M D Dalhousie Univ, Halifax, N S, Canada *Prof of Physiology* (1, 1936)
- Weld, Mrs Julia T Columbia Univ College of Physicians and Surgeons, 630 W 168th St, New York City *Research Assoc in Pathology* (6, 1920)
- Welker, William H, Ph D 534 N Elmwood Ave, Oak Park, Ill *Prof Emeritus of Biological Chemistry, Univ of Illinois College of Medicine* (2, 1906)
- Weller, Carl Vernon, M D 1130 Fair Oaks Parkway, Ann Arbor, Mich *Prof and Chairman of Dept of Pathology, Univ of Michigan*, (4, 1923)
- Wells, Herbert S, M D Univ of Minnesota, Minneapolis 14 *Prof of Clin Physiology* (1, 1932)
- Wells, Joseph Albert, M D, Ph D Northwestern Univ Med School, Chicago, Ill *Assoc Prof of Pharmacology* (3, 1944)
- Welsh, John H, Ph D Harvard Univ, Biological Labs, 16 Divinity Ave, Cambridge 38, Mass *Assoc Prof of Zoology* (1, 1945)
- Wendel, William B, Ph D Tulane Univ School of Medicine, 6501 St Charles Ave, New Orleans 15, La *Prof and Head of Dept of Biochemistry* (2, 1932)
- Werber, Erna A, Ph.D 44 W 83rd St, New York City *Dir of Research Lab, Jewish Hospital of Brooklyn* (6, 1948)
- Werkman, C H, Ph D Iowa State College, Science Hall, Ames *Prof and Head of Dept of Bacteriology* (2, 1942)
- Werle, Jacob M, M D 2000 W 25th St, Cleveland 13, Ohio *Surgeon* (1, 1943)
- Werner, Harold W, Ph D The Wm S Merrell Co, Lockland Station, Cincinnati, Ohio *Dir Pharmacology Research* (3, 1942)
- Wertemberger, Grace E, Ph D Univ of Indiana, Dept of Physiology, Bloomington (1, 1943)

- Werthessen, Nicholas T, Ph D Shrewsbury, Mass Worcester Foundation for Exper Biology *Sr Fellow* (1, 1946)
- Wescow, W Clarke, M D Cornell Univ Med College, 1300 York Ave, New York City 21 *Instr in Pharmacology* (3, 1949)
- Wesson, Laurence G, Jr, Ph D New York Univ College of Medicine, Dept of Physiology, New York City 16 *Asst Prof* (1, 1949, 2, 1929, 3, 1932)
- West, Edward S, Ph D Univ of Oregon Med School, Portland *Prof of Biochemistry* (2, 1925)
- West, Harold D, Ph D Meharry Med College, Nashville 8, Tenn *Prof and Head of Dept of Biochemistry* (2, 1946)
- Westerfeld, Wilfred Wiedey, Ph D Syracuse Univ College of Medicine, Syracuse 10, N Y *Prof of Biochemistry* (2, 1944)
- Westfall, B A, Ph D Univ of Missouri School of Medicine, Dept of Physiology and Pharmacology, Columbia *Prof of Pharmacology* (1, 1949)
- Weston, Raymond E, M D, Ph D Montefiore Hospital, Med Div, New York City 67 *Asst in Medicine* (1, 1947)
- Weymouth, Frank W, Ph D Stanford Univ, Calif *Prof and Exec of Dept of Physiology* (1, 1917)
- Wheeler, George W, M D New York Hospital, 235 E 73rd St, New York City *Asst Dir* (6, 1920)
- Wheeler, Mary W, M A New York State Dept of Health, Div of Labs and Research, Albany *Assoc Bacteriologist* (6, 1933)
- Wheeler, Homer, M S, M D Univ of Washington, Seattle, Wash *Prof of Medicine* (1, 1919)
- Whipple, George H, M D, Sc D Univ of Rochester, Rochester, N Y *Prof of Pathology and Dean of School of Medicine and Dentistry* (1, 1911, 4, 1913)
- White, Abraham, Ph D Univ of California School of Medicine, Los Angeles 24 *Prof and Chairman of Dept of Physiological Chemistry* (2, 1934, 5, 1937)
- White, Alan G C, Ph D Tulane Univ, Dept of Biochemistry, New Orleans, La *Asst Prof of Biochemistry* (2, 1949)
- White, Colin, B S Univ of Pennsylvania School of Medicine, Dept of Physiology, Philadelphia *Asst Prof* (1, 1949)
- White, Florence R, Ph D % Dr Julius White, Natl Insts of Health, Natl Cancer Inst, Bethesda 14, Md *Biochemist* (2, 1946)
- White, Frank D, Ph D Univ of Manitoba, Faculty of Medicine, Bannatyne Ave, Winnipeg, Manitoba, Canada *Prof of Biochemistry, Biochemist, Winnipeg General Hospital* (2, 1931)
- White, Harvey Lester, M D Washington Univ Med School, St Louis 10, Mo *Prof of Physiology* (1, 1923)
- White, Julius, Ph D Natl Insts of Health, Natl Cancer Inst, Bethesda 14, Md *Head Chemist* (2, 1937)
- White, Paul Dudley, M D Massachusetts General Hospital, Boston, Mass *Physician (in charge of Cardiac Clinics and Lab), Lecturer in Medicine, Harvard Med School* (3, 1921)
- Whitehead, Richard W, M A, M D Univ of Colorado School of Medicine, 4200 E Ninth Ave, Denver *Prof of Physiology and Pharmacology* (1, 1933, 3, 1928)
- Whitehorn, William V, M D Univ of Illinois College of Medicine, Chicago *Asst Prof of Applied Physiology* (1, 1947)
- Whittenberger, James L, M D Harvard School of Public Health, Dept of Physiology, 55 Shattuck St, Boston 15, Mass *Asst Prof and Head of Dept of Physiology* (1, 1949)
- Wiener, Alexander S, M D 64 Rutland Rd, Brooklyn, N Y *Bacteriologist and Serologist to Office of Chief Med Examiner of New York City, Head of Transfusion Div, Jewish Hospital of Brooklyn* (6, 1932)
- Wiersma, Cornelis A G, Ph D California Inst of Technology, Pasadena *Assoc Prof of Physiology* (1, 1941)
- Wiggers, Carl J, M D, Sc D Western Reserve Univ Med School, Cleveland, Ohio *Prof and Dir of Physiology* (1, 1907, 3R, 1909)
- Wiggers, Harold C, Ph D Union Univ, Albany Med College, Dept of Physiology and Pharmacology, Albany 3, N Y *Prof and Chairman of Dept* (1, 1938)
- Wigodsky, Herman S, Ph D, M D Natl Research Council, Committee on Atomic Casualties, Div of Med Sciences, Washington 25, D C *Professional Assoc* (1, 1943)
- Wikler, Abraham, M D USPHS Hospital, Lexington, Ky *Surgeon (R)* (3, 1944)
- Wilber, Charles G, Ph D St Louis Univ, 1402 S Grand Blvd, St Louis 4, Mo *Dir of Biological Labs* (1, 1947)
- Wilde, Walter S, Ph D Tulane Univ School of Medicine, Station 20, New Orleans, La *Assoc Prof of Physiology* (1, 1944)
- Wilder, Russell M, Ph D, M D Mayo Clinic, Rochester, Minn *Prof of Medicine, Mayo Foundation, Univ of Minnesota* (1, 1921, 4R, 1924, 5, 1933)
- Wiley, Frank H, Ph D Federal Security Agency, Food and Drug Admin, Washington 25, D C *Chemist* (2, 1933)
- Wilhelmi, Alfred E, Ph D Yale Univ School of Medicine, 333 Cedar St, New Haven 11, Conn *Assoc Prof of Physiological Chemistry* (2, 1942)

- Wilhelmj, Charles Martel, M D Creighton Univ School of Medicine, Omaha, Neb *Prof of Physiology* (1, 1931)
- Wilkerson, Vernon A , M D , Ph D Med Arts Bldg , 61 K St , N W , Washington, D C *Biochemical Consultant* (2, 1936)
- Williams, Carroll M , Ph D , M D Harvard Univ , Biological Labs , Cambridge, Mass *Asst Prof of Zoology* (1, 1947)
- Williams, Harold H , Ph D Cornell Univ , Savage Hall, Ithaca, N Y *Prof of Biochemistry* (2, 1938, 5, 1936)
- Williams, Horatio B , M D , Sc D Box 893, Greenwich, Conn *Dalton Prof Emeritus of Physiology, Columbia Univ* (1, 1912)
- Williams, J W , Ph D Univ of Wisconsin, Chemistry Bldg , Madison *Prof of Chemistry* (2, 1944)
- Williams, Ray D , M S , M D 6834 Waterman St , St Louis, Mo *Asst Prof of Clin Medicine, Washington Univ* (5, 1941)
- Williams, Robert Hardin, M D Univ of Washington, Dept of Medicine, Seattle *Prof and Exec Officer* (4, 1940)
- Williams, Robert R , D Sc 297 Summit Ave , Summit, N J *Dir of Grants, Research Corp* (2, 1919, 5, 1941)
- Williams, Roger J , Ph.D Univ of Texas, Dept of Chemistry, Austin *Prof of Chemistry, Dir of Biochemical Inst* (2, 1931)
- Williams, W Lane, Ph D Univ of Minnesota Med School, Minneapolis 14 *Assoc Prof of Anatomy* (1, 4, 1947)
- Wills, J H , Ph D Med Div , Pharmacology Section, Army Chemical Center, Md (1, 1943)
- Wilson, Armine T Alfred I DuPont Inst , Wilmington 99, Del (6, 1949)
- Wilson, David Wright, Ph D Univ of Pennsylvania Med School, Philadelphia *Benjamin Rush Prof of Physiological Chemistry* (1, 1915, 2, 1915)
- Wilson, Eva D , Ph D 256 E Irvin Ave , State College, Pa (5, 1947)
- Wilson, Frank N , M D Univ Hospital, Ann Arbor, Mich *Prof of Medicine, Univ of Michigan* (4, 1925)
- Wilson, J Walter, Ph D Brown Univ , Dept of Biology, Providence 12, R I *Chairman of Dept of Biology, F L Day Prof of Biology* (1, 1949)
- Wilson, John W , Ph D Wright-Patterson Air Force, Aero-Med Lab , Dayton, Ohio *Research Physiologist* (1, 1948)
- Wilson, Karl M , M D Univ of Rochester School of Medicine, Rochester, N Y *Prof of Obstetrics and Gynecology* (4, 1927)
- Wilson, P W , Ph D Univ of Wisconsin, Dept of Agricultural Bacteriology, Madison *Prof in Agricultural Bacteriology* (2, 1939)
- Wilson, Robert H , Ph D U S Dept of Agriculture, Western Regional Research Lab , 800 Buchanan St , Albany, Calif *Pharmacologist* (3, 1937)
- Winder, Claude V , Sc D Parke Davis and Co , Detroit, Mich *Research Pharmacologist* (1, 1938, 3, 1948)
- Windle, William Frederick, Ph D Univ of Pennsylvania Med School, Philadelphia *Prof and Chairman of Dept of Anatomy* (1, 1937)
- Winkenwerder, Walter LaF , M D 1014 St Paul St , Baltimore, Md *Assoc in Medicine, Johns Hopkins Med School* (6, 1938)
- Winnick, Theodore, Ph D Univ of Iowa Med School, Iowa City *Assoc Prof of Biochemistry* (2, 1946)
- Winter, Charles A , Ph D Merck Inst for Therapeutic Research, Rahway, N J *Research Assoc* (1, 1940)
- Winter, Irwin Clinton, Ph D , M D G D Searle and Co , P O Box 5110, Chicago 80, Ill *Dir of Clin Research* (3, 1941)
- Winternutz, M C , M D Yale Univ School of Medicine, New Haven, Conn *Anthony N Brady Prof of Pathology* (4, 1913)
- Winters, Jet C , Ph D Univ of Texas, Austin *Prof of Home Economics* (5, 1933)
- Wintersteiner, Oskar, Ph D Squibb Inst for Med Research, New Brunswick, N J *Member, Head, Div of Organic Chemistry* (2, 1930)
- Wintrobe, Maxwell Myer, M D , Ph D Salt Lake County General Hospital, Dept of Internal Medicine, 2033 S State St , Salt Lake City 5, Utah *Prof and Head* (4, 1940)
- Winzler, Richard J , Ph D Univ of Southern California Med School, Dept of Biochemistry, Los Angeles 7 *Assoc Prof of Biochemistry* (2, 1946)
- Wiseman, Bruce Kenneth, M D Ohio State Univ , Kinsman Hall, Columbus *Prof and Chairman of Dept of Medicine, Asst Dir of Med Research* (4, 1932)
- Wislocki, George B , M D Harvard Univ Med School, 25 Shattuck St , Boston, Mass *Parkman Prof of Anatomy* (1, 1924)
- Wissler, Robert W , M D , Ph D Albert Merritt Billings Hospital, 950 E 59th St , Chicago 37, Ill *Asst Prof of Pathology, Univ of Chicago* (4, 1949)
- Witebsky, Ernest, M D Buffalo General Hospital, 100 High St , Buffalo, N Y *Prof and Head of Dept of Bacteriology and Immunology* (6, 1935)
- Wittich, Fred W , M D 401 LaSalle Med Bldg , Minneapolis 2, Minn *Sec -Treas of American College of Allergists, Chairman of Exec Commit-*

- tee, *International Assoc of Allergists* (6, 1944)
- Wolbach, S Burt**, M D Children's Hospital, Boston, Mass *Shattuck Prof Emeritus of Pathological Anatomy, Harvard Med School, Dir of Nutritional Research, Children's Hospital* (4, prior to 1920)
- Wolf, Abner**, M D Columbia Univ College of Physicians and Surgeons, New York City 32 *Assoc Prof of Neuropathology* (4, 1948)
- Wolf, Arnold Veryl**, Ph D Union Univ, Albany Med College, Albany, N Y *Assoc Prof of Physiology and Pharmacology* (1, 1946)
- Wolf, Stewart**, M D Cornell Univ Med College, New York City *Asst Prof of Medicine* (1, 1948)
- Wolff, Harold G**, M D, M A New York Hospital, 525 E 68th St, New York City *Assoc Prof of Medicine, Cornell Univ Med College, Assoc Attending Physician, New York Hospital* (1, 1930, 3, 1942)
- Wolff, William A**, Ph D Wake Forest College, Bowman Gray School of Medicine, Winston-Salem 7, N C *Asst Prof of Biochemistry* (2, 1947)
- Womack, Madelyn**, Ph D U S Dept of Agriculture, Agricultural Research Admin, Foods and Nutrition Div, Washington 25, D C *Biochemist* (5, 1948)
- Wood, Earl H**, Ph D, M D Mayo Aero-Med Unit, Mayo Foundation, Rochester, Minn *Assoc Prof of Physiology, Grad School, Univ of Minnesota, Consultant in Physiology, Mayo Clinic* (1, 1943, 3, 1948)
- Wood, Harland G**, Ph D Western Reserve Univ, Dept of Biochemistry, Cleveland 6, Ohio *Prof of Biochemistry* (2, 1944)
- Wood, John L**, Ph D Univ of Tennessee School of Biological Sciences, 875 Monroe Ave, Memphis 3 *Assoc Prof of Chemistry* (2, 1947)
- Woodbury, Robert A**, Ph D, M D Univ of Tennessee, Dept of Pharmacology, Memphis *Prof of Pharmacology* (1, 1936, 3, 1941)
- Woods, Alan C**, M D Johns Hopkins Hospital, Wilmer Ophthalmological Inst, Baltimore, Md *Dir of Inst and Ophthalmologist-in-Chief, Acting Prof of Ophthalmology, Johns Hopkins Univ* (6, 1918)
- Woods, Ella**, Ph D Univ of Idaho, Moscow *Home Economist, Exper Station* (2, 1925, 5, 1933)
- Woods, Lauren A**, M D, Ph D Univ of Michigan, Dept of Pharmacology, Ann Arbor *Instructor* (3, 1949)
- Woodward, Alvalyn E**, Ph D Univ of Michigan, Ann Arbor *Asst Prof of Zoology* (1, 1932)
- Woodyatt, Rollin T**, M D 237 E Delaware Place, Chicago, Ill *Prof of Medicine, Rush Med College, Univ of Chicago* (2, 1912)
- Wooley, Jerald G** Natl Insts of Health, Exper Biology and Medicine Inst, Bethesda, Md (6, 1949)
- Woolley, D Wayne**, Ph D Rockefeller Inst for Med Research, 66th St and York Ave, New York City *Member* (2, 1946, 5, 1941)
- Woolpert, Oram C**, M D, Ph D Camp Detrick, Frederick, Md *Tech Dir of Biological Div, Chemical Corps* (6, 1947)
- Woolsey, Clinton N**, M D Univ of Wisconsin, Service Memorial Insts, Madison *Charles Sumner Slichter Research Prof of Neurophysiology* (1, 1938)
- Wortis, S Bernard**, M D New York Univ College of Medicine, Dept of Psychiatry, New York City 16 *Prof and Chairman of Dept of Psychiatry, Dir of Psychiatric Div of Bellevue Hospital* (1, 1947)
- Wright, Angus**, M D Univ of Southern California Med School, 657 S Westlake Ave, Los Angeles *Pathologist, California Hospital* (4, 1935)
- Wright, Arthur W**, M D Albany Med College, New Scotland Ave, Albany, N Y *Prof of Pathology and Bacteriology* (4, 1941)
- Wright, Charles Ingham**, Ph D Natl Insts of Health, Bethesda, Md *Sr Pharmacologist, USPHS* (1, 1935, 3, 1936)
- Wright, George G**, Ph D Camp Detrick, Frederick, Md *Chief, Special Procedures Branch, S-Div* (6, 1943)
- Wright, Harold N**, Ph D Univ of Minnesota, Minneapolis *Assoc Prof of Pharmacology* (3, 1933)
- Wright, Lemuel D**, Ph D Sharp and Dohme, Inc, Med Research Div, Glenolden, Pa, *Dir of Nutritional Research* (2, 1946, 5, 1946)
- Wright, Sydney L**, Ph D Endsmeet Farm, Wyncote, Pa (2, 1933)
- Wulff, V J**, Ph D Univ of Illinois, Dept of Zoology and Physiology, Urbana *Asst Prof of Physiology, Special Research Asst* (1, 1949)
- Wyckoff, Ralph W G**, Ph D USPHS, Natl Insts of Health, Bethesda, Md *Sr Scientist* (6, 1940)
- Wyman, Jeffries, Jr**, Ph D Harvard Univ, Biological Labs, Cambridge, Mass *Assoc Prof of Zoology* (1, 1928)
- Wyman, Leland C**, Ph D Boston Univ, College of Liberal Arts, 675 Commonwealth Ave, Boston, Mass *Prof of Biology* (1, 1927)
- Wynne, Arthur M**, Ph D Univ of Toronto, Dept of Biochemistry, Toronto, Ontario, Canada *Prof of Biochemistry* (2, 1940)
- Yonkman, Frederick F**, Ph D, M D Ciba Pharmaceutical Products, Inc, Summit, N J *Dir of Research at Ciba, Lecturer in Pharmacology, Columbia Univ College of Physicians and Surgeons* (3, 1931)
- Youmans, John B**, M D Univ of Illinois, Chicago, Ill *Dean and Prof of Medicine, College of Medicine, Med Dir of Research and Educational Hospitals* (5, 1948)

- Youmans, William Barton, Ph D , M D Univ of Oregon Med School, Portland *Prof of Physiology* (1, 1939, 3, 1949)
- Young, A G , Ph D , M D 520 Commonwealth Ave , Boston, Mass *Asst Prof of Therapeutics, Boston Univ School of Medicine, Med Dir of Corey Hill Hospital, Brookline* (3, 1925)
- Young, E G , Ph D Dalhousie Univ , Halifax, N S , Canada *Prof of Biochemistry* (2, 1925)
- Youngburg, Guy E , Ph D Route 2, Box 268, Mesa, Ariz (2, 1927)
- Yuile, Charles L , M D , C M Univ of Rochester School of Medicine and Dentistry, Rochester, N Y *Assoc Prof of Pathology* (4, 1941)
- Zarrow, M X , Ph D Purdue Univ , Dept of Biological Sciences, Lafayette, Ind (1, 1949)
- Zechmeister, L , Dr Ing California Inst of Technology, Pasadena *Prof of Organic Chemistry* (2, 1941)
- Zeckwer, Isolde T , M D Univ of Pennsylvania School of Medicine, Philadelphia *Assoc Prof of Pathology* (1, 1934, 4, 1927)
- Zeidman, Irving, M D Univ of Pennsylvania School of Medicine, Dept of Pathology, Philadelphia *Instructor* (4, 1949)
- Zeldis, Louis J , M D Brookhaven Natl Lab , Med Dept , Upton, Long Island, N Y *Pathologist, Div of Pathology and Brookhaven Natl Lab Hospital* (4, 1945)
- Zilversmit, D B , Ph D Univ of Tennessee, Dept of Physiology, Memphis 3 *Instructor* (1, 1949)
- Zimmerman, Harry M , M D Montefiore Hospital, Gun Hill Rd , New York City 67 (4, 1933)
- Zirkle, Raymond E , Ph D Univ of Chicago, Chicago, Ill *Prof of Botany and Dir of Inst of Radiobiology and Biophysics* (1, 1948)
- Zittle, Charles A , Ph D U S Dept of Agriculture, Eastern Regional Research Lab , Philadelphia 18, Pa *Chemist, Protein Div* (2, 1946)
- Zucker, Marjorie B , Ph D New York Univ School of Dentistry, Dept of Physiology , 209 E 23rd St , New York City 10 (1, 1947)
- Zweifach, Benjamin W , Ph D New York Hospital and Cornell Univ Med College, Dept of Medicine, 525 E 68th St , New York City 21 *Asst Prof of Physiology* (1, 1945)
- Zwemer, Raymund L , Ph D 5003 Battery Lane, Bethesda 14, Md *Exec Sec , Natl Academy of Sciences and Natl Research Council* (1, 1930)

SUMMARY OF MEMBERSHIP

The American Physiological Society	1155
American Society of Biological Chemists	826
American Society for Pharmacology and Experimental Therapeutics	410
The American Society for Experimental Pathology	333
American Institute of Nutrition	347
The American Association of Immunologists	375
Total membership of Federation	3446

DECEASED MEMBERS

Addis, Thomas (1, 1922) June 4, 1949	Michaelis, Leonor (2, 1929) October 9, 1949
Baxter, J G (2, 1946) April 18, 1949	Petroff, S A (6, 1926) November 26, 1948
Becker, Theodore J (3, 1944) August, 1947	Porter, W T (1, 1891) February 16, 1949
Bookman, Samuel (2, 1912) October 8, 1946	Renshaw, Birdsey (1, 1941) November 23, 1948
Brewer, George (1, 1937) June 13, 1949	Ries, Fred A (1, 1933) August 1949
Brooks, Sumner C (1, 1923) April, 1948	Schnedorf, Jerome J (1, 1941) December 26 1948
Cameron, A T (2, 1914) September 25, 1947	Sinclair, R Gordon (2, 1931) August 17, 1949
Dann, W J (2, 1937, 5, 1938) December 5, 1948	Soley, Mayo (1, 1943) June 21, 1949
Fraser, Alexander MacLeod (3, 1939) 1949	Taylor, Alonzo E (6, 1933) May 20, 1949
Krichesky, Boris (1, 1948) August 28, 1949	Tisdall, Frederick F (2, 1922, 5, 1933) April 23, 1949
Krogh, August (1, 1946) September, 1949	West, Randolph (2, 1931) May 20, 1949
McCullagh, D Roy (2, 1932) September 17, 1949	

LIST OF MEMBERS BY LOCATION

Alabama

AUBURN *Alabama Polytechnic Inst* R W Engel, 5, E L Hove, 5, W D Salmon, 2, 5
BIRMINGHAM *Baptist Hosp* A E Casey, 4
Hillman Hosp T D Spies, 3, 4, 5 *Univ of Alabama* R D Baker, 4, J M Bruhn, 1, E B Carmichael, 1, 2, P Hitchcock, 3, J S McLester, 5, J F A McManus, 4, R S Teague, 3, Elizabeth N Todhunter, 5
GREENSBORO *Pan American Sanitary Bureau* R A Lambert, 4

Arizona

TUCSON *Univ of Arizona* A R Kemmerer, 5
Unclassified A L Lieberman, 1, G E Youngberg, 2

Arkansas

CAMDEN *Grapette Co* J T Skinner, 2
FAYETTEVILLE *Univ of Arkansas* M C Kik, 5, B Sure, 2, 5
LITTLE ROCK *Univ of Arkansas* J E Davis, 1, 3, P L Day, 2, 5, F E Emery, 1, A Nettleship, 4, J R Totter, 2

California

ALBANY *Western Regional Lab USDA* A M Ambrose, 3, B Axelrod, 2, A K Balls, 2, F DeEds, 2, 3, O H Emerson, 2, H L Fraenkel-Conrat, 2, J D Greaves, 2, E F Jansen, 2, J C Lewis, 2, H Lineweaver, 2, H P Lundgren, 2, H S Olcott, 2, S Schwimmer, 2, R H Wilson, 3
BERKELEY *Cutter Labs* W E Ward, 6 *State Dept Pub Health* J B Enright, 6, E H Lennette, 4, 6 *Univ of California* F W Allen, 2, H A Barker, 2, L L Bennett, 1, Matilda M Brooks, 1, M Calvin, 2, F H Carpenter, 2, I L Chaikoff, 1, M Doudoroff, 2, S S Elberg, 6, H M Evans, 1, C R Grau, 5, D M Greenberg, 2, 5, W Z Hassid, 2, H B Jones, 1, P L Kirk, 2, C A Knight, 2, A P Krueger, 4, 6, S Lephovsky, 2, 5, C H Li, 2, G Meiklejohn, 6, Agnes F Morgan, 2, 5, Ruth Okey, 2, 5, J M D Olmsted, 1, N Pace, 1, H S Reed, 2, Miriam E Simpson, 1, W M Stanley, 2, E S Sundstroem, 2, H Tarver, 2, T Winnick, 2 *Unclassified* L J Bogert, 2, H C Bradley, 1, 2
DAVIS *Univ of California* H H Cole, 1, H Goss, 2, 5, M Kleiber, 1, 5, F H Kratzer, 5, S A Peoples, 3
LOS ANGELES *Bio Service Labs* O J Golub, 6
Cedars of Lebanon Hosp N B Friedman, 4, H Goldblatt, 1, 4, E Haas, 2, M Prinzmetal, 3
Children's Hosp R E Knutti, 4 *Coll of Med Evangelists* H A Davis, 4, M Levine, 6 *Co Hosp* H E Pearson, 6, H Schwerma, 1, Daisy G Simonsen, 2 *Gooch Labs* B W Beadle, 2
Univ of California T H Bullock, 1, W G Clark, 1, 3, F Crescitelli, 1, M S Dunn, 2, H B Friedgood, 1, T J Haley, 3, T L Jahn, 1, S Roberts, 1, Clara M Szego, 1, C L Taylor, 1, A White, 2, 5
Univ of So California W H Bachrach, 1, F M Baldwin, 1, H J Deuel, Jr, 1, 2, 5, D R Drury, 1, P O Greeley, 1, C Hyman, 1, Jessie Marmors-ton, 6, W Marx, 2, J W Mehl, 2, L E Morehouse, 1, M D Nathanson, 3, B O Raulston, 3,

S S Sobin, 1, C H Thienes, 3, L E Travis, 1, R J Winzler, 2, A Wright, 4
V A J O Erickson, 6, R L Libby, 6 *Unclassified* W M Cahill, 2, P A Cavelti, 6, H H Mitchell, 6

MODESTO *Grange Co* H J Almquist, 2, 5
OAKLAND *Alameda County Inst* R J Parsons, 4 *Permanente Fndn* F R Goetzl, 1
OLIVE VIEW *Olive View Sanatorium* Marie C D'Amour, 1

PALO ALTO *Stanford Univ* W H Manwaring, 4, 6, J R Slonaker, 1R

PASADENA *Alles Labs* C H Ellis, 1 *California Inst of Tech* G A Alles, 1, 3, H Borsook, 2, D H Campbell, 6, J W Dubnoff, 2, N H Horowitz, 2, C G Niemann, 2, A Tyler, 6, A Van Harreveld, 1, C A G Wiersma, 1, L Zechmeister, 2

SAN DIEGO *County Hosp* H A Ball, 4 *Rees-Steady Med Research Fund* B F Stimmel, 2
Scripps Inst of Oceanography N W Rakestraw, 2
Scripps Metabolic Clin E M MacKay, 1

SAN FRANCISCO *Laguna Honda Home* Nellie Halliday, 5, M B Shimkin, 4 *Mt Zion Hosp* G R Biskind, 4, S O Byers, 1, M Friedman, 1
Stanford Univ A L Bloomfield, 3, 4, A J Cox, Jr, 4, W C Cutting, 3, R H Dreisbach, 3, P J Hanzlik, 1, G Lu, 3, H W Newman, 3, L A Rantz, 3, D A Ryland, 3 *Univ of California* H H Anderson, 3, W L Bostick, 4, J L Carr, 4, J J Eiler, 2, H W Elliott, 3, N E Freeman, 1, L D Greenberg, 2, W M Hammon, 4, 6, C H Hine, 3, W J Kerr, 3, H I Kohn, 1, B Libet, 1, S Lindsay, 4, S R Mettler, 4, K F Meyer, 4, 6, E W Page, 1, H J Ralston, 1, J F Rinehart, 4, P P T Sah, 3, E L Way, 3 *Unclassified* H E Foster, 6, J J Sampson, 1, A B Stockton, 3

SANTA BARBARA *Cottage Hosp* F E Bischoff, 2, 5, A E Koehler, 2 *Sahyun Research Lab* M Sahyun, 2

SANTA MONICA *Kabat-Kaiser Inst* O L Hudleston, 1

STANFORD UNIVERSITY *Stanford Univ* J P Baumberger, 1, J M Crismon, 1, P M Dawson, 1R, G A Feigen, 1, C S French, 1, 2, F A Fuhrman, 1, V E Hall, 1, H S Loring, 2, J M Luck, 2, S Raffel, 6, E W Schultz, 4, 6, R E Swain, 2, E L Tatum, 2, F W Weymouth, 1

UNCLASSIFIED W S Lawrence, 3, F H Scharles, 5, E L Walker, 3

Colorado

DENVER *Univ of Colorado* F A Cajon, 2, 5, H J Corper, 2, W B Draper, 1, 3, H H Gordon, 5, R M Hill, 2, J H Holmes, 1, R C Lewis, 2, 5, B B Longwell, 2, C A Maaske, 1, J B McNaught, 4, R M Mulligan, 4, R W Whithead, 1, 3 *Univ of Denver* Broda O Barnes, 1, F D'Amour, 1 *Unclassified* Gladys K Lewis, 5, W J Tomlinson, 4, R Virtue, 2

FORT COLLINS *Colo A & M Coll* F X Gas-sner, 1

UNCLASSIFIED Helen Bourquin, 1

Connecticut

EAST HARTFORD *United Aircraft Corp* S L Pond, 1

GREENWICH *St Luke's Convalescent Hosp* A A Albanese, 2 *Unclassified* H L Amoss, 4, 6
HARTFORD *Conn State Dept of Health* Olive R Benham, 6 *Inst of Living* W T Liberson, 1 *St Francis Hosp* S J Martin, 1

MIDDLETOWN *Wesleyan Univ* R A Gortner, 5, E G Schneider, 1R, 2

NEW HAVEN *Conn Agric Exper Sta* Rebecca B Hubbell, 2, 5, H B Vickery, 2 *Yale Univ* R J Anderson, 2, G A Batsell, 1, D H Barron, 1, W Bergmann, 2, F G Blake, 4, 6, C I Bliss, 3, E J Boell, 1, D M Bonner, 2, D D Bonnycastle, 3, J R Brobeck, 1, C H Bunting, 4, H G Cassidy, 2, G R Cowgill, 1, 2, 5, E C Curnen, 6, D C Darrow, 2, J English, 2, J S Fruton, 2, J F Fulton, 1, S Gelfan, 1, R H Green, 6, H S N Greene, 4, H W Haggard, 1, 2, R G Harrison, 1, L P Herrington, 1, D I Hitchcock, 2, H D Hoberman, 2, Marion E Howard, 4, 6, W A Krehl, 2, 5, D M Kydd, 5, H Lamport, 1, C S Leonard, 3, C N H Long, 1, 2, Evelyn B Man, 2, E L McCawley, 3, J L Melnick, 2, 6, W R Miles, 1, L N Nahum, 1, J S Nicholas, 1, J R Paul, 4, 6, J P Peters, 2, P D Rosahn, 4, Jane A Russell, 1, W T Salter, 1, 3, 5, Sofia Simmonds, 2, G H Smith, 6, Edna H Tompkins, 4, H P Treffers, 6, Gertrude Van Wagenen, 1, A E Wilhelm, 2, M C Winternitz, 4

NEW LONDON *US Navy Submarine Base* F L Dey, 1 *Unclassified* Katharine Blunt, 2

ROCKVILLE *Eastern State Farmers' Exchange* W E Anderson, 2, 5

STAMFORD *American Cyanamid Co* J T Litchfield, Jr, 3, R O Roblin, Jr, 2, 6 *Unclassified* M R Thompson, 3

STORRS *Univ of Connecticut* V Groupe, 6
UNCLASSIFIED H Louise Campbell, 5, N L Cressey, 6, W J Elser, 6, M C Pease, 6, H F Pierce, 1, A J Wakeman, 2, J B Watson, 1

Delaware

WILMINGTON *Alfred I DuPont Inst* L E Farr, 4, P B Hamilton, 2, A T Wilson, 6 *Unclassified* D Marine, 4

District of Columbia

Dept of Health H Kabat, 1,
Food and Agric Org Charlotte Chatfield, 5
George Washington Univ E C Albritton, 1, A Griffin, 6, C E Leese, 1, Zelma B Miller, 2, L W Parr, 4, Mary L Robbins, 6, J H Roe, 2, 5, Elizabeth R B Smith, 2, P K Smith, 2, 3, C R Treadwell, 2, 5

Georgetown Univ R A Cutting, 1, E de Savitsch, 4, W C Hess, 2, T Koppányi, 1, 3, C F Morgan, 1, 3, A M Shanes, 1, M X Sullivan, 2, E B Tuohy, 3

Harris Research Labs M Harris, 2
Howard Univ W M Booker, 1, 3, S A Corson, 1, J L Johnson, 1, A H Maloney, 3, E G Weir, 1, V A Wilkerson, 2

Natl Research Council M O Lee, 1, 5, H C Nicholson, 1, L Voris, 5, H S Wigodsky, 1, R L Zwemer, 1

Pa Cent Airlines L G Lederer, 1
U S Govt AEC P B Pearson, 2, 5 *Army Inst of Pathol* H M Dixon, 4, H G Grady, 4, H F Smetana, 4 *Army Med Ctr* H C Batson, 6, M Hilleman, 6, Elizabeth B Jackson, 6, J F Kent, 6, L R Kuhn, 6, H L Ley, Jr, 6, J E Smadel, 4, 6 *Army Med Dept, Field Duty* N S R Maluf, 1, G J Stein, 6, J M Werle, 1 *Army Med Dept, SGO* C J Koehn, Chief, 5

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TRAVEL AWARDS

18th International Physiological Congress

The Executive Committee of the Federation of American Societies for Experimental Biology has authorized the granting of five or more Travel Awards to younger scientists in the physiological field desiring to present papers before the 18th International Physiological Congress in Copenhagen, Denmark, August 15 to 18, 1950. Awards will be in the amount of \$500 each, and will be made to those scientists, not older than 35 years, who submit for presentation the best contributions in physiology, broadly defined, and give the best evidence of creative productivity. Membership in one of the Federated Societies will not influence selection for an award.

Applicants may address letters to Dr. M. O. Lee, Federation Secretary, 2101 Constitution Avenue, Washington 25, D. C., enclosing seven copies of an abstract of the paper proposed for presentation, and seven copies of a curriculum vitae and bibliography. The applicant should also include a list of two to four references, scientists competent to evaluate the applicant's work.

Applications will be received until January 20, 1950.

A Federation Committee, consisting of the Presidents of the six Societies, will select the individuals to receive the travel awards.

FEDERATION PROCEEDINGS

Published quarterly by the
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for EXPERIMENTAL BIOLOGY

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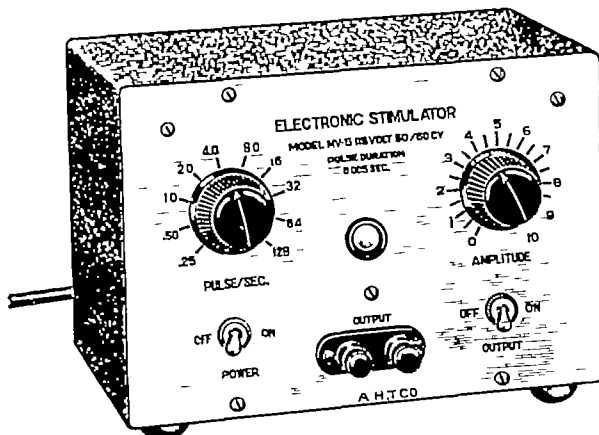
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American Society for Pharmacology and Experimental Therapeutics

The American Society for Experimental Pathology

American Institute of Nutrition

The American Association of Immunologists

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FEDERATION PROCEEDINGS is published quarterly by the Federation of American Societies for Experimental Biology. The *March* issue consists of the program (Part II) of the Annual Meeting of the Federation, and the Abstracts (Part I) of the papers presented at the scientific sessions. The program includes an author index. The abstracts are arranged alphabetically according to the first author and segregated as to Societies. The *June* and *September* issues contain symposia and other special papers presented at Federation meetings as selected by the Editorial Board. The *December* issue contains the membership list and other matters pertinent to the Constituent Societies of the Federation.

Price Schedule

The subscription price is \$4.50 (\$5.25 foreign) payable in advance. Single issues may be purchased, if ordered in advance, at the following prices: No. 1 (Part I March, Abstracts), \$3.00; No. 1 (Part II, March Program), 50¢; Nos. 2 and 3 (June and September), \$1.50 an issue; No. 4 (December), \$2.00. Subscriptions and orders should be sent to the Federation of American Societies for Experimental Biology, 2101 Constitution Ave., Washington 25, D. C.

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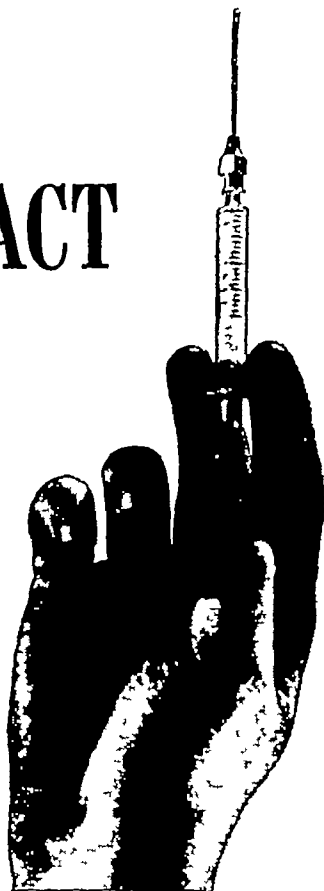
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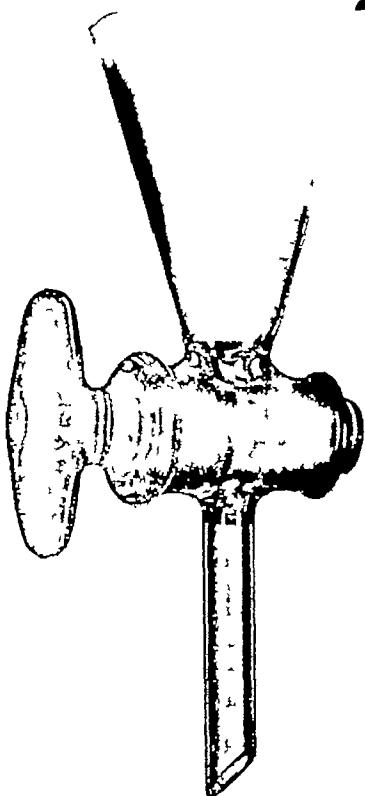
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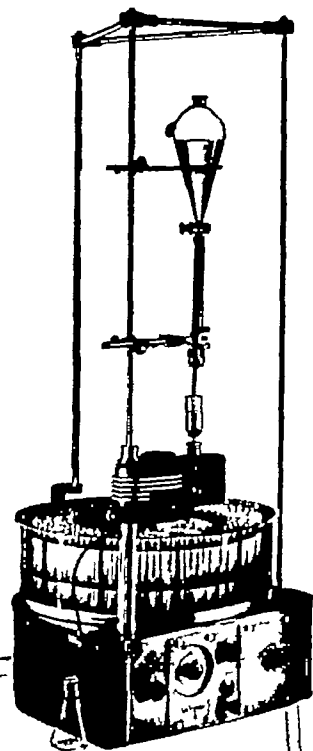
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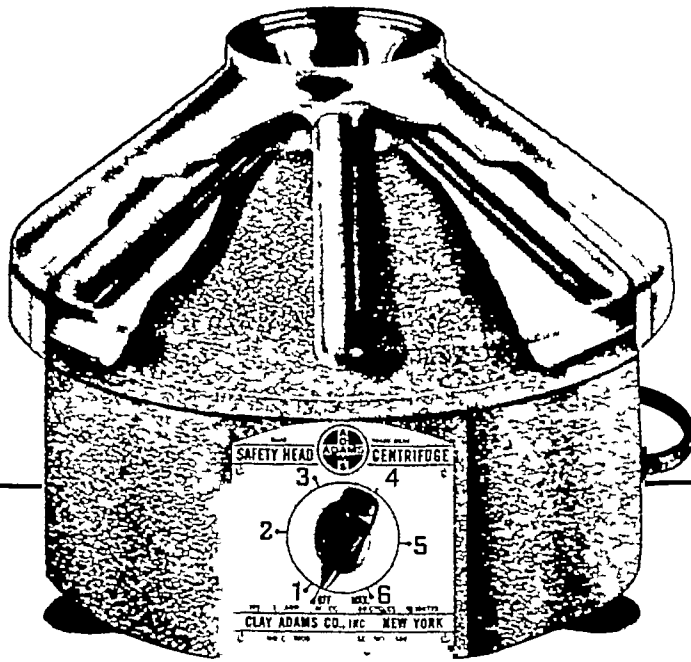
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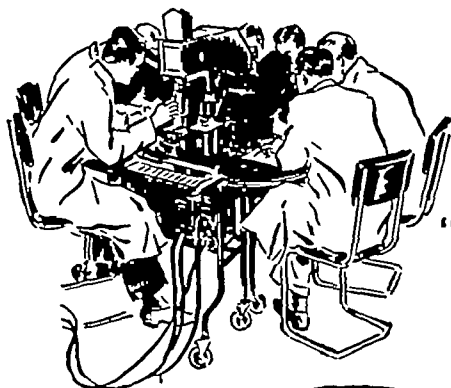
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Federation Proceedings

VOLUME 8

June 1949

NUMBER 2

NOTES ON THE DETROIT CONVENTION OF THE FEDERATION

April 18 to 22, 1949

The thirty-third annual meeting of the Federation of American Societies for Experimental Biology was held at Detroit, Michigan, April 18 to 22, 1949. Headquarters hotels were the Book-Cadillac for three Societies (Biochemistry, Pathology and Immunology) and the Statler for three Societies (Physiology, Pharmacology and Nutrition). All of the scientific sessions of the Societies were held in the Masonic Temple, as was also the Joint Session of the Federation on Tuesday evening, April 19. One motion picture session was presented, at the Detroit-Leland Hotel. The Federation 'Mixer' was held Wednesday evening, April 20, at the Hotel Statler.

Dr. Harry P. Smith, President of the American Society for Experimental Pathology, was Chairman of the meeting and presided at the Joint Session. One hundred and thirteen scientific sessions were held at which 1304 papers were read and 170 papers were read by title. Symposia were presented by the American Society of Biological Chemists, the American Society for Pharmacology and Experimental Therapeutics, the American Institute of Nutrition and the American Association of Immunologists.

The total registered attendance was 3517, including guests (wives of members) and exhibitors. As in previous years, the attendance was about equally divided between Society members and non-members.

Forty-four technical exhibits, occupying 60 booths, were shown.

FEDERATION ACTIONS

The following actions of the Federation Executive Committee are of general interest.

1 The plan to hold the 1950 meeting of the Federation in Atlantic City, New Jersey, April 17 to 21, was confirmed.

2 The Federation assessment for the year beginning July 1, 1949, was set at \$3.00 per member of each Society (all members to be in-

cluded, whether retired, honorary or members of more than one Society). The assessment includes subscription to *FEDERATION PROCEEDINGS*.

3 It was decided to permit the sale of reprints of abstracts published in the March 1950 issue of *FEDERATION PROCEEDINGS*; provided orders and payment are sent with the abstracts.

4 The Societies approved in principle the proposal for drafting a Constitution, revision of the By-Laws and incorporation of the Federation, the Constitution and By-Laws to be referred to the Societies for approval.

5 The Executive Committee voted that the form of the listing of members in the December issue of *FEDERATION PROCEEDINGS* be revised and that each entry contain only the following information: name of member, highest earned degree (when applicable both the M.D. and Ph.D. degrees, or, when applicable, the highest earned graduate-school degree and the M.D. degree), mailing address, title of position held (not more than two), and the Society affiliation.

6 The Executive Committee approved the proposal that upon adoption of the new Constitution and By-Laws the Control Committee for *FEDERATION PROCEEDINGS* be replaced by an Editorial Committee composed of the Society Secretaries and the Managing Editor.

7 The American Institute of Nutrition will act as the host Society for the 1950 meeting and will arrange the program of the Joint Session.

INTERNATIONAL CONGRESSES

Two International Congresses of interest to members of the Federation are scheduled for 1949 and 1950.

a) The First International Congress of Biochemistry will be held August 19 to 25, 1949, at Cambridge, England.

b) The 18th International Physiological Congress will be held August 15 to 18, 1950, at Copenhagen, Denmark.

CORRECTIONS OF ABSTRACTS IN MARCH ISSUE

Page 21 CAMPBELL AND DAVIDSON The last sentence of the abstract, beginning "The liver slices ,," should be omitted

Page 86 KLÜVER In line 1, the name "Henrich" should read "Heinrich" In line 3, the word "prophyrins" should read "porphyrins"

Page 141 SCHWARTZ, SCHACHTER AND FREINKEL In line 19, the equation should read " $Z = D - UV - P_{w_{in}} P_{w_v}$ "

Page 144 SELYE AND STONE Column 1, line 22 from the top of the page, should read, "granules, which is normally produced by such stimuli as folliculoid hormones"

Page 230 MILLER AND BALE Insert the following table before the last sentence of the abstract

C^{14} DISINTEGRATIONS/MINUTE/MILLIMOL $\times 10^{-4}$

	TOTAL	RELEASED BY NIN HYDRIN	RELEASED FROM NINHY- DRIN RESIDUE
Liver			
L-Lysine	4 49		
L-Glutamic acid	0 750	0 145	0 472
		0 147	0 453
L-Aspartic acid	0 21	0 154	0 02
Plasma protein			
L Lysine	5 28		
L-Glutamic acid	0 607	0 150	0 425
L-Aspartic acid	0 338	0 24	0 02

Page 335 SWINYARD AND TOMAN Line 16, insert the following table preceding the sentence beginning, "Maximum potency "

R	TD ₅₀ mg/kg	ELECTROSHOCK TEST		METRAZOL TEST	
		ED ₅₀ mg/kg	P I	ED ₅₀ mg/kg	P I
H (a)	740 (550-1000)	40 (36-44 5)	18 5 (13 6-25 2)	212 (137-324)	3 5 (2 1-5 9)
H (b)	1620 (1095-2400)	170 (111-260)	9 5 (5 3-17 1)	>1600	<1 0
CH ₃ (a)	173 (124-242)	17 5 (12 2-25 2)	9 9 (5 2-19 0)	59 (38-92)	2 9 (1 7-5 1)
CH ₃ (b)	332 (256-432)	16 (13 0-19 7)	20 8 (14 8-29 2)	203 (151-272)	1 6 (1 1-2 4)
C ₂ H ₅ (a)	170 (129-224)	18 (14 4-22 5)	9 5 (6 6-13 5)	160 (105-243)	1 1 (0 6-1 8)
C ₂ H ₅ (b)	119 (98-144)	10 8 (7 8-15 0)	11 0 (7 5-16 4)	62 (51-76)	1 9 (1 5-2 5)
C ₆ H ₅ (a)	No activity up to 1600 mg/kg				
C ₆ H ₅ (b)	>3200	30 (20-45)	>100	>3200	<1 0

() 95 per cent confidence limits

Page 335 TABOR Throughout the abstract the abbreviation mm should read micromol

Page 344 WAX, ELLIS AND LEHMAN Line 8, the word "was" should read "were" In line 15, the word "jejunum" should read "intestine" Line 21 (new sentence) to the end of the article should read, "Increased absorption area may have resulted in increased tissue concentration, but did not definitely influence total absorption The percentage absorption was not markedly affected by the concentration of IPA Absorption of IPA was 99 per cent complete at the end of 2 hours, the greatest part occurring within the first 30 minutes Previous absorption of IPA did not significantly influence subsequent absorption from adjacent loops Ethyl alcohol administered intravenously perhaps decreased the intestinal absorption of IPA"

Page 404 HAUROWITZ AND ETILI Line 2 from the bottom should read "per antigen-antibody bond or ~ 0.47 kcal per \AA^2 results"

Joint Session of the Federation

Detroit, Michigan, April 19, 1949

Chairman H. P. SMITH

AMINO ACID METABOLISM IN MUTANT STRAINS OF MICROORGANISMS

E. L. TATUM

From the Department of Biological Sciences, Stanford University

STANFORD, CALIFORNIA

THIS discussion of some aspects of amino acid metabolism in microorganism is necessarily limited in scope. It will deal primarily with metabolic relations which have been found to be of general significance in both higher and lower organisms and with certain new relations, probably also of general significance, which have been suggested by studies with microorganisms. In both instances, the discussion will center around the results obtained with the help of mutant strains.

Mutations which have as biochemical consequences defective biosyntheses of essential cellular constituents, such as vitamins and amino acids, are being produced in steadily increasing numbers of types of microorganisms. These now include representative bacteria, yeasts and fungi.

In order to give some idea of how nutritionally-deficient mutant strains of microorganisms are produced and isolated, a brief summary of various techniques now available is given in table 1. Basically, mutations in a microorganism are produced by exposing the culture to the action of a mutagen, γ -rays, ultra-violet radiation, or chemicals such as mustard-gas. Pure genetic lines of the treated material are derived from single spores with fungi, or from single vegetative cells with bacteria, by growing them on a 'complete' medium which supplies a variety of growth-factors, both known and unknown. A nutritionally deficient mutant among these cultures is detected by its failure to grow in a 'minimal' synthetic medium, and its defective biosynthesis is identified by testing its growth in variously supplemented synthetic media. This method has been used with *Neurospora* (1) and with *E. coli* (2) and other microorganisms. Several less laborious methods for selecting mutants from large popula-

tions have recently been developed. These are also summarized in table 1. All of these modifications are based on the failure of a mutant to metabolize and grow normally without its specific essential supplement. When this supplement is added after a period of incubation, mutant cells of bacteria only then form colonies which at a given time are smaller than those from normal cells (3). Fries has successfully increased the proportion of mutant spores of the mold *Ophrostoma* in mixtures by removing the germinated normal mycelia by filtration (4). With *Ophrostoma* (4) and with *Neurospora* (5) mutants have been visually selected from populations of spores cultured on minimal agar media on the basis that a mutant fails to grow or grows very slowly under these conditions. Even more efficient are methods based on the more rapid death of normal cells due in one case to exhaustion of endogeneous reserves in a minimal medium lacking a growth-factor required by the normal strain (6) and due in the other instance to the growth and consequent sterilization of normal cells in minimal medium containing penicillin (7, 8).

If the B-vitamins and the amino acids are metabolically essential for microorganisms, and if random mutation in nature or in the laboratory results in defective syntheses of these substances, we would expect to find a close correlation between the growth factor requirements of strains as isolated from nature and mutant strains produced experimentally. This expectation is fairly well fulfilled.

The requirements of mutant strains which have now been found in bacteria and fungi do include most of the known water soluble vitamins and the amino acids. This widespread range is summarized most easily by listing those sub-

stances not yet so represented. It can be seen from table 2 that deficiencies in mutant strains of fungi include riboflavin, pantothenic acid,

TABLE 1 TECHNIQUES FOR ISOLATING NUTRITIONAL MUTANTS OF MICROORGANISMS

DESCRIPTION OF METHOD	ORGANISM USED	REFERENCE
Isolation on complete medium, testing on minimal	<i>Neurospora</i>	(1)
Incubated in minimal, later supplemented as desired	<i>E coli</i>	(2)
Incubated in minimal, non-mutants removed by filtration	<i>E coli</i>	(3)
Incubated on minimal, mutants selected by observation	<i>Ophiostoma</i>	(4)
Incubated in minimal, with selective survival of mutants	<i>Ophiostoma</i>	(4)
Incubation in minimal with penicillin, with selective survival of mutants	<i>Neurospora</i>	(5)
	<i>Ophiostoma</i>	(6)
	<i>E coli</i>	(7, 8)

TABLE 2 COMPARISON OF NUTRITIONAL REQUIREMENTS OF MUTANT AND WILD-TYPE STRAINS OF MICROORGANISMS

FILAMENTOUS FUNGI		BACTERIA	
Strains from nature	Mutant strains	Strains from nature	Mutant strains
B-vitamins not required			
B ₂	B ₁₂	Inositol	B ₂
Pantothenic acid	Folic acid	Choline	Inositol
Nicotinic acid			Choline
p - amino benzoic acid			B ₁₂
Choline			Folic acid
B ₁₂			
Folic acid			
Amino acids not required			
?	Alanine	Alanine	Alanine
	Hydroxyproline	Hydroxyproline	Hydroxyproline

nicotinic acid, p-aminobenzoic acid and choline, which are not required by any filamentous fungi yet isolated from nature. So far as is known, folic acid and vitamin B₁₂ are not required either

by fungi from nature or by mutant strains. For the bacteria, inositol is not yet known to be required but all the other vitamins except riboflavin, folic acid, B₁₂ and choline are included in the requirements of mutant strains.

A similar comparison for amino acids suggests that in nature few spontaneously arising amino acid-requiring mutants of non-pathogenic fungi survive. However, mutant strains of fungi have been found with requirements for all the well-established amino acids except alanine and hydroxyproline. The same is true for strains of both classes of bacteria.

There is therefore available a wealth of experimental material in mutant strains of bacteria and fungi. In the strains to be considered, for example, single reactions in the biosyntheses of amino acids are blocked by gene mutations in a more specific manner than possible with enzyme inhibitors. The use of mutant strains as tools in the biochemical analysis of synthetic mechanisms is evident. However, before discussing specific examples of this, I want to mention briefly a phenomenon which has been found fairly frequently in mutant strains requiring amino acids. This phenomenon is one of a specific and often extreme susceptibility to growth inhibitions caused by certain amino acids, usually not the ones required for growth. These inhibitions are important both because they may indicate previously unsuspected interrelations between amino acids, and because they must be taken into account in the interpretation of studies of biosyntheses. To illustrate the diversity of antagonistic amino acid relations in *Neurospora* some examples are shown in table 3. Similar antagonisms have been invoked by Bonner to explain the double requirement of a mutant strain of *Neurospora* for isoleucine and valine (15). In this case an accumulated precursor of isoleucine appears to inhibit the synthesis of valine, and both are therefore required.

I now want to discuss some of the biochemical interrelations which have been found in amino acid metabolism in microorganisms. Three general considerations should be emphasized for this discussion: first, the validity of the viewpoint of comparative biochemistry, that the biochemical processes of microorganisms, higher plants, and animals are fundamentally similar; secondly, the contributions of studies with mutant strains of microorganisms to the analysis of the biochemical steps involved in reactions and relations between amino acids; thirdly, the im-

portance of mutant strains of microorganisms in discovering hitherto unknown reactions and unsuspected amino acid interrelations

Since amino acid metabolism has been most thoroughly studied in mutants of *Neurospora* and of *E. coli*, the examples used in this discussion for the most part will come from work with these two microorganisms

As shown in figure 1, one of the best examples of the essential similarity of amino acid metabolism in different organisms is the synthesis of arginine, in which the same steps of the ornithine cycle are involved in mammals, in *Neurospora* (16), in *Penicillium* (17), and in bacteria (18, 19)

TABLE 3 SOME AMINO-ACID ANTAGONISMS IN *NEUROSPORA*

GROWTH-FACTOR	GROWTH INHIBITOR	REFERENCE
Isoleucine + valine	Excess of either	(9)
Lysine	Arginine	(10)
D- α -amino adipic acid	Arginine, asparagine, glutamic acid	(11)
Glycine or serine	Asparagine	(12)
Methionine or threonine	Excess methionine	(13)
None (reversed by arginine)	Canavanine	(14)
Histidine	'Complete' medium	(5)
Adenine	Indole	(5)

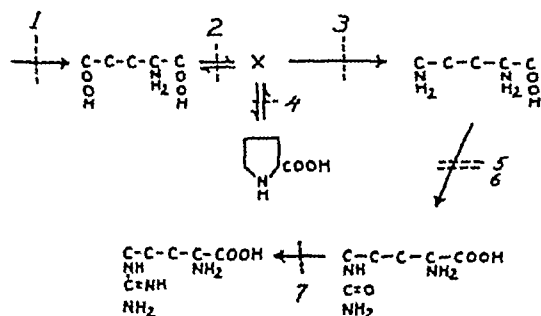


FIG 1

thine cycle are involved in mammals, in *Neurospora* (16), in *Penicillium* (17), and in bacteria (18, 19) Further probable relations of arginine to glutamic acid and to proline are indicated by reactions 1, 2, and 4 One mutant strain of *E. coli* is apparently blocked at step 1 and another at 4, resulting in requirements for glutamic acid or proline, and for proline, respectively (2) A block at reaction 2 in *Penicillium* apparently explains a requirement for proline or for arginine (17)

Another excellent example of the essential similarity in different organisms is in the metab-

olism of the sulfur-containing amino acids As shown in figure 2, the relation of cystathionine to cysteine and methionine first established for higher animals by du Vigneaud and collaborators holds also for *Neurospora* (20) and *E. coli* (21) Some minor differences exist, however In *Neurospora* serine as such seems not to be formed from cystathionine (13) In *E. coli* the reactions leading from methionine to cysteine apparently do not operate in that direction, and this bacterium differs from *Neurospora* in being able to use D-allo-cystathionine as a source of methionine (21)

Investigations with mutant strains of *Neurospora* have also indicated unsuspected relations between homoserine and threonine (13), as pic-

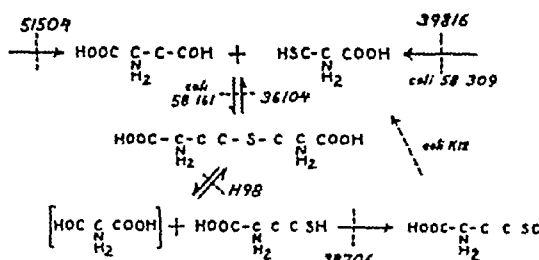


FIG 2

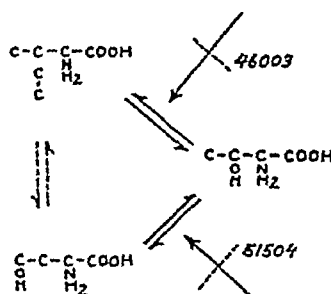


FIG 3

tured diagrammatically in figure 3 Strain 51504 grows with either amino acid A similar relation has been found in mutant strains of *B. subtilis* by Teas (22) An even more surprising relation is suggested by a mutant strain of *Neurospora* with an alternative requirement for isoleucine or threonine (22) These intriguing amino acid interconversions remain to be elucidated

The metabolic relation between lysine and α -amino adipic acid found in the rat by Borsook and collaborators (23) and shown in figure 4, has been found also in *Neurospora* since α -amino adipic acid has recently been shown to be a precursor of lysine for strain 33933 (11)

Although antranilic acid, indole, and tryptophane have for some time been known to be met-

abolically related, convincing evidence that anthranilic acid is a precursor of tryptophane was first obtained with mutant strains of *Neurospora* (24). This is shown in figure 5. The mechanism of the conversion of indole to tryptophane has likewise been analyzed with *Neurospora*, and has been shown to involve a hitherto unsuspected condensation of indole with the amino acid serine (25). There seems to be at least two steps involved in the conversion of anthranilic acid to indole, since two genetically different mutants are unable to carry out this reaction. A clue to the mechanism of this conversion has been given by

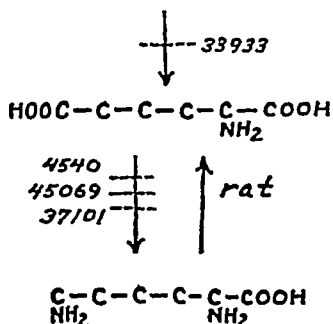


FIG 4

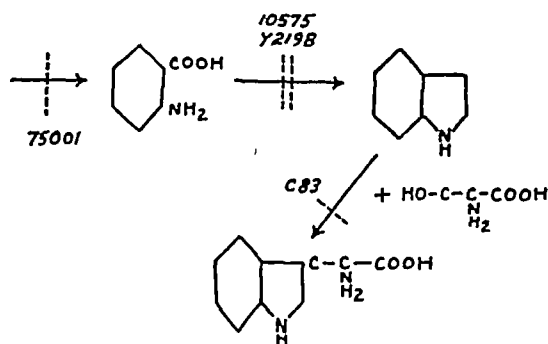


FIG 5

Nyc *et al* (26) who have found by isotopic techniques that the carboxyl carbon of anthranilic acid is not present in the formed tryptophane. The conversion of indole to tryptophane has been shown to be an enzymatic reaction involving pyridoxine (27). Recently a mutant strain of *Neurospora* which requires tryptophane and which apparently lacks this enzyme has been described (28). This evidence provides excellent supporting evidence that a gene-enzyme relation is involved in the genetic control of biosynthetic reactions.

An interesting relation between an amino acid and a vitamin is the conversion of tryptophane to nicotinic acid. This relation has been substan-

tiated and clarified with mutant strains of *Neurospora*. As shown in figure 6, some strains of *Neurospora* are known which require either tryptophane or nicotinic acid. This conversion is most readily explained by steps involving kynurenine, the hypothetical compound hydroxykynurenine and the established intermediate 3-hydroxyanthranilic acid. This latter compound is converted to nicotinic acid by strains 40008 and 65001 and by strain Y31881 (29-31). The production of this last intermediate by strain 4540 has been demonstrated by Bonner (31). The evidence obtained with these strains of microorganisms thus lends excellent support to the relation of tryptophane and nicotinic acid suggested first by animal studies. In addition, this conversion has been shown to involve several new

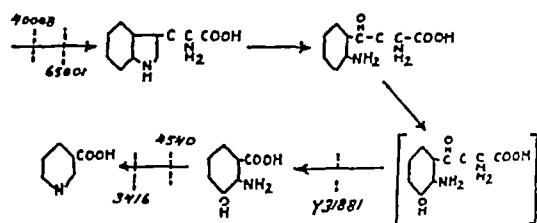


FIG 6

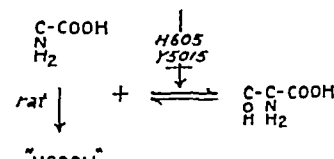


FIG 7

biochemical reactions of considerable general significance.

Another interesting relation which exists both in animals and in microorganisms is that of the interconversion of glycine and serine. Strains of bacteria (18, 19) and of *Neurospora* (12) are known which have alternative requirements for either glycine or serine. The relation of these two amino acids has been demonstrated in higher animals by Shemin (32) and the mechanism illustrated diagrammatically in figure 7 has been recently suggested by Sakami (33). Sakami's evidence, based on the use of marked glycine, suggests that glycine gives rise to formate, which then reacts with another molecule of glycine to form serine. This reaction may be involved in the interconversion of these amino acids in microorganisms. The arrow marked with the two strain numbers on the figure represents the reac-

tion chain leading into this series. There seem to be at least two steps which have been genetically blocked in the synthesis of glycine and serine. Evidence now available suggests that the rat and *Neurospora* may metabolize glycine in somewhat different fashions. Shemin has presented evidence that in the rat glycine is not particularly active in transamination reactions. In *Neurospora*, glycine apparently very readily supplies amino nitrogen for the formation of several other amino acids, including tryptophan, histidine, aspartic acid, and glutamic acid, as indicated by the use of nitrogen labelled glycine with a glycine requiring strain (34).

Some other extremely interesting relations between amino acids have been found which deal with the aromatic compounds phenylalanine, tyrosine, anthranilic acid and para-aminobenzoic acid. The precise mechanisms of these interrelations remain to be investigated. As diagrammatically represented in figure 8, mutant strains with re-

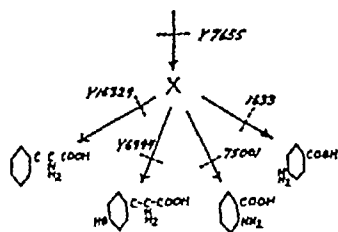


FIG 8

quirements for each one of the indicated aromatic compounds are known in *E. coli* (18, 19, 35) and in *Neurospora* (24, 36, 37). In *E. coli* recovery experiments have indicated that phenylalanine and tyrosine are synthesized independently, perhaps from a common precursor (35). The synthesis of all four compounds from a common precursor (x) is suggested by the existence of a mutant strain of *Neurospora*, Y7655, which requires all four substances (36) and by the existence of similar multiple-requiring mutants in *E. coli* (19). The relations existing in these mutant strains provide extremely interesting possibilities for further biochemical investigations of the biosynthesis of aromatic compounds.

Shemin (32) has suggested that the non-essential amino acids may be synthesized in the rat by amino acid interconversions rather than by amination of their keto acid analogues. Recent investigations with *Neurospora* have suggested that the keto acid of at least one essential ali-

phatic amino acid may not be directly involved in its biosynthesis. Working with a mutant strain of *Neurospora* which requires isoleucine and valine (9, 15) E. A. Adelberg, in our laboratory, has recently isolated and identified a new intermediate in the biosynthesis of isoleucine. The structure of this compound and a tentative suggestion of its part in the synthesis of isoleucine are indicated in figure 9. The keto acid analogue of isoleucine is perhaps only indirectly related to the biosynthesis of isoleucine in microorganisms. The evidence for this includes the inactivity of the keto acid in releasing the valine inhibition of growth of wild-type *E. coli*. In addition, some mutant strains of bacteria have been found capable of using the dihydroxy or the keto-acid analogue instead of isoleucine, while other mutant strains can use only the dihydroxy analogue in place of isoleucine. This evidence apparently provides a new clue to the mechanism of synthesis of aliphatic α -amino acids.

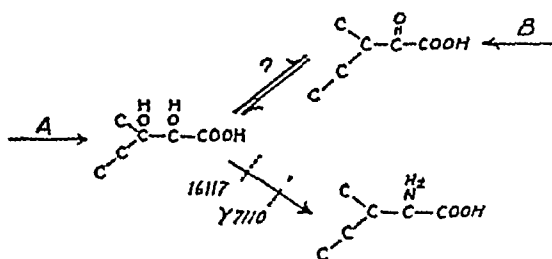


FIG 9

As mentioned earlier, there is now a considerable body of evidence suggesting that many amino acids may be synthesized by interconversions rather than directly from their keto acid analogues. In addition, evidence is now available that some keto acids are biologically inactive in certain microorganisms. These two lines of evidence cast some doubt on the general significance of direct amination reactions as terminal steps in the bio-syntheses of certain amino acids in microorganisms. Some examples are summarized in table 4. In the first group of examples listed, the most probable syntheses are by way of mechanisms other than amination of the keto acids, perhaps those indicated in the last column of the table. In several instances the keto acids have been found to be inactive for mutant strains of microorganisms. It might be concluded that perhaps the only amino acids which are formed *de novo* by direct amination or transamination are those directly related to known intermediates in

carbohydrate metabolism, notably alanine, aspartic acid and glutamic acid

Some of the known amino acid relations which have been found to be common to both mi-

TABLE 4 KETO-ACID AND AMINO ACID RELATIONS IN MUTANT STRAINS OF MICROORGANISMS

AMINO ACID	ACTIVITY OF KETO-ACID ANALOGUE	PROBABLE SYNTHESIS FROM
Arginine	?	Citrulline (16)
Lysine	?	α -aminoadipic acid (11)
Serine	?	Glycine (12)
Glycine	?	Serine (12)
Cysteine	?	Cystathionine (20)
Methionine	+	Cystathionine (20)
Isoleucine	-, +	Dihydroxyacid analogue
Tyrosine	\pm (10%)	Phenylalanine or common precursor (35)
Aminoadipic acid	- (11)	?
Tryptophane	-	Indole + serine (25)
Histidine	?	
	- (Hydroxy- acid, 34)	

TABLE 5 REACTIONS COMMON TO MICROORGANISMS AND ANIMALS

Ornithine \rightarrow citrulline \rightarrow arginine
 cystathionine \rightleftharpoons homocysteine + [serine]
 cystathionine \rightleftharpoons homoserine + cysteine
 glycine \rightleftharpoons serine
 α -aminoadipic acid \rightleftharpoons lysine
 tryptophane \rightarrow nicotinic acid
 tryptophane \rightarrow kynurenine

TABLE 6 REACTIONS AND INTERRELATIONS ESTABLISHED WITH MICROORGANISMS

indole + serine \rightarrow tryptophane
 threonine \rightleftharpoons homoserine
 anthranilic acid ($-\text{CO}_2 + ?$) \rightarrow indole
 kynurenine \rightarrow 3-hydroxyanthranilic acid
 3-hydroxyanthranilic acid \rightarrow nicotinic acid
 α, β , dihydroxy, β -ethyl-butyrlic acid \rightarrow isoleucine

croorganisms and animals are summarized in table 5. These reactions, which have already been discussed, include those of the ornithine cycle, the relation of cystathionine to methionine and cysteine, the interconversion of glycine and serine, the relation of alpha-aminoadipic acid to lysine,

and the conversion of tryptophane to nicotinic acid, and to kynurenine

As the result of examinations of the mechanisms of these and other biosynthetic reactions with mutant strains of microorganisms, a number of new reactions and relations have been established. These include the reactions summarized in table 6. The involvement of serine in the synthesis of tryptophane has been discussed. The relationship of threonine to homoserine suggested by studies with microorganisms remains to be elucidated. Similarly, the exact steps in the conversion of anthranilic acid to indole are as yet unknown. The essential outline of the biochemical steps involved in the conversion of kynurenine to 3-hydroxyanthranilic acid, and of this compound to nicotinic acid has been suggested. Finally, a role of α, β -dihydroxy- β -ethylbutyric acid in the biosynthesis of isoleucine has been fairly adequately established by studies with microorganisms.

It may be expected that future investigations with microorganisms, particularly with mutant strains, will reveal other new and unsuspected relations between amino acids, and that these strains will prove highly useful tools for the elucidation of the mechanisms of reactions involved in the biosyntheses of these amino acids. It may also be anticipated that further studies correlating enzyme activity with gene mutation will add additional information regarding the role of the gene and of gene mutations in determining enzyme specificity. The first clear-cut example of this relationship in *Neurospora* is the enzyme involved in tryptophane synthesis from indole and serine. Information is now available which should permit the extension of this analysis of gene-enzyme relations to other biosyntheses. These include the syntheses of the amino acids arginine, proline, glycine, and serine. It may be expected that other reactions will become available for such studies as the precise mechanisms of the biosyntheses of other amino acids are established.

In summary, we have discussed various aspects of amino acid metabolism in the examination of which microorganisms have been of primary value. Judging from past experience we may anticipate that additional discoveries coming from studies from microorganisms will be of general metabolic significance. The principles of comparative biochemistry thus adequately justify the use of microorganisms in investigations of amino acid

metabolism In these investigations mutant strains of microorganisms should prove of significant value in future developments

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VIRAL MULTIPLICATION

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WHEN a virus and a susceptible cell come together, there occurs an increase in the amount of the virus. An increase in the concentration of a virus has not been obtained as yet in cell-free media nor in the absence of living cells. It is now well established that viruses are discrete particles with definite morphology which can be visualized with the electron microscope (1). An increase in viral concentration is associated with an increase in the number of viral particles of like amount, there is a close correlation between the number of particles and the number of infective units (2).

The increase in the amount of virus which occurs in the presence of susceptible cells is generally considered to result from viral multiplication. The validity of this concept was indicated by the early classic work of Woodruff and Goodpasture (3). Apparently, viruses do not carry out demonstrable metabolic activities (4, 5). Moreover, they have not been shown to contain known enzyme proteins (2). If this is true, it seems important to inquire: How do viruses undergo multiplication, where do they acquire the substance and energy necessary for their synthesis, what orients and directs the process? Most workers now think that during their multiplication viruses derive the needed mass and energy from the host cell. But the directional, orienting influence appears to derive from the virus itself even though it may operate through the indispensable partner, the host cell.

Because viruses apparently are multiplied within the host cell and give little evidence of their presence until they burst forth, it is not simple to learn what goes on. Studies with bacteriophages, which are often termed bacterial viruses, have yielded important information. The development by Burnet (6) of a method for measuring the yield of virus from individual bacteria and the development of the one-step growth experiment and other techniques by Ellis and Delbrück (7) made possible close analysis of the multiplication of bacterial viruses. The process is considered as a series of step-wise events.

Chance collisions bring the viral particle and bacterial cell together. Attractive forces lead to adsorption of virus at the surface of the cell. One viral particle is all that is required to initiate multiplication, but the cell can adsorb a number of particles equal to the number it can produce (8). Adsorption is rapid and may be essentially completed in a few minutes (9). Adsorption co-factors such as univalent cations (10), tryptophane (11) or calcium may be required. The virus enters the cell, which then rapidly becomes altered with the result that other viruses usually cannot multiply within it (12). At this point mysterious events take over. The virus seems, in effect, to have disappeared as is indicated by the experiments of Cohen and Anderson (13) and Foster (14). This puzzling state, which is termed the latent or silent period, continues for 13 to 40 minutes, depending on the virus employed, when suddenly the virus reappears, i.e., bursts from the cell in large numbers (8). As Delbrück (15) has shown, one viral particle leads to the synthesis of 100 to 400 new viral particles in this short interval. Multiplication has occurred, of this there is no doubt, but it happened during the latent period and its stages were unobserved. The results of the numerous elegant experiments which have been carried out provide data about the kinetics of the interaction between bacterial virus and cell, the preliminary events and the final results, but shed no clear light upon the real problem: the biochemical mechanism of viral multiplication.

Results which are closely analogous have been obtained with certain animal viruses, especially the influenza group and mumps. Adsorption by susceptible cells (16-18), the probable nature of certain cell receptors (19), the duration of the latent period and the approximate number of viral particles liberated (20) have been demonstrated. The only striking difference from the bacterial viruses appears in the length of the latent period. With influenza viruses, Henle, Henle and Rosenberg (20) found that this period

is of the order of 6 to 9 hours, with mumps virus it appears to be considerably longer (21)

The riddle of multiplication has puzzled many workers since viruses first were recognized. Various hypotheses have been proposed. With the larger viruses, the psittacosis group as example, the life cycle hypothesis has its proponents, and changes in the size, staining properties and number of intra-cellular viral particles have been correlated with multiplication of the agents (22, 23). Growth and division, as with bacteria, is a possibility but many facts do not fit such a theory. Self-reduplication or autocatalytic processes have been invoked, chiefly since the crystallization of some plant viruses was achieved (24). These processes are hardly less mysterious than viral multiplication itself and do not provide a satisfactory solution to the problem (25). A virus precursor hypothesis has been put forward. According to this view (26) the precursor is present in the cell before infection and is transformed to virus by an autocatalytic process. Another suggested mechanism is the template hypothesis: virus is synthesized by the cell in conformity with the models (the templates) it presents to the intracellular enzymes (25).

The phenomenon of viral interference, perhaps more than any other, has called forth a turn in current ideas. Infection with one virus often precludes infection with another, one virus may act as inhibitor of the multiplication of another. The characteristics of the reaction (27, 28) are consistent with the idea that it is competitive. That it is not dependent upon competition for cell receptors appears established (27, 29), as is the fact that it can be obtained with inactivated virus (30, 31). Taken together, the existing data suggest that the competition may be for a metabolic system in the host cell and that both the integrity and availability of this system are essential for viral multiplication.

If all pairs of viruses showed interference, it could be supposed that all required similar metabolic pathways in the host cell. But numerous pairs of viruses do not show interference, some are capable of simultaneous multiplication in a single cell (32, 33). Because competition does not occur in these instances, it seems probable that non-interfering viruses demand different metabolic pathways of the cell. How many pathways for different viruses there may be is not known but in the cells lining the allantoic sac of the chick embryo it appears there are at least three (21, 29).

Studies on interference have raised the possi-

bility that identifiable metabolic steps may be demonstrable. More detailed information concerning cyto-chemical processes would be of value in unravelling their nature. In the absence of adequate information regarding cellular synthetic processes, attempts have been made to find substances which block viral multiplication and act as specific inhibitors. Knowledge of the chemical nature and structural configuration of such substances may help in identifying the metabolic steps which they block.

Certain acridine compounds have been found to inhibit the multiplication of certain bacterial viruses (34, 14) as well as influenza viruses (35). Foster (14) showed that proflavine concentrations which do not inhibit bacterial growth, inhibit completely the multiplication of T_4 and T_6 viruses. The concentration of inhibitor required is inversely related to the length of the latent period. Of importance is the finding that when introduced during the latent period proflavine effectively inhibits viral multiplication. Inhibition of T_2 and T_4 is obtained when the drug is added 12 to 15 minutes after infection, a period about equal to one-half of the latent period. When proflavine is added later during the period of multiplication, decreased yields of virus are obtained, the extent of inhibition is an inverse function of the time of addition of the drug. Apparently, proflavine blocks some late step in the process of multiplication but does not block earlier steps since removal of the drug during the first part of the latent period permits normal multiplication. Infected bacteria in which viral multiplication is inhibited by proflavine undergo lysis at the end of the normal latent period but do not yield any infective virus (14).

Cohen and Anderson (13) demonstrated that the multiplication of T_2 is inhibited by 5-methyl tryptophane. The inhibition is reversed by the addition of tryptophane (36). As with proflavine, complete inhibition is obtained only if the substance is introduced during the first half of the latent period. These findings raise the possibility that no infective virus is present within the cell during the first half of the period required for multiplication (14). Moreover, they suggest that multiplication is a discontinuous process during which virus particles are built up in steps and that only in the final stage are complete or mature, infective particles developed.

There are three means by which the presence of bacterial viruses can be demonstrated dependent upon their infectivity, specific antigenicity

and characteristic morphology, respectively. If multiplication occurs in steps, then antigens characteristic of the virus might be present before infective particles are evolved. There is no evidence available on this point with bacterial viruses. However, recent studies by Wyckoff (37) with the electron microscope showed that during the latent period distinct particles are present in the bacterial cytoplasm. At first they are extremely small. They increase in size and number rapidly until they replace much of the cytoplasmic material, but only just before the end of the latent period do they show the sperm-like morphology which typifies the mature, infective virus. These findings support the theory of a step-wise mechanism of viral multiplication.

With influenza virus there is evidence pointing in the same direction. This agent, like a number of other animal viruses, is capable of agglutinating red blood cells and its concentration can be measured by the hemagglutination reaction *in vitro* (38). Gard and von Magnus (39), in studies on the interference phenomenon, obtained startling results which indicate that at least two varieties of viral particles are produced. One variety is incomplete or immature, capable of agglutinating red cells but non-infective and of small particle size with a sedimentation constant of 380S. The other is the classical virus, infective and capable of causing hemagglutination and of usual particle size with a sedimentation constant of 660S. Thus, it appears that influenza virus, during one stage in its multiplication, occurs in a state which is almost entirely non-infective; computations indicate that only about 0.01 per cent of the particles were actually infective (39). These latter particles, however, may be of critical importance and probably are alone capable of initiating the process which results in the development of incomplete, non-infective viral particles. In earlier work Friedewald and Pickels (40) showed that influenza virus preparations contained a component which, although devoid of infectivity, caused hemagglutination and was smaller than the complete virus.

Recently, Hoyle (41) found that antigenic components characteristic of influenza virus, but of small size, are present in infected tissue well before mature, infective viral particles appear. Henle (42) has obtained similar results suggesting that with influenza virus, as with bacterial virus, multiplication may be a step-wise process.

The completed viral particle fails to emerge until a series of stages in its synthesis has been completed.

With certain plant viruses equally unorthodox evidence is accumulating. Markham, Matthews and Smith (43) showed that crystalline preparations of turnip yellow mosaic virus contained two components which could be separated by centrifugation. One component is a nucleoprotein and infective, a classical plant virus. The other is non-infective, smaller by 50 per cent than the complete virus and devoid of nucleic acid. Both components react identically with specific anti-viral serum, have identical electrophoretic mobilities, crystallize as octahedra and are spheres of similar size. The proteins of both have the same size and shape and contain the same amino acids in similar proportions. The infective virus contains 28 per cent pentose nucleic acid, the non-infective particle, none. It appears that the presence of combined nucleic acid is essential for the multiplication of the virus. It should be emphasized that no infective virus has been shown to be free of nucleic acid (1, 25).

These observations are indicative of what may happen to viral particles during the process of intracellular multiplication. However, they do not provide information as to the indispensable role of the host cell in the process. We are still confronted by the problems: How does the virus obtain the mass and energy necessary for synthesis, how does it orient and direct cellular metabolic processes?

Cohen and Anderson (44) showed that bacteria infected with a virus stop growing but maintain a constant rate of O_2 consumption. Ultraviolet inactivated T_2 stops the multiplication of *E. coli*. Moreover, ultraviolet inactivated influenza virus inhibits the development of the chick embryo (45). Infected bacteria carry out metabolic processes which give evidence of being abnormal. The most definite abnormalities appear in the synthesis of nucleic acids. Cohen (25) found that *E. coli* infected with T_2 synthesizes desoxyribose nucleic acid exclusively, the nucleic acid which is characteristic of the virus. Protein is synthesized from the beginning of infection, DNA synthesis does not begin for 8 to 10 minutes after infection. The available evidence (Cohen 25) suggests that the synthesis of viral peptides precedes and may be essential for the synthesis of viral nucleotides. In Cohen's view (25) the "energy and substance for virus synthesis are

supplied in entirely normal fashion" by the infected cell and the virus "organizes a specific enzymatic environment for its own multiplication." Tests of the hypothesis have not yet been possible with animal or plant viruses.

Studies on the biochemical mechanism of multiplication of animal viruses are beset with serious difficulties. As yet only a very few chemical inhibitors of multiplication have been discovered. Certain complex carbohydrates, polysaccharides, inhibit the multiplication of some animal viruses. A number of polysaccharides derived from bacteria, as well as some obtained from other sources, appear to stop the multiplication of pneumonia virus of mice (PVM) (46). The capsular polysaccharides of Friedländer bacilli are most active of those tested, 20 micrograms per mouse is effective. Friedländer polysaccharides also inhibit the multiplication of mumps virus in the allantoic sac, 5 micrograms per egg is sufficient (47). Inhibition of multiplication of either PVM or mumps virus results when the carbohydrate is introduced as long as 4 days after infection.

Friedländer polysaccharides do not cause inactivation of either virus, do not combine with them and do not prevent their adsorption by susceptible cells (18, 46, 47). On the other hand, the polysaccharides are taken up by host cells and remain fixed in them for long periods (21). The extent of inhibition of multiplication is directly related, although not strictly proportional, to the quantity of polysaccharide injected. Moreover, the extent of inhibition is inversely proportional to the amount of multiplication which has occurred, or to the time which has elapsed, before polysaccharide is given. Polysaccharide does not cause any reduction in the concentration of virus present in infected tissue, but further multiplication of the virus is inhibited (46, 47).

Three lines of evidence raise the possibility that inhibition of viral multiplication by Friedländer polysaccharides results from blockade of a metabolic pathway in the host cell. 1) Inhibition of either PVM or mumps virus multiplication is obtained when polysaccharide is introduced as late as 4 days after infection is initiated. At this time the concentration of virus is so high there can be no reasonable doubt that all susceptible cells are already infected (47). This fact and the finding that inhibitory polysaccharide does not prevent adsorption of virus by susceptible cells appear to exclude the possibility that an extracellular effect is responsible for the inhibition. 2)

The multiplication of mumps virus and PVM is inhibited by Friedländer polysaccharides, whereas the multiplication of influenza and Newcastle disease viruses is not (47). Mumps virus interferes with the multiplication of PVM and, moreover, influenza and Newcastle disease viruses reciprocally interfere with each other, but neither mumps virus nor PVM prevents the multiplication of influenza viruses (29). Thus, viruses which are inhibited by a polysaccharide cause interference with each other but do not cause interference with viruses which are unaffected by the same polysaccharide. 3) Treatment of Friedländer polysaccharide with alkali results in a product which is not capable of inhibiting viral multiplication. However, such altered polysaccharide blocks the inhibitory effect of the native polysaccharide (21). The blocking action of the altered carbohydrate shows characteristics which are consistent with the idea that it acts as a competitive antagonist relative to the native polysaccharide.

It should be emphasized that only a few viruses have been employed in recent studies on the mechanism of viral multiplication and in each instance the evidence is largely indirect and far from complete. Nonetheless, there are suggestive similarities in the findings with certain bacterial, plant and animal viruses which raise the possibility that they undergo multiplication in like manner. In each instance it appears probable that the viral particle itself may be developed through steps or stages and that the complete or mature, infective particle is chemically more complex than its incomplete predecessors. Of most importance are the indications that only the complete infective particle is capable of initiating the process which results in the development of further viral particles. Speculation as to the identity of the metabolic pathways of host cells apparently required by viruses probably would be unrewarding. But it may be that one step in such a pathway is indicated by the results of studies with chemical inhibitors. As has been pointed out, certain complex carbohydrates inhibit the multiplication of some animal viruses. If these substances are indeed structural relatives, are analogues, of an intracellular substance required in viral multiplication, then it may be that a step in the metabolic pathway which is controlled by polysaccharide is essential. Such an hypothesis has merit only in that it is not contrary to the available data and may provoke further study of the problem.

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THE PHYSIOLOGY OF THE INDIVIDUAL AS AN APPROACH TO A MORE QUANTITATIVE BIOLOGY OF MAN¹

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THE fact of individual variation is frequently taken to be merely an impediment to biological research. The customary approach is to emphasize those features that are common to all individuals and hope thereby to discern basic relations and general mechanisms. Individual variability is eliminated or minimized by taking enough individuals to get a 'good average'. With laboratory animals the numerical requirement is reduced by using inbred strains or litter mates. With man, where such expedients are almost never available, the task of sampling the whole population would be so great—and actually impossible in view of the unknown distribution of major biological characteristics—that it is really never attempted. We are confined to selected groups which may not represent any large and easily identified segment of mankind and may not even be homogeneous in those respects which are important to the problem. The most popular human guinea pigs, college students, are not necessarily very homogeneous except in regard to chronological age. In any case, the emphasis is on averages without critical definition as to source, on the one hand, and to application, on the other.

This is in sharp contrast with the practice of medicine and the general problem of evaluating individuals as in selecting airplane pilots or professors of physiology. The physician asks how his patient differs from normality, the Air Forces also ask about normality but with a different definition. And, presumably, college presidents have their own inscrutable standards.

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Dr. Olaf Mickelsen, now at the Division of Nutrition, U. S. Public Health Service, Washington, D. C., supervised the work on cholesterol in the year 1947-48. Miss Erma V. O. Miller, Assistant Scientist, made all of the cholesterol determinations.

It is easy enough to smile at the intuitive and empirical procedures used in medical management and in the selection of candidates for specific jobs. It is easy to be impatient with the non-discriminatory premium rates of insurance companies which presume that all 'non-diseased' persons, except for the obese, have equal disease expectations. As scientists, we should insist that there must be sound bases of a physico-chemical nature which have not been developed and utilized for such judgments. There is a very large area of human affairs in which the day-to-day operations, though concerned with human characteristics, proceed with a minimum of reference to knowledge of physiology, biochemistry, and the kindred sciences represented in the Federation. In our work there seems to have been, so far, relatively little of value for individual prediction.

This may be right and proper, perhaps these applied problems should be of no concern to us. There are three objections to this view. In the first place, if it is agreed that the physico-chemical characteristics of the individual should have predictive value, then those who are most expert at measuring and working with those characteristics should be best qualified to interpret the data. In other words, someone must be concerned with the scientific problem of the individual and in the technical area of physiology the physiologist should take the responsibility.

In the second place, consideration of the individual forces attention on the interrelations between variables, at least some of which must be hidden in the pooled data which refer to the common denominators of all dogs or all men. This means that the scrutiny of the individual should be an instructive point of departure for the study of general relations between variables in physiology and allied fields.

Finally, the experimental biologist, like the clinician, is constantly faced with the problem of deciding what is 'normal'. When does physiology end and pathology begin? But 'normal' function

may obviously be achieved by a variety of combinations of quantitatively different conditions. Moreover, values for a given characteristic which would be normal for one individual may be abnormal, that is pathological or predictive of future disorder, in another individual, depending on other characteristics and conditions. Once the problem of normality is seriously raised, logic demands successively sharpening and narrowing definitions until one comes to the individual. In essence, the problem is to place, quantitatively, the operating machinery of the individual—function and structure—in the spectrum of possibilities afforded by the species.

The simplest form in which the problem arises is in the evaluation of normality of a measurement. Having measured some characteristic of a person, the question follows: Is the person 'normal' with regard to this characteristic? To illustrate, we may consider the cholesterol in the blood, about which there is considerable interest at the moment. Basal blood samples drawn last December from two members of the laboratory staff, *Mr X* and *Dr Y*, were found to contain, respectively, 250 and 310 mg of total cholesterol per 100 cc of serum. Are these 'normal'?

Neither cursory nor exhaustive searches of the literature provide the basis for a satisfactory answer, but data recently obtained in the Laboratory of Physiological Hygiene may be analyzed for this purpose. We have values, obtained by duplicate analyses on basal blood samples, from each of 541 'normal' white men, who were leading an ordinary life in a gainful occupation not involving manual labor and who were pronounced free of detectable disease on the basis of medical examination. Their ancestral backgrounds, like that of *Mr X* and *Dr Y*, stemmed from the British Isles and Northern Europe. All measurements were made by the same methods and analysts in Minneapolis in the fall and winter months of the year. It will be observed that this material is characterized by an unusual degree of homogeneity as to source—species, sex, race, occupation, freedom from apparent disease, season, geographical location, physiological conditions and analytical method.

Surely it might be concluded that these data should provide a suitable reference standard for *Mr X* and *Dr Y*. The mean value for the 541 men is 218.7, with a standard deviation of 50.6 mg. *Mr X*'s value of 250 mg is only 62 per cent of one standard deviation above the mean and

would be expected, on the basis of normal probability, to be exceeded by about 25 per cent of the men in any normal sample like the reference group. *Dr Y*'s value of 310 mg, however, is 161 per cent of one standard deviation above the mean and a value as high as this would be expected to occur in only 5 per cent of the cases. Quantitative placement of *Mr X* and *Dr Y* has been made in good statistical terms and the first conclusion is that, with regard to blood cholesterol, *Mr X* is 'normal' and *Dr Y* has moderate hypercholesterolemia.

But, having made use of normal probabilities, it is well to check the normality of the distribution of the reference material before closing this chapter. A simple frequency-distribution plot of the data shows a reasonable form but when a true normal curve, calculated from the mean and

TABLE 1 TOTAL SERUM CHOLESTEROL, IN MG/100 ML, IN BASAL REST, IN 'NORMAL' MALES

AGE		NUMBER	CHOLESTEROL	
Mean	Range		Mean	S.D.
19	17-20	109	173.2	±30.2
22.5	21-25	92	179.5	±36.7
32	27-35	17	198.2	±25.3
43	39-44	16	222.4	±25.4
46	45-47	87	242.5	±37.5
49	48-50	98	249.1	±42.8
52.5	51-55	102	251.9	±43.0
68	63-74	20	233.9	±37.4
All ages		541	218.7	±50.6

standard deviation, is superimposed, it appears that the peak is too broad, it is 'platykurtic', and the suspicion is created that the data contained in it are not homogeneous. Obviously, the attempt should be made to segregate the 541 men into more closely defined groups.

Age was not originally considered because the literature generally disclaims a relation between age and blood cholesterol in adults (1-5). However, Brüger and Möbius (6) reported a distinct age trend. In any case, the result of segregating by age groups is given in table 1. Clearly, there is a very large effect of age. Low values in early manhood rise progressively with age, but apparently reach a plateau in the fifties, and slowly decline in the sixties. The implications are numerous and intriguing but are beside the present point which is that, for adult men, it is necessary

to specify age in considering the meaning of a particular cholesterol value

Mr X, who is 20 years old, now turns out to have a cholesterol level which is 76.8 mg above the mean of 109 men in his age group. With a standard deviation of 30.19 mg, such a value would be reached by only 0.5 per cent of the reference population and Mr X must now be labelled as definitely abnormal.

Dr Y's case is less definite, he is 50 years old and the mean value for 98 men in his age group is 249.1 mg, S.D. = 42.8. A value as high as his (310 mg) may be expected in only 7.6 per cent of normal men of his age. It is of interest to see whether a more definite result can be obtained by still closer scrutiny of his reference group.

TABLE 2 B.M.R. IN ML O₂/MIN/M² OF BODY SURFACE, AND TOTAL SERUM CHOLESTEROL, IN MG/100 ML FOR 'NORMAL' MEN 48-55 YRS OF AGE

B.M.R. CLASS	NO OF MEN	B.M.R. MEAN	CHOLESTEROL	
			Mean	S.D.
Higher (118.2-149.3 ml)	64	126.5	244.5	39.1
Medium (109.4-118.2 ml)	64	113.6	253.2	40.1
Lower (90.4-109.1 ml)	63	103.2	256.3	48.4
All (90.4-149.3 ml)	191	114.5	251.3	42.8

Since we have recorded many characteristics of these men, there is almost an embarrassment of possibilities. As a start, we may consider the basal metabolism, on the ground that blood cholesterol is often high in hypothyroidism. None of these men has myxedema but they do cover a considerable range in basal metabolism—from 90.4 to 149.3 ml of oxygen/minute/square meter of body surface in the men from 48 to 55 years of age. When the 191 men of this age, for whom full data are available, are arranged in 3 classes according to B.M.R., the result is as summarized in table 2. It is indicated that there is an inverse relation between B.M.R. and the blood cholesterol.

It would be of much interest to continue to sharpen the definition of the 'normal' standard groups, but in spite of the fair size (541) in the original total, the size of the sub-groups is getting small for the present type of analysis. For the next stage we may consider the combined medium and lower B.M.R. classes on the ground that they differ only slightly in their cholesterol averages. These 127 men have been arranged accord-

ing to relative fatness and divided into two groups with the results shown in table 3.

The criterion of fatness used was the thickness of the skin fold at a specified point on the abdomen, this choice being dictated from the results of rather extensive studies on the estimation of fatness in man. The result would be similar if only relative body weight were used, since this is closely but not perfectly correlated with the skin fold thickness. In any event, in agreement with

TABLE 3 RELATIVE FATNESS, AS ESTIMATED BY THICKNESS OF ABDOMINAL SKIN FOLD, IN MM, ('SKIN'), AND TOTAL SERUM CHOLESTEROL, FOR 'NORMAL' MEN 48-55 YRS OF AGE WHOSE B.M.R. IS BETWEEN 90.4 AND 118.2 ML O₂/M²/MIN

	NO OF MEN	SKIN MEAN	B.M.R. MEAN	CHOLESTEROL	
				Mean	S.D.
Thinner men (Skin' 9.5-24.6 mm)	64	19.2	109.0	249.8	45.7
Fatter men (Skin' 24.8-60.0 mm)	63	33.7	108.0	259.7	42.6

TABLE 4 BLOOD CHOLESTEROL AND EYE COLOR IN 127 MEN CONSIDERED IN TABLE 3 (48-55 YRS, B.M.R. MEDIUM TO MODERATELY LOW)

GROUP	N	MEAN	S.D.
Fatter men			
Brown eyes	22	265.8	±37.7
Blue eyes	41	256.4	±45.0
Thinner men			
Brown eyes	27	252.8	±31.9
Blue eyes	37	247.6	±50.4
All men with brown eyes	49	258.6	±38.5
All men with blue eyes	78	252.2	±47.0

advance suspicion, it is indicated that the fatter men tend to have the higher cholesterol values even at the same age and with substantially the same basal metabolic rates.

For a further breakdown it is at least amusing to try one more classification, using an item about which we have no preconceived notions. Eye color is known for all of these men so it may be used to classify further the same 127 men considered in the fatness breakdown summarized in table 3. The results are given in table 4.

In both fatter and thinner men of this age group

the brown-eyed men have slightly higher average cholesterol values than the blue-eyed men. Since both numbers and differences are rather small, we cannot yet conclude that there are real differences in the blood cholesterol related to eye color, but the values given in table 4 are the best estimates so far available for the particular classes of men. Such as they are, they must be used as reference standards in lieu of better ones. We may now return to the evaluation of *Dr Y* on this basis.

It so happens that *Dr Y* has a B M R of 106 cc of oxygen/square meter/minute, and an abdominal skin fold thickness of 32 mm, and his eyes are brown. His blood cholesterol value of 310 mg is only 44.2 mg above the mean for his group (first line of table 4) and that mean has a standard deviation of 37.7 mg. According to normal probability, some 12.1 per cent of men in his group may be expected to equal or surpass his blood cholesterol level and he must be judged fully 'normal' in this respect.

The foregoing is an illustration of the result of attempting to evaluate a characteristic of an individual. In the process it was necessary to give more than usual precision to the term 'normal' in the sense of 'average' and free from apparent disease. At the outset no real judgment could be made about *Mr X* and *Dr Y* because there were no real standards, much the same could be said about almost any characteristic besides blood cholesterol which might be chosen. In the first phase of the analysis, erroneous judgments were made because sources of variation in the standards were not segregated. The second, third and fourth phases of the analysis revealed a progressively changing picture. Because of exhaustion of the reference standard material, no more discrimination is possible, but it cannot be doubted that the tentative evaluation could be further improved by considering more characteristics in a larger sample. Moreover, it is conceivable that some other characteristics might be more significant and greater discrimination could have been achieved than with the characteristics tested here.

The value of refined standards such as developed here can scarcely be doubted. Equally important is the discovery of relations previously unknown or only suspected. Obviously it is now suggested that more specific research be undertaken with regard to the relations between blood cholesterol and the items considered here, namely basal metabolism, obesity and eye color. The attempt to evaluate the individual provides guidance for other research on mechanisms, though

nowhere in this analysis was there any direct consideration of those problems.

Of course, questions of mechanism were in the background when the initial question was asked as to the normality of the blood cholesterol values for *Mr X* and *Dr Y*. Currently, such a question is apt to be asked because of the belief that the level of blood cholesterol may influence or participate in the production of atherosclerosis. What *Mr X* and *Dr Y* want to know is whether their blood cholesterol indicates anything about their personal ageing processes and inclinations to earlier or later atherosclerosis. The analysis here says nothing about this but we hope that the long range program at the Laboratory of Physiological Hygiene will eventually contribute to this particular prediction problem.

Although the data are not at hand to attempt a prediction analysis regarding cholesterol and atherosclerosis, the general problem may be indicated with another example. On the International High Altitude Expedition to Chile (7) there were 9 men for whom various characteristics were recorded before they went to make a prolonged stay in the high Andes. Though all of the men were 'normal' at sea level, they showed great and consistent differences in their ability to acclimatize to altitudes above 12,000 ft (3660 meters). Were these strikingly different responses related to the differences in the recorded characteristics of the men at sea level? The analysis of this question may be summarized here because it reveals some of the essentials of the general prediction problem (8).

The differences in the individual characteristics at sea level were reliably established. At high altitude the men lived, ate and worked together, so 'external' circumstances were constant and the large differences in acclimatization must have been predetermined by inherent differences which already existed in the men at sea level. But the items we measured at sea level were not then known to be related to the factors which subsequently produced the different responses at high altitude, there were no clear clues as to which items we should measure. In the end, however, there were two sets of data, one on sea-level characteristics and one on high altitude responses, and the problem was to discover what, if any, were the relations between these.

Each item of measurement at sea level can be separately considered in the first phase of the analysis. Some of the characteristics, including vital capacity, blood pressure, muscle strength

and the Schneider cardiovascular fitness index, appeared to have little or no relation to the ability to acclimatize. But suggestive correlations were obtained with 7 of the variables as indicated in table 5. Ability to acclimatize would seem to be directly related to the alkaline reserve and to the partial pressure of carbon dioxide in the alveolar air at rest, and inversely related to age, to basal pulse rate, to body weight, to the hemoglobin concentration in the blood, and to the partial pressure of oxygen in the alveolar air.

Several of these indicated relations may be surprising but, *ex post facto*, they are physiologically reasonable. For example, it may be startling at first glance to suggest that a low hemoglobin at sea level should be predictive of high ability to acclimatize to high altitude. But the person who normally maintains a high hemoglobin concentra-

TABLE 5 CORRELATIONS BETWEEN OBSERVED ABILITY TO ACCLIMATIZE AT HIGH ALTITUDE AND VARIOUS CHARACTERISTICS RECORDED AT SEA LEVEL BEFORE GOING TO HIGH ALTITUDE. DATA FROM KEYS ET AL (8)

SEA LEVEL CHARACTERISTIC	CORREL COEFF
Basal pulse rate	-0.61 ±0.13
Alveolar pO ₂	-0.59 ±0.14
Alveolar pCO ₂	+0.63 ±0.13
Arterial blood alkaline reserve	+0.60 ±0.14
Blood oxygen capacity	-0.59 ±0.15
Age	-0.53 ±0.18
Body weight	-0.42 ±0.18

tion to meet the sea-level condition of oxygen supply can be considered to have a reduced margin to meet the more difficult situation at high altitude. Similarly, the person who habitually gets along at sea level at a ventilation level which amounts to relative 'underbreathing', may be considered to have a more efficient, or at least more economical, respiratory characteristic. This man will, of course, tend to have, at sea level, a low alveolar pO₂ and a high pCO₂.

In any case, these correlations indicate that each of these 7 variables has some predictive value for ability to acclimatize. We may inquire as to whether they may be combined so as to provide a more perfect index or measure of the factors which determine the ability to acclimatize. This would seem likely since at least some of the measured variables are not strongly intercorrelated. Four of the characteristics which have the great-

est independence may be combined in a least-squares solution for the prediction of acclimatization. The result is

$\text{Acclim} = K + 8.89 \text{ Alk Res} - 2.15 \text{ pO}_2 - 1.82 \text{ age} - 1.63 \text{ weight}$ (9). The acclimatization ability predicted from this equation is compared, in table 6, with that actually observed. The agreement is good and is statistically significant with the 9 men on whom it was tested.

It could be suggested that, since there were 7 sea-level characteristics which seemed to be related to later acclimatization, a better prediction equation could be evolved by combining all of them in a similar least-squares solution. This may be done, of course, and the final result of computation is given in table 6 (7 factors). But though the 'fit' is slightly better, the computational proce-

TABLE 6 OBSERVED RELATIVE ABILITY TO ACCLIMATIZE TO HIGH ALTITUDE AS COMPARED WITH VALUES PREDICTED FROM SEA LEVEL CHARACTERISTICS. DATA FROM KEYS, ET AL (8) AND FISHER (9)

SUBJECTS	OBSERVED	PREDICTED	
		7 Factors	4 Factors
B	59	61	60
C	32	32	30
D	34	34	32
E	46	45	46
F	65	65	73
H	10	9	11
K	90	89	84
Mc	26	27	28
T	50	51	50

dures are more laborious and the statistical significance is actually lessened because of the larger number of coefficients (9).

There would seem to be several values in this analysis: 1) unsuspected relations between variables were revealed, 2) suggestions were made as to critical items for study in regard to the mechanism of acclimatization, 3) it was shown that some variables may be profitably combined though otherwise we should not think of them as being associated, 4) a prediction formula was obtained for trial with other groups. It is reasonable to believe that a like outcome would emerge from a similar systematic attack on other problems of prediction besides the high altitude example cited here.

The two examples used in this discussion, deal-

ing with blood cholesterol and with acclimatization to high altitude, were chosen in part because in both cases there was a minimum of guidance available from considerations of cause and effect and qualitative biological theory. The deliberate choice was made of questions for which frontal attack on the actual questions asked could be attempted, at the present stage of knowledge, only by methods which are largely empirical in a biological sense. Unfortunately, it seems that the development of a true quantitative biology is still so rudimentary that the majority of questions pertaining to the physiology of the individual must be attacked with a similar emphasis on empirical methods, normal cholesterol standards and high altitude acclimatizations are by no means exceptional in this regard. This is not to decry in the least the virtue of a more theoretical approach. Given enough knowledge of theory and quantitative information on the mechanisms, both of the problems discussed here could probably be more easily and surely solved. The point is that such knowledge is now lacking and, as a matter of fact, a good deal of empirical gathering of data, plus rigorous analysis, is necessary to create the wanted body of knowledge.

It should be observed that even when a relation has been mathematically established between two variables, or between a present characteristic and a future development, this does not necessarily mean there is any direct cause and effect relationship. While this is a serious limitation, the fact of some kind of a relationship offers both a challenge and a clue to further study. Moreover, the habit of reasoning solely in terms of likely mechanisms is also objectionable unless supported by direct quantitative evidence. A simple example may be taken from the cholesterol data discussed here.

Lately, the suggestion has been made repeatedly that the development of atherosclerosis may be reduced or delayed by a reduction in the dietary intake of cholesterol on the ground that with a smaller intake there would be less cholesterol in the blood available for deposition in the arteries (10). A good many middle-aged people are being advised by their physicians to avoid eggs, butter, milk and fat meats on this account. Now it so happens that we have data on the habitual cholesterol intake of 312 of the men whose blood cholesterol levels have been examined. These men have been grouped according to their habitual cholesterol intakes and the groupings have been separated according to age.

The results are summarized in table 7. Neither in the younger (18-25 years) nor older (45-55 years) men is there any relation between blood cholesterol and the habitual intake of cholesterol, in spite of the fact that the differences in the latter are large.

The purpose of this discussion has not been to present data or even theories, but only to call attention to a point of view and an area of problems. It should be particularly observed in connection with the cholesterol data used to illustrate the argument that it is not, for the present, important whether the relations observed in the men studied at Minneapolis will apply precisely to other groups of apparently similar men. Using the Minneapolis data as a reference—and we must use these, at least temporarily, in default of

TABLE 7 BLOOD SERUM TOTAL CHOLESTEROL, IN MG/100 ML, AND HABITUAL DIETARY INTAKE OF CHOLESTEROL, IN GM/WK, IN NORMAL MEN 18-25 ('YOUNGER') AND 45-55 ('OLDER')

Younger men					
Number	32	30	30	32	32
Intake limits	1 9	1 9-2 5	2 6-2 9	3 0-3 4	3 4
Intake mean	1 49	2 18	2 64	3 15	4 20
Serum mean	175	180	165	176	184
Older men					
Number	32	30	30	32	32
Intake limits	2 1	2 1-2 5	2 6-3 1	3 7-3 9	3 9
Intake mean	1 62	2 27	2 83	3 56	4 81
Serum mean	242	258	249	253	251

any other—the best judgment of normality about *Dr Y* is made from the mean value, and standard deviation, for brown-eyed fat men, aged 45-55, who have medium to low rates of basal metabolism. We have not here asked any questions about the statistical significance of the apparent differences between the various groups segregated according to B M R, fatness, age and eye color. These questions are currently being examined, with a larger body of data, at the Laboratory of Physiological Hygiene.

The exploration of the frontiers of useful knowledge in the biological sciences increasingly demands a more quantitative approach which is apparent when specific questions are asked about individuals. Whenever individuals are concerned, it is necessary to consider their variation. When ever individual variation is examined it is clear

that there are such important interrelations between characteristics that the classical approach of isolating variables into single pairs (dependent and independent) must be used with care. The assumption of homogeneity is too readily made and the custom of dealing only with averages delays the recognition of the total truth.

The goal of all natural science is accurate, quantitative prediction. In the area of human health and disease, this begins with the problem of normality and normal standards. With the extension to development with time, these become more than devices for present classification of individuals and populations, they become the essential apparatus for prediction. But, because of the interdependence of most biological characteristics, simultaneous multi-variable analysis is required. This imposes the burdens of more rigorous selection and characterization of the

particular animals and men studied and the use of larger numbers and more complicated mathematical analysis. With all this, the need for true cause-and-effect understanding is not lessened. Pure empiricism has its limitations, no matter how systematic it may be.

We all realize that the development of the biological sciences is still in an early phase and a true quantitative science of biology remains for the future. I have tried to indicate that work on problems of individual physiology must be an essential part of the coming age of quantitative biology. One may approach these problems from the basis of questions about normality or about the prediction of future characteristics, the logical result is substantially the same. Finally, it may be agreed that the fact of individual variability represents far more than a technical obstacle to research, it offers a difficult but intriguing field for research in its own right.

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SOME COMMENTARIES ON ELECTRON MICROSCOPY AS APPLIED IN BIOLOGY

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IT is now some fifteen years since biological objects were first observed in the electron microscope. In this brief period, during which electron microscopes were made available commercially and a number of important preparative techniques were developed, it has become clear that electron microscopy is destined to play an important role in future biological and medical research. It is the purpose of this paper to discuss some of the technical procedures upon which these advances depend and to mention briefly a few of the contributions which electron microscopy has already made in the biological field. Finally, since this symposium concerns problems of experimental pathology, a few observations will be made concerning the application of electron microscopy in pathology.

RESOLVING POWER AND RESOLUTION

The resolving power of a particular electron microscope is determined by its construction and proper operation. The degree to which this inherent resolving power can be realized in the examination of a particular preparation depends importantly upon the nature of the material in the preparation. For example, if an electron microscope has a limiting resolving power of 25 Å, resolution of this order may be realized if the particles to be examined are of high electron density (e.g., colloidal gold). However, if the same instrument is used in the examination of relatively large protein fibers the resolution obtainable may not exceed 100–200 Å. In this case the large size of the particles and the low electron density of their constituent atoms may make it possible to realize but a fraction of the resolving power of the instrument. If the resolution (distance between centers of the closest particles which can be clearly distinguished as separate) is correctly determined the danger of drawing unwarranted conclusions as to the significance of images seen in electron micrographs will be reduced. There has been some tendency among biologists to infer

resolutions considerably greater than the micrographs justify. Indeed, one of the problems of the biologist is to prepare his specimens so as to obtain the maximum possible resolution of the objects of special interest in the specimen.

Some Technical Developments

The procedures of particular interest to the biologist are those which permit him a) to obtain the constituent of interest in a form which is most amenable to electron microscopic examination and b) to obtain information about thickness and detailed structure of the constituent. Some of the more useful of these may now be discussed.

Thin sectioning. Early attempts (1–3) to obtain sections of cells and tissues sufficiently thin for useful examination with the electron microscope (0.05–0.1 μ) were cumbersome and yielded but a small fraction of suitable sections. The development of high-speed sectioning (4–6) aroused considerable interest among biologists. A number of papers have been published showing electron micrographs of normal and pathological tissues sectioned by this method. However, the results left much to be desired, either because of relatively low resolution or because of poor preservation of the tissue structure.

The theoretical basis for the necessity of high velocity impact of the knife on the specimen has never been convincingly developed. Indeed, it has recently been shown by Pease and Baker (7) that quite satisfactory thin sections may be obtained with the conventional rotary microtome which has been fitted with a simple device to permit very small cutting increments. These authors have obtained electron micrographs of liver cells, muscle and other tissue which are of a quality at least as high as those obtained with the high-speed microtome though in few instances outstandingly superior to those obtained with the light microscope.

As in conventional cytology, the success of thin sectioning in the electron microscopic examination of tissue structure depends very largely

upon the preliminary fixation and embedding. Conventional methods are not suitable and much development will be required since the criteria of success are different than in the case of light microscopy. Doubtless special methods will have to be developed for each type of material. The mere fact that sections may be obtained which are sufficiently thin to be penetrated by the electron beam does not necessarily mean that the electron micrograph obtained will reveal considerably more information at a magnification of 10,000 than was revealed by conventional cytological methods at a magnification of 1,000. This is partly due to the fact that the process of fixation may precipitate materials upon the structures to be examined in a manner such as to prevent resolution of the details of structure within the object.

Thus the problems of fixation may plague the new generation of morphologists who use the electron microscope even more than it did their predecessors who used the light microscope. However, it is to be hoped that the mechanisms of fixation itself may now be amenable to a much more penetrating analysis since the electron microscope is capable of reaching down to the molecular level in visualizing the alterations which proteins and other organic substances undergo when treated with fixatives. At this level the study of fixation is the study of the composition and structure of the protein molecules themselves, a goal worthy of intensive effort.

Fragmentation methods. It was more or less generally assumed that significant advances in the electron microscopy of tissues could be made only by the development of thin sectioning techniques. However, it has already been demonstrated that the use of appropriate fragmentation techniques may yield highly significant results.

In the case of striated muscle it was demonstrated that from formalin fixed tissue fragmented with a blender, or with sonic oscillations, bits of myofibrils could be prepared which are susceptible of very profitable examination with the electron microscope (8). In such preparations it was possible to resolve the individual actomyosin filaments and to demonstrate that they course in parallel bundles through both the anisotropic (A) and isotropic (I) bands. This disproved the concept that the relative isotropy of the I bands is due to disorientation of the filaments in these regions. It was also shown that, in contraction, the filaments thicken and shorten but are not

thrown into coils or folds, hence the contractile elements are considerably thinner than the actomyosin filaments (ca 150 Å). There is also evidence for the existence of a periodic variation of density, thickness, or both, of the filaments in the axial direction. The period is of the order of 400 Å, which agrees with that obtained by x-ray diffraction studies of muscle (9) and of actin (10, 11). The manner in which this period varies with contraction or tension production has not yet been established with clarity, it is possible that further study of this aspect may throw important light on the contractile mechanism.

Another example of the use of fragmentation methods is provided by recent studies of the nerve axon (12). Frozen sections of formalin fixed nerves were fragmented with sonic oscillations. Fibrous structures were observed in the supernate. The fibrils characteristically have dark edges which suggested the term 'neurotubule'. After staining with phosphotungstic acid or after shadowing with chromium the fibrils manifest an axial periodicity in the form of cross-bands. The period averages about 650 Å, which is identical with that of collagen fibrils. This poses a difficult problem. Peripheral nerve fibers are invested with collagen fibers and, since the specimens are prepared by fragmentation of whole nerve it was necessary to demonstrate that the fibrils actually occur in the axon and are not merely extra-axonic collagen fibers which have acquired dark edges by the method of preparation, perhaps by adsorption of dispersed myelin material. The view that the fibrils are axonic in origin was supported by the fact that they were observed in fragmented axons isolated from the giant fibers of the squid and by the fact that after nerve degeneration the fibrils could no longer be observed (13).

This example illustrates the difficulty of localizing structures obtained after fragmentation of whole tissues. The difficulty happens to be particularly emphasized in the case of nerve because of the close similarity of the presumably intracellular structures with those of the extracellular collagen fibrils. The sampling difficulty is very prominent in this problem.

Tissue cultures. Another method of observing intracellular structures without the use of sectioning is that of tissue cultures. The cells are grown in a thin film so that they tend to flatten out and remain quite thin. When the culture has reached the desired stage the cells are fixed and transferred to metal grids for observation in the electron microscope. Various types of struc-

tures have been observed, including the limiting cell membrane, mitochondria, particulates of different kinds and, in certain malignant cells, dense particles which may be viruses (14-16) Fixation is also critical in such work not only as to the kind of fixative but also the duration of fixation, the structure may be strikingly different after 15 minutes' fixation in osmic acid from that after fixation for 45 minutes

With the current development of tissue culture techniques, particularly with respect to synthetic media, it is probable that the number of cell types and problems which may be studied by this method with the electron microscope will be greatly increased

Staining One of the most useful techniques employed by the histologist is that of elective staining by means of which he is able not only to achieve heightened contrast between tissue constituents but also to obtain information about the chemical nature and electric charge of the structures which combine with the stain

Stains are also valuable in electron microscopy However, in this case the heightened contrast depends on the electron density of the stains rather than their color, they are essentially 'electron stains' They are useful to the extent that they can attach molecules containing atoms of large weight to specific tissue constituents or to localized regions in these constituents Biological materials are composed predominantly of atoms of low weight Therefore some stains customarily used in histology may be useful as electron stains because of the presence of a few atoms of higher weight These stains have not yet been extensively explored Rather, emphasis has been placed on substances which contain atoms of great weight (17) Particularly useful are the coordination compounds of the heteropolyacids Osmic acid, which was early used in electron microscopy, is also an effective stain Phosphotungstic acid (PTA) has been extensively used in this laboratory though phosphomolybdic and uranyl phosphotungstic acids are also effective

Electron stains have been used very successfully in bringing out the fine structure of fibrous proteins Particularly striking is the case of the fibrils of the adductor muscles of molluscs Unstained fibrils show usually only an irregular, mottled appearance After staining with PTA, a periodic cross-banding, with spots occurring in a regular array along the bands, was observed (18) This structure agrees remarkably closely with

that deduced from small-angle x-ray studies (19) Detailed intraperiod banded structure was also observed in collagen fibrils after staining with PTA

Electron stains may function both in a specific and a non-specific manner Thus PTA may combine with specific basic groups in proteins depending on pH, concentration and so on To what extent such specific affinities may reveal the location of particular chemical groups in the structure stained remains to be seen As usually used, electron stains probably act in a relatively non-specific manner, essentially revealing local differences of thickness Detailed intraperiod structure with six or more bands can be observed in collagen fibrils stained with PTA (20-22) However, under favorable conditions, similar structure though with low contrast, can be seen in unstained fibrils From metal-shadowed specimens it was concluded that the bands which stain with PTA are regions of relative thickness It is possible therefore that the heightened contrast obtained with PTA is due in part at least to a non-specific volume effect

It seems quite possible that certain of the cytochemical procedures developed for the localization of specific chemical groupings, enzymes and so on, may be applicable in modified form in electron microscopy The writer has been investigating this possibility in the case of the acid phosphatase reaction which involves the formation of an insoluble lead salt about the structure containing the enzyme In this case the heavy metal would constitute an electron stain for this specific enzyme Success in such investigations will probably depend on the degree to which the reaction can be made truly specific

Heavy metal shadowing This technique, developed by Williams and Wyckoff (23, 24), has proven enormously valuable in biological as well as in most other types of electron microscopy When properly applied, shadowing reveals extremely small details of structure Indeed, already in the early days of its application in the study of viruses, thin protein filaments and even globular protein molecules themselves, the inherent structure of the film on which the specimen is deposited became a limiting factor The search for a film which is essentially structureless to within the resolving power of the electron microscope is still in progress In the meantime, film difficulties may be partially avoided by depositing the material in sufficient thickness to cover the

film completely and observing the discontinuities on the surface of the material. Precaution must be taken to avoid aggregation of the metal atoms by the action of the electron beam during examination in the electron microscope. Thus gold has a strong tendency to aggregation and this has given rise to erroneous conclusions in some instances where this metal was employed. Such difficulties are minimized by the use of metals like chromium, platinum and uranium.

Replicas Plastic replicas were originally developed for the study of the surface structure of metals and other opaque objects. Excellent electron micrographs have been obtained of the structure of tooth and bone (25-27). With the early successes with replicas it was hoped that the method might be applied to sections of tissues. However, thus far little has been accomplished in this direction, the chief difficulty is in successfully removing the plastic film from the tissue.

In its application to the study of the finest details of structure the replica technique suffers somewhat because of the finite size of the plastic particles and because of alterations which occur either to the specimen or to the replica at one or another of the steps in the procedure. Resolutions greater than 50-100 Å are seldom achieved. By shadowing the replica film very slight differences in elevation are brought out.

While it is impossible to observe biological structures in their normal aqueous environment in the electron microscope, it is possible to obtain replicas of moist material and in this way to get some information of the surface structure. This has been done in the case of bacteria in their culture medium (28-30) and also with collagen fibrils (31). Replicas of dried collagen fibrils show the characteristic periodic surface elevations corresponding to the cross-bands. Replicas of slightly moist fibrils show a smooth contour. This is consistent with the x-ray diffraction results (32) which indicate that, unlike dry collagen, the moist collagen fibril is essentially a smooth cylinder, loss of water from localized regions probably gives rise to the periodic axial contour of the dried fibrils. In applying the moist replica technique it is of course important to control accurately the water content of the specimen.

The replica technique which, like that of metal shadowing, has no counterpart in standard techniques of histology, will probably prove more and more useful in biological problems as technical difficulties are overcome.

SOME CONCEPTUAL CONTRIBUTIONS

To what extent has the infant field of electron microscopy made significant contributions to our concepts of the molecular organization of cells and their constituents? It is obvious that observations of importance to the physiologist or pathologist may be made at resolutions only two or three times greater than that obtainable by the light microscope (500-1,000 Å). However, it is in the colloidal range of about 25-100 Å that the most significant advances may be expected in the next decade.

The direct visualization of animal, plant and bacterial viruses has been of great academic and practical value. Thus far relatively little progress has been made in the study of the detailed internal organization of viruses though x-ray studies (33, 34) offer some clues in the case of the plant viruses. The details of the process by which viruses undergo self-duplication have not yet been penetrated though a beginning has been made in this important problem by Wilhams and Backus (unpublished).

The investigation of chromosome structure has fared less well probably because chromosomes are difficult to prepare in a condition best adapted to electron microscope study at high resolution. Recently, from a study of thin sections of the chromosomes of *Drosophila*, Pease and Baker (35) identified certain particles with the genes. However, until more definitive reasons for this identification are adduced and higher resolution obtained, such claims must be considered with caution.

Electron microscopy has contributed substantially to our knowledge of the structure of the fibrous proteins. Since fibrous arrays play a dominant role in many vital cellular processes, the investigation of their structure, their origin in cells and tissues and their alterations in physiological processes stand high in the priority list of problems in this field.

One of the most intriguing of the properties of protein fibers is their large repeating periods which may extend to hundreds of Angstrom units both in the axial and transverse directions. X-ray and electron microscope evidence for such periods has been obtained for collagen, keratin, myosin, actin, fibrin, protozoan trichocysts and several other proteins.

These regular repeating periods serve as 'finger-prints' by which particular fibrous proteins may be identified in tissues. However, the period in

itself may suffice to characterize only the *class* of protein. To identify the particular member of the class, it may be necessary to study the detailed intraperiod structure. Thus a number of proteins, differing widely from each other in chemical composition, belong to the 'collagen class' (36). From small-angle x-ray studies (37) it has been shown that, while members of this class all have approximately the same large axial period, the intraperiod structure varies from one to the other member of the class. This discovery may prove of great interest in attempts to devise a 'molecular taxonomy,' and perhaps 'phylogeny,' of the proteins.

Aside from their identifying value the repeating patterns may be expected to reveal something about the internal organization of the fibrous proteins. Since the bands and sub-bands repeat with very great regularity, there must be an underlying regularity of molecular organization.

From present evidence it appears that collagen is composed of a parallel packing of extremely thin filaments, possibly the polypeptide chains themselves represent the unit fibrous structures. The filaments have the inherent pattern of axial periodicity, to the extent that the neighboring filaments in a fibril are in register laterally with respect to the periodic structure, the whole fibril will appear to be cross-stratified.

The keratin-myosin-fibrin class of fibrous proteins appears to have a somewhat different structure. Thus fibrous actin is made of a linear aggregation of globular actin molecules (10, 11, 38, 39). The reversible transformation from the fibrous to the globular modification is not only of great physical chemical interest in itself but may be involved in muscle contraction (40).

The structure of fibrin is of unusual interest in connection with the theory of the causes underlying the production of long repeating periods. Physical chemical measurements indicate that fibrinogen molecules have dimensions of about 35×700 Å. Yet fibrin fibrils have a very accurately repeating pattern of about 250 Å (42) and fine structure has been observed within the bands (42). In aggregating to form fibrin, how do fibrinogen molecules give rise to a repeating period bearing no obvious relation to molecular length or composition? It seems clear that such phenomena depend upon interactions of sub-molecular units. Hall (42) has suggested that such interactions must involve colloidal forces, e.g. forces exerted over considerable distances. This type of spatial adjustment of small particles so

as to produce a macro-pattern of great regularity is fundamental in the production of structure in protoplasm. Alterations in these adjustments, due to changes in chemical environment, are probably at the basis of many physiological phenomena. The underlying physical forces which determine regular inter-particle spacings, as in tactoids, have been under investigation and some clarification of the various possibilities has been achieved (43).

ELECTRON MICROSCOPY IN PATHOLOGY

The rapidity with which electron microscopy will be profitably applied in the study of the structure of abnormal tissues and of the phenomena underlying pathological alterations will of course depend upon the development of new techniques which will have to be specially devised for the purpose. However, assuming that adequate techniques were at hand, it should be stressed that before abnormalities can profitably be studied, the investigator must be thoroughly familiar with the structure of the normal tissue. Such familiarity will probably require years of work with each special tissue. Particularly vexing is the sampling problem when working at such high magnifications. Unless one is able to show that adequate sampling of the specimen has been achieved, and some rough quantitative relations established, it is difficult to demonstrate with conviction that a particular difference of structure exists between the normal and abnormal specimens. 'Normal' specimens usually show a fairly wide range of variability, depending on conditions of preparation. The extent of these variations must be at least roughly known.

Reference was made above to the fairly complex technical aspects of electron microscopy. It should not be supposed that useful application of electron microscopy to biological and medical problems can be achieved only by physicists fully conversant with electron optical theory. On the other hand it should be clear that the biologist entering the field should be prepared to spend the time required to become thoroughly familiar not only with the operation of the instrument and with the accessory techniques, but also to learn the criteria by which his results may be correctly evaluated and interpreted.

Though the difficulties to be surmounted by the biologist in applying this technique to his problems may appear to be great, the rewards that await the deeper penetration of the structural organization of protoplasm are correspond

ingly great. The expected advances will be accelerated to the extent that the lessons of the past century of histology, with its uninterpretable

artifacts, are needed and, wherever possible, the full knowledge of modern biophysics and biochemistry is brought to bear on the problems.

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THE extensive breakdown of the teeth and their supporting structures among modern populations represents a real challenge to investigators in the broad field of public health. Some idea of the extensive destruction and loss of teeth produced by these two major causes of dental disability can be obtained from the annual cost of dental services in the United States. In 1945, approximately \$652,000,000 was spent for dental care, almost all of which was for restorative work (1), this figure actually represents 48.7 per cent of the cost for physicians' services and 13.1 per cent of the total outlay for all medical services. The Veterans Administration alone, in the last three months of 1948, spent a total of \$11,519,000 for restorative dentistry in World War II veterans (2). Since estimates have been made that the 83,000 practicing dentists in this country can care for little more than 25 per cent of the existing dental needs, the above figures represent only a part of the true picture (3, 4). Urgently as these figures indicate the need for preventive knowledge, their message seems all too weak when one is confronted in the clinic with a child in early adolescence with such rampant caries that complete extraction and full dentures are indicated.

Although technical procedures in dentistry developed through several centuries have attained a high degree of restorative perfection, not one of these gives the least promise of effecting a reduction in the incidence or rate of progress of

dental disease. Undoubtedly, the greatest hope for the prevention or retardation of either tooth decay or diseases of the supporting structures lies in the collection of fundamental knowledge about the various factors which influence the development and maintenance of the dental tissues. A wide variety of investigations need to be conducted to study the effect of various systemic conditions on 1) the differentiation, development and maturation of the teeth and their supporting structures (the gingival margin, the epithelial attachment of the gingiva to the enamel, the cementum, the periodontal membrane and the alveolar bone), 2) the metabolic processes in the mature, hard dental structures, in the supporting structures, and in the pulp and, 3) the rate of secretion, quantity and composition of saliva. A wide variety of the experimental tools and methods in the field of nutrition are readily applicable to study the relation of nutritional influences to the above dental problems. In order to evaluate the results of these investigations, full cognizance must be taken, wherever possible, not only of the length of the experiment and character of the diet during the experiment but, also of the state of development and condition of the teeth and their supporting structure, the previous history of these tissues, the ability of the experimental subject to utilize the food provided, and the hereditary constitution and the physical well-being of the control and experimental individuals, regardless of whether they are human subjects or laboratory animals.

Time does not permit an extensive historical

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résumé of the early literature on the relation of nutrition to dental caries, but it is of interest to comment on a few of the important investigations conducted in the past with human subjects. Early research in nutrition clearly related the nutritional value of the diet consumed during the developmental period to tooth structure. These findings prompted much speculation and research on the relation of nutrition, in particular of vitamin D, both during tooth development and after their emergence into the oral cavity on the development of tooth decay in children. The experiments conducted by Mellanby in the early 30's (5) indicated that the administration of vitamin D to institutionalized preadolescent children resulted in the development of significantly lower numbers of new carious lesions than were observed in the control children. These results have since been confirmed by other investigators (6, 7). The only negative results were obtained in experiments where older children were used (6, 8, 9).

Greater reductions in the dental caries attack rate were observed by Boyd and co-workers in diabetic and in normal children fed adequate diets over long periods (10). These reductions were attributed by the investigators to the nutritional adequacy of the diet. Certain experiments pointed strongly to the maintenance of a favorable calcium and phosphorus retention (11, 12). Careful dietary counselling of outpatients has been reported by Howe, White and Elliott (13), and by Becks and Jensen (14) to give reductions in the occurrence of new lesions by a magnitude of 60 to 70 per cent. Several investigations with children have been conducted in which the results were interpreted as indicating that increases of the soluble carbohydrate content of the diet resulted in increases in the initiation and rate of development of new carious lesions in fully formed and in recently erupted teeth (15, 16). The latter investigators have felt that the reductions in dental caries experience observed by Boyd and co-workers should be attributed to the low carbohydrate content of the experimental diet and not to their nutritional adequacy. From these conflicting backgrounds arose the current controversy as to the primary cause and methods of controlling tooth decay to which there is presently no complete answer.

Much more knowledge about the effects of nutrition during tooth development and maturation upon the susceptibility to dental caries

is needed in order to explain numerous exceptions to the current hypotheses concerning the susceptibility to tooth decay. A valuable demonstration of a developmental relationship between diet and caries resistance is provided in a large volume of dental statistics which have been methodically collected in the school clinics of Europe. Sognnaes (17) has assembled and analyzed available reports on the trends in dental caries experience among European children during the past forty years. A total of 27 surveys were evaluated which included observations on three-fourths of a million children from eleven European countries, Czechoslovakia, Denmark, England, Finland, France, Germany, Holland, Norway, Scotland, Spain and Sweden. Most surveys contained data which had been collected by trained examiners at yearly intervals over a period of several years and which had been tabulated by narrow age groups for limited geographical districts. Thus, the dental caries experience of the children of a given age in any year in these communities could be compared to that of the children of the same age in any other year. These excellent aggregations of material were studied for possible effects of dietary changes which were enforced on entire populations during World Wars and in the recovery periods thereafter. Because of the long duration of some surveys, the relation of dietary changes to the susceptibility to tooth decay could be evaluated in much greater detail than would be possible in the best controlled human experiments of shorter duration. In the summary of the trends in dental caries experience recorded in these surveys, Sognnaes concluded that the teeth of European children showed a definite and relatively uniform tendency to a decreased incidence of tooth decay toward the latter part of and following both World War I and II. The reductions in tooth decay were most significant in young children and in those teeth of older children which developed and/or matured during the war years. After the initiation of wartime dietary regimens, there seemed to be several years' delay before an appreciable reduction in the susceptibility of individuals to tooth decay was observed. Following the first World War there was an even greater delay in the return to the prewar susceptibility to tooth decay. Insufficient time has elapsed since the recent war to determine how long the present low dental caries attack rate will continue.

As an illustration of the time relationship between dietary changes and susceptibility to tooth decay, the dietary and dental data collected by several groups of Norwegian investigators during World War II permits the best analysis of the difference in reduction of tooth decay noted between those teeth which developed during war years and those which had erupted before, or shortly after, the beginning of hostilities. The annual sugar consumption of Norway was listed for 1936 as 36.3 kilograms (kg) per person. In 1939 before Norway's entrance into the war, sugar was rationed in Norway to 17.9 kg per person per year. This 50 per cent reduction apparently did not significantly reduce the susceptibility of the permanent teeth of 7-year-old children to caries. Through the years 1941 to 1945, inclusive, the average consumption per year was relatively constant at about 13.5 kg, a reduction of about 60 per cent below the prewar level. In addition to the sugar, large amounts of highly refined white flour were consumed by the Norwegian peoples in the prewar period. An estimate was made that 1242 calories of the average daily caloric consumption by an adult were supplied by refined white flour, and an additional 357 calories from sugar, i.e. more than 50 per cent of the daily calories were provided by refined carbohydrates. With the beginning of the occupation, there was a complete substitution of high extraction flour for the refined white flour and, simultaneously, drastic reductions in the amount of meat, dairy products, eggs, fruits and some vegetables. The loss of calories from these sources was largely made up by cheaper vegetables, mostly kohlrabi and potatoes, wartime bread, and fish when and where available.

The dental statistics for the Norwegian survey are summarized in table 1. For the purposes of the present comparison, the best age for observations of the initiation of carious lesions in the deciduous teeth was at 2½ to 3 years of age at which time the deciduous teeth have recently erupted into the oral cavity, a similarly suitable age for observation of the caries incidence in permanent teeth was in the 7-year-old group where the only permanent teeth were ones which had recently erupted. Unfortunately, data on the incidence of caries in the deciduous teeth of the 2½- to 3-year-old group were not collected for the years 1940 to 1943, inclusive. However, by 1944 there had been a 65 per cent reduction in

the number of carious teeth and a 44 per cent reduction in the number of children with dental caries. In 1945 and 1946 there were, respectively, 80 and 88 per cent reductions in the number of carious deciduous teeth, and 66 and 80 per cent reductions in the number of children with dental caries of the deciduous teeth. A more detailed evaluation is available for the permanent teeth of the 7-year-old children (figure 1). Negligible reductions in the number of carious teeth were observed during the first 2 years of the war.

TABLE 1¹ TIME LAG BETWEEN RATIONING OF REFINED CARBOHYDRATES AND REDUCTION IN DENTAL CARIES OF NEWLY ERUPTED TEETH (EXPOSED TO A SIMILAR ORAL ENVIRONMENT FOR A COMPARABLE LENGTH OF TIME)²

Norway									
YEARS OF WAR		PERCENTAGE REDUCTION IN CARIES FROM 1939 LEVEL				PERCENTAGE REDUCTION FROM THE PREWAR CONSUMPTION OF			
		Reduction in Carious Teeth		Reduction in Children with Dental Caries		Flour ³		Sugar ⁴	
		Deciduous teeth (2½-3 yr)	Permanent teeth (7 yr)	Deciduous (2½-3 yr)	Permanent (7 yr)				
		%	%	%	%	%	kg	%	kg
	1939	—	—	—	—	—	(120)	50	(18)
1	1940	(?)	6	(?)	0	35	(78)	70	(10)
2	1941	(?)	7	(?)	0	39	(73)	62	(13)
3	1942	(?)	23	(?)	0	39	(73)	62	(13)
4	1943	(?)	42	(?)	0	39	(73)	62	(13)
5	1944	65	57	44	7	39	(73)	62	(13)
6	1945	80	75	66	8	24	(91)	62	(13)
	1946	88	(?)	80	(?)	(? on the increase)			

¹ From SOGNAES, R. F. *Am J Dis Child* 75:792, 1948.

² Figures for the caries are averages from the youngest age groups recorded in Norwegian surveys 18, 20, 22, 23 and 24 shown in original table 1.

³ The use of refined flour was restricted before the invasion, and limited to babies and sick people after the occupation (April 1940). The extraction of the flour was increased from 69% before the war to 85% already in 1939 and soon after 95% of the grain was utilized for flour production.

⁴ The percentage reduction of sugar is calculated from the 1936 consumption of 36.3 kg per individual per year. The severest rationing 10 kg per individual (i.e. 70% below prewar), came into effect late in 1940. From then on until 1945 Toverud (by adding periodic increases) has estimated a weekly consumption of 260 gm (i.e. 13.5 kg per year or an average of 62% reduction from the prewar level).

Then major consecutive reductions were observed in 1942, 1943, 1944 and 1945, amounting to reductions of 23, 42, 57 and 75 per cent below the 1939 figure. In other words, the reduction in carious permanent teeth in 1945 after 6 years of war was higher than in any other previous war year. No reduction in the percentage of children with dental caries of the permanent teeth was noted until 1944 and 1945 when there were

7 and 8 per cent less children with tooth decay. A comparison for deciduous teeth can be made from the Norwegian data of Toverud collected in the towns of Blaker and Fet. The reductions in dental caries in the deciduous teeth of 2-year-old, 4-year-old and 6-year-old children are tabulated for the years 1939, 1944, 1945, 1946 and 1947 in table 2. Data were not available for 1940 to 1943, inclusive. The children in these three age groups examined in 1944 had the least reduc-

ed in 1945 and 1946 had 64 per cent less carious deciduous teeth than 4-year-old children in 1939. In 1947 there was a slightly lower reduction (55%). In the 6-year-old groups of children where the deciduous teeth have been exposed to the oral cavity for 4 to 5 years, the least reduction (21%) in dental caries experience was observed in 1944. In 1945, 1946, 1947 the reduction in carious deciduous teeth had increased to 44, 47, and 56 per cent, respectively. The

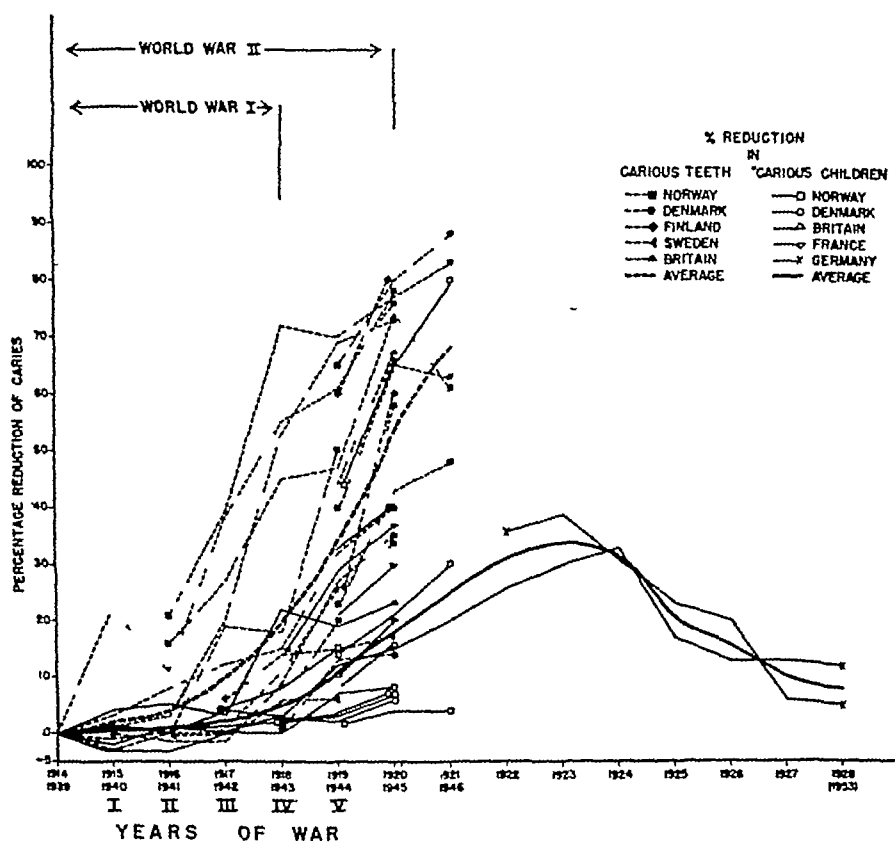


FIG 1 YEAR-TO-YEAR REDUCTION of caries-susceptible teeth and carious children during the two World Wars (From SOGNAES, R. F., *Am J Dis Child* 75:792, 1948)

tions in dental caries experience even though their deciduous teeth had been exposed to the oral environment induced by wartime diets for 1, 3 and 5 years, respectively. In the 2-year-olds maximum reduction (87%) in caries-affected teeth was observed in the children examined in 1946, i.e. born in 1944 where the teeth developed in 1944 and 1945. By 1947, there was a slight increase in the dental caries attack rate. Even in 1947, the 2-year-old children had 74 per cent less carious deciduous teeth than in 1939, this despite a gradual return toward prewar diets in the two years following the cessation of hostilities. In the 4-year-old groups, the children ex-

amined in 1944 had been developed in prewar years and appeared to have profited little by wartime dietary changes. However, those teeth in 6-year-old children examined in 1947 were formed in the years 1941-42 and had profited greatly even though the last 2 years of their oral exposure had been influenced by post-war diets.

The question arises as to the adequacy of the diet of the Norwegian children throughout the war years. Relatively good data have been collected by Stoltenberg (18) who reported that the caloric requirements of the children from birth to the age of 7 years were adequately provided

From 7 to 20 years of age there was an increasing caloric deficit from an average of 370 for the youngest to 1260 in the oldest. All public school children were stated to be taller in 1943 than in 1935. Underweight children were observed in prewar years at a rate of 16 per cent of the child population, this figure had only increased to 24 per cent in 1943. Therefore, it appears unlikely that the reductions in tooth decay were due to any restriction in the caloric

TABLE 2¹ DEVELOPMENT AND POSTERUPTIVE CONDITIONS OF DECIDUOUS TEETH OF NORWEGIAN CHILDREN DURING AND AFTER WORLD WAR I²

AGE	YEAR EXAMINED	YEAR OF BIRTH	YEARS OF DENTAL DEVELOPMENT	YEARS OF ORAL EXPOSURE	CHILDREN WITH CARIES	REDUCTION IN CARIES AFFECTED	
						Children	Teeth
					%	%	%
2	1939	1937	1937-1938	1938-1939	75	—	—
2	1944	1942	1942-1943	1943-1944*	42	44	85
2	1945	1943	1943-1944	1944-1945	25	67	78
2	1946	1944	1944-1945	1945-1946	15	80	87
2	1947	1945	1945-1946	1946-1947	34	54	74
4	1939	1935	1935-1936	1936-1939	100	—	—
4	1944	1940	1940-1941	1941-1944*	86	14	38
4	1945	1941	1941-1942	1942-1945	79	21	64
4	1946	1942	1942-1943	1943-1946	70	30	64
4	1947	1943	1943-1944	1944-1947	74	26	55
6	1939	1933	1933-1934	1934-1939	100	—	—
6	1944	1938	1938-1939	1939-1944*	97	3	21
6	1945	1939	1939-1940	1940-1945	95	5	44
6	1946	1940	1940-1941	1941-1946	95	5	47
6	1947	1941	1941-1942	1942-1947	87	13	56

¹ From SOGNAES R. F. *Am J Dis Child* 75: 792, 1948.
² Interpretation of chart by Toverud from examinations at Blaker and Fet.

* The groups whose teeth after eruption have been exposed to an oral environment favored by the greatest wartime reduction in refined carbohydrates. Yet these are the groups which show the least reduction in caries. A "developmental" explanation is suggested in text.

consumption. In addition, Ström (19) studied the food habits of 102 families in Oslo and two smaller localities who were able to maintain a daily average of 2,847 calories (2708 to 3046) per person per day throughout 1942 to 1945, inclusive.

The marked reductions in dental caries experience among European children during the latter years of World Wars I and II cannot be explained entirely on the basis of an oral environmental effect produced by a reduction in the refined carbohydrate content. The sudden onset of restrictions in the refined carbohydrates

available for distribution and the constant amounts available for rationing in Norway throughout the years 1940 to 1944, inclusive, eliminates any argument that the reduction in dental caries incidence was gradual and prolonged because of a gradually increasing restriction in refined carbohydrates. If the oral environment, as influenced by the composition of the diet, had been the preeminently important factor in determining the dental caries experience of these children, the reduction in tooth decay should have been evident within a year or two in all children with recently erupted teeth regardless of whether the teeth were formed before, at or after the initiation of the wartime dietary regimen. In view of the long delay in the production of a reduction in dental caries experience, Sognaes (17) believed that the wartime diets appeared to have permitted the formation and maturation of teeth which were less susceptible to the development of carious lesions. Even when these teeth were exposed to the oral environment produced by post-war diets, they were highly resistant to decay. If the delay in caries reduction had only been observed in Norway in World War II, reasonable doubt might be cast upon this interpretation, since similar trends of rather uniform magnitude had been observed in ten other European countries during World War I and II, the relationship of wartime diets during tooth development to an increased resistance to dental caries appears to be well established. Despite the conclusiveness of the above relationship, there has been an almost universal tendency on the part of the European investigators who collected these dental statistics to interpret them as strong evidence of oral environmental changes without preliminary influences on the tooth structures.

There is a real need to continue to collect survey data of this type in the hope of determining how long the reduction in caries continues and whether any future change can be correlated with dietary alterations. Parallel to the collection of more human statistics, many investigations with laboratory animals are needed to evaluate the exact role of diet during tooth development. During the period from 1910 to 1945, inclusive, animal experimentation in the dental field did not keep pace with human investigation in quantity and much less in quality. The white rat was used frequently in experiments designed to test human findings with unpredictably confusing

and conflicting results. In the middle of this era, Hoppert, Webber and Canniff (20) observed that various lesions developed readily in the molar teeth of the white rat only if the experimental diet were composed largely of coarse particles. When the coarse particles were ground finely, dental caries did not develop. Thereafter investigators demonstrated that the coarse particles caused a mechanical injury to the enamel surfaces followed by tooth decay (21). When the molars of the upper jaw were extracted,

foodstuffs. Two years later, the molar teeth of cotton rats which had been maintained for periods of 10 to 14 weeks on purified rations were found to have been susceptible to the initiation and development of carious lesions (25). The composition of a typical caries-producing, purified ration is given in table 3. The incidence of tooth decay was particularly high in the third molars which were largely developed during the experimental period and which did not erupt until late in the experiment. Tests on the effect of masticating this ration made by the extraction of opposing molars indicated that there was a much less important effect of mastication upon the initiation and progress of carious lesions in this species than had been observed previously in the white rat fed the caries-producing, Hop-

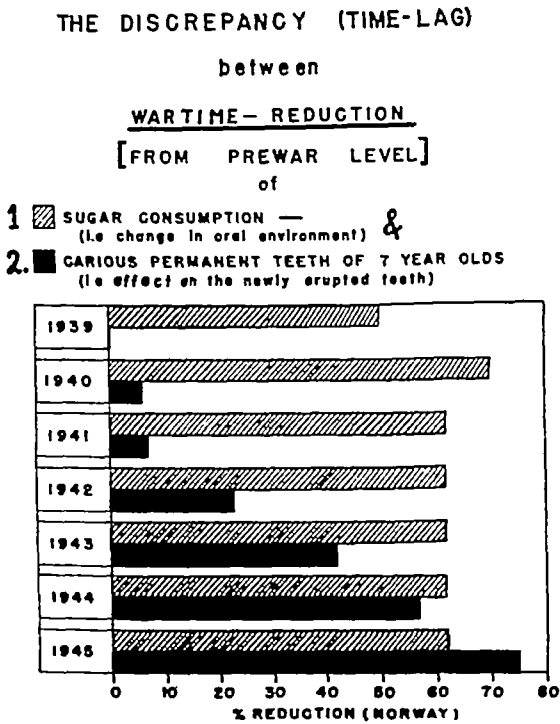


FIG 2

prior to administration of the coarse particle diet, practically no lesions developed in the lower molars (22, 23). Since human caries is not believed to result from such mechanical causes, this type of regimen was untenable.

In the last seven years, satisfactorily controlled experimental circumstances have been devised for the study of tooth decay in four species of experimental animals: the Syrian hamster (*Cricetus auratus*), the cotton rat (*Sigmondon hispidus hispidus*), the common laboratory rat (*Mus norvegicus*) and the rhesus monkey (*Macaca mulatta*). In 1942, Arnold (24) observed that carious lesions developed in the molar teeth of hamsters which were fed finely ground, high carbohydrate diets composed largely of natural

TABLE 3. A TYPICAL CARIES-PRODUCING, PURIFIED RATION

	gm		mgm
Sucrose	67	Thiamine	0.35
Casein	24	Riboflavin	0.35
Corn oil	5	Pyridoxine	0.35
Salts	4	Calcium pantothenate	2.0
1:20 liver concentrate		Niacin	2.5
powder	2	Choline chloride	100.0
Whole liver substance		Inositol	100.0
		p-aminobenzoic acid	30.0
	104	β-carotene	1.1
		α-tocopherol	5.0
		2-methyl 1,4-naphthoquinone	0.6
		Irradiated ergosterol	1 U
			310

pert-Webber-Canniff diet (22, 23). Further evidence that the carious lesions in cotton rat molars were not preceded by mechanical injury was obtained through the examination of ground sections of teeth and jaws after varying periods of experimental exposure. In addition, if all ingredients in the purified ration were finely powdered, the rates of initiation and development of carious lesions were not altered (26).

Examples of the need for careful control of all possible variants in caries investigations with laboratory animals are presented in tables 4 and 5. In the former, an effect of caloric restriction to produce a reduction in the initiation and development of carious lesions is recorded (27). In the latter, the results of two experiments to study if there were any effect of the type of cage and bedding upon the dental caries experience are tabulated (26). Those cotton rats housed

in screen-bottom cages had a higher dental caries attack rate than littermates maintained in cages where the sheet metal floors were covered with shavings. On the contrary, cotton rats housed in cages where the sheet metal floors were covered with paper clippings did not have any different caries experience than littermates kept in cages with screen bottoms. The

TABLE 4 EFFECT OF CALORIC RESTRICTION

AMT OF RATION 100	NO OF RATS	WT GAIN	NO OF CARIOUS LESIONS		EXTENT OF LESIONS	
			Mean	C R ²	Mean	C R ²
A) Controls fed ad libitum	9	gm 73	26.1 ± 2.2 ¹	3.1 (a,b)	79 ± 9	4.0 (a,b)
B) 65% of amt controls received	11	32	17.5 ± 1.8	5.2 (a,c)	35 ± 6	0.6 (a,c)
C) 50% of amt controls received	6	16	12.0 ± 1.5	2.4 (b,c)	20 ± 3	2.1 (b,c)

¹ Standard error of mean ² Critical ratio

TABLE 5 AVERAGE NUMBER AND EXTENT OF CARIOUS LESIONS OBSERVED IN COTTON RATS MAINTAINED FOR 14 WEEKS IN CAGES WITH DIFFERENT TYPES OF FLOORS AND BEDDING

TYPE OF BEDDING	NO OF COTTON RATS	AV NO OF CARIOUS LESIONS	S.E.M. ¹	C.R. ²	AV EXTENT OF CARIOUS LESIONS	S.E.M. ¹	C.R. ²
None	7	33.7	1.3	6.0	110+	6+	6.1
Wood shavings	10	17.8	2.3		39+	8+	
None	9	27.2	2.8	0.9	80+	12+	0.9
Paper clippings	10	24.1	2.7		67+	8+	

¹ Standard error of mean ² Critical ratio

causes of the effects on dental caries experience occasioned by caloric restriction and by the type of bedding are unknown, but the effects are sufficiently great as to necessitate careful control. Sognnaes (28) has studied the caries-conducive effect of the above purified diet when fed to rodents during, as well as after, tooth development. If weanling rats, hamsters and mice born to females fed laboratory chow throughout the reproductive cycle were fed the chow diet for periods of as long as 9 months after weaning,

practically no carious lesions developed in the molar teeth. When weanling white rats and hamsters born to mothers maintained on chow throughout pregnancy and lactation were fed the purified ration for 4 to 8 months, a few carious lesions developed in their molar teeth. In contrast, when female rats and hamsters were changed from chow to the purified ration at parturition and their offspring maintained on the latter diet for 3 to 5 months after weaning, the molar teeth of the offspring developed a rather high number of carious lesions. A still higher rate of dental caries experience was observed in offspring of female rats and hamsters which had been fed the purified ration throughout pregnancy and lactation. The increases in number of molars and number of areas affected by the

Experimental Rations During		
PREGNANCY	LACTATION	POSTERUPTIVELY
stock	stock	stock
stock	stock	sucrose
stock	sucrose	sucrose
sucrose	sucrose	sucrose

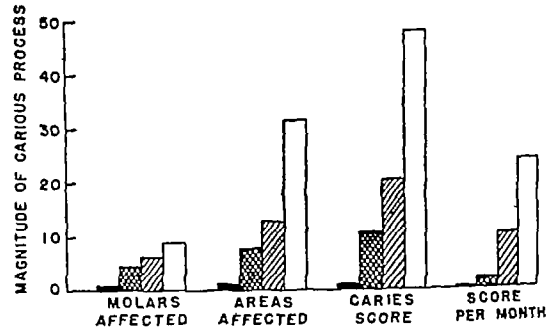


FIG. 3 CARIOGENIC EFFECT of a purified experimental diet (labelled sucrose ration) during tooth development (Syrian hamster) (From SOGNNÆS, R. F. J. Am. Dent. A., 37: 676, 1948)

carious process, and the increase in caries score which were observed for the Syrian hamster are presented in figure 3. In the mouse similar but less striking developmental effects of the purified ration during tooth development were observed. There is a very strong parallel between these studies with the rodents and the above interpretation of the trends in dental caries experience of European children. The white rat and the hamster appear to be suitable experimental animals for the evaluation of dietary influences during tooth development. One possible drawback to the use of these species is the fact that the crowns of the first and second molars are in an advanced state of development at birth and the third molars are largely formed at weaning. Thus, any

dietary effect on these stages of development has to be mediated through the maternal organism via the placenta and/or the milk. This does not seem to be a serious difficulty in view of the qualitative effect of diet during tooth development already described.

One advantage of the cotton rat in developmental studies is the early weaning age (14 days) at which time the third molar crowns are in an early stage of development. The remainder of their formation can be influenced by diets begun at weaning. Another illustration of the inadequacy of the purified diet for the development of the dental structures has been observed through this method of studying the developmental rate of the third molar in the cotton rat. Offspring maintained for 100 days after weaning on the purified diet had a retarded rate of eruption and root formation in the third molars. Among 854 cotton rats killed at this age, the upper third molars of 77 had not pierced the mucosa, an additional 81 had emerged into the oral cavity but were only partially erupted. In contrast, the upper third molars of all cotton rats maintained on natural diets until the 54th postnatal day, or longer, were fully erupted. A similar but less severe retardation in the development of the lower third molars of cotton rats fed the purified diet was observed (26).

Various studies have been made of methods for altering the initiation and rate of development of carious lesions in fully developed molar teeth of weanling cotton rats and common laboratory rats which had been bred for high caries-susceptibility. When representatives of the susceptible strains of both species were maintained for appropriate periods after weaning on the basal purified ration, a high caries attack rate was observed. If diets were fed in which the level of fat, or protein, or both were increased at the isocaloric expense of sucrose, appreciable reductions in dental caries experience were observed (26, 29). If the sole source of nutrients were mineralized whole milk, no carious lesions developed in either species of highly susceptible rodents, as much as 10 per cent of sucrose or other soluble carbohydrates by weight could be dissolved in the mineralized whole milk without any appreciable increase in the dental caries experience (26, 29). At least part of the reduced development of carious lesions observed when milk diets were fed has been shown to be due to its fluid nature (30). Thus, there are numerous dietary procedures in rodents by which develop-

ment of dental caries after tooth formation is largely completed can be influenced.

As an extension of these studies to primates, species of monkeys have been sought which were susceptible to dental caries. Although the rhesus monkey maintained on natural diets throughout life has an extremely low incidence of tooth decay, representatives of this species maintained on purified diets for long periods have been found to develop a moderate number of lesions (31). The following experiment has been conducted to determine whether certain factors which influence the development of tooth decay in rodents would have the same effect in primates. Twelve rhesus monkeys, purchased from an animal dealer after capture in their native environment, have been maintained from the time they were about 1½ years of age for an additional 3½ years on highly purified rations (32). The 6 monkeys maintained on ration I, which contains 73 per cent sucrose, have not developed any extensive degree of caries. In 2, no caries has been found by oral examination and radiographs. Four have small, slowly progressing carious lesions in the first permanent molars which were almost completely formed at the beginning of the experiment. The second permanent molars and the first and second bicuspids have been formed to a large degree while the monkeys were maintained on the purified ration and have recently erupted. Insufficient time has elapsed since their eruption to permit development of detectable carious lesions. The 3 monkeys maintained on ration II, in which all sucrose has been replaced by fine dextrin, have only developed a few minute lesions. One is caries-free, and 2 have slowly progressing lesions in the first permanent molars. The three monkeys maintained on ration III, in which one-half of the sucrose has been replaced isocalorically by lard, have as yet developed no carious lesions.

In contrast to the above monkeys, 4 rhesus monkeys were transferred to purified ration IV containing 64 per cent sucrose at a much younger age and have in a much shorter period developed definite carious lesions even in the generally less susceptible deciduous dentition. Two were born in captivity in the colony of the Obstetrics Department of the Yale Medical School. Both developed definite carious lesions in the deciduous teeth within a few months after the purified ration was first fed. The other two, one male and one female, while born in the wild were transferred to the purified ration at the time of weaning. In

both cases, the second deciduous molars have definite carious lesions after a year's maintenance on the purified ration, during which time their weight has doubled

The demonstration of the above contrast seems to merit considerable further investigation. The fully formed teeth of the older monkeys, reared in the wild, appear to have a great caries-resistance, which even the high sucrose ration with the demonstrated ability to produce a high dental caries attack rate in caries-susceptible rodents, has been unable to fully overcome in 3½ years. True, even the older monkeys maintained on this ration exhibited more tooth decay than comparable monkeys maintained on natural diets. However, none of the older monkeys attained the degree of caries observed in the 4 younger monkeys which were transferred to the purified ration regimen at an earlier period of development.

In summary, there appears to be little question about the importance of nutrition during tooth development and maturation in determining the susceptibility to dental caries. At the present stage of the investigations, there is insufficient data to justify speculation as to the identity of the active factor, or factors, which mediate this effect or the mechanism of operation. In most future studies, it will be necessary to define the stage of development of the teeth under investigation and wherever possible, the dietary circumstances which preceded the experimental period. Both human surveys and animal experimentation justify the conclusions: 1) teeth can be developed by proper nutritional regimentation which are highly resistant to tooth decay under extreme caries-producing circumstances, and 2) teeth which are highly caries-susceptible at the time of emergence into the oral cavity can be protected from the carious process to some degree by dietary methods. It is scarcely necessary to emphasize that our present knowledge in this subject can be considered as little more than a fundamental introduction into a complex and controversial problem. The prevalence of dental caries and its sequelae, inflammation of the pulp, cysts, abscesses and further focal and systemic infections indicate the necessity for extensive exploration of the relationship of nutrition during dental development, maturation and maintenance.

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AMINO ACID REQUIREMENTS OF MAN¹

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THE isolation and identification of threonine (1, 2) rendered it feasible, for the first time, to support growth upon diets carrying appropriate mixtures of highly purified amino acids in place of proteins. By the successive removal from such diets of single amino acids, the nutritive role of each was established (3). The results demonstrated that, for the growing rat, ten amino acids are essential dietary components. These are valine, leucine, isoleucine, threonine, methionine, phenylalanine, tryptophan, lysine, histidine, and arginine. The exclusion from the food of any one of these, other than arginine, leads to a profound nutritive failure, loss in weight, diminished appetite, and eventual death. In contrast to these spectacular effects, arginine deprivation merely decreases the rate of gain (4). This is accounted for by the fact that arginine can be synthesized by the rat (5), but not at a rate which keeps pace with the needs of the organism for maximum growth. This behavior distinguishes arginine from the amino acids of the non-essential group. The latter apparently can be manufactured in sufficient amounts to meet fully the requirements of the animal.

The above experiments provide much information concerning the types of synthetic reactions which the animal organism can and cannot accomplish. However, the ultimate objective throughout our investigations has been the establishment of the amino acid requirements of man. It was anticipated that time and expense might be saved by first conducting the animal tests, and then applying the results thus obtained to the more difficult and costly studies upon human subjects. Subsequent events have fully justified this supposition.

Investigations in the human species were first undertaken in this laboratory during the autumn

of 1942, and since then have been in progress continually. Publication of the detailed findings has been postponed deliberately in order to permit the accumulation of a large number of data upon a comparatively large number of individuals. It was hoped thereby to diminish the probability of errors in interpretation. The results now appear to be sufficiently numerous and definite to warrant positive conclusions.

Healthy male graduate students have served as the experimental subjects. In formulating the diets, only those articles of food were selected which are virtually devoid of nitrogen of an unknown nature. These consisted of corn starch, sucrose, butterfat (which had been melted and centrifugated to remove particles of protein), corn oil, inorganic salts, and vitamins. The starch, corn oil, inorganic salts, and parts of the sugar and butterfat were mixed with water and baked into wafers. The remaining portions of butter and sugar were consumed separately, the former as a spread for the wafers, and the latter as a flavoring agent for the amino acids. The vitamins were supplied in the form of daily doses of cod liver oil, thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, nicotinamide, ascorbic acid, calcium *D*-pantothenate, α -tocopherol, 2-methyl-1,4-naphthoquinone, and a concentrate of liver (6) equivalent to 5 gm of Wilson's 'liver powder 1:20'. The amino acid mixtures furnished 6.7 to 10 gm of nitrogen daily, and were taken in solution flavored to taste with filtered lemon juice and sugar. From such a diet, the daily intake of nitrogen of an unknown nature amounts to 0.30 to 0.35 gm.

The results of the *qualitative* tests have already been summarized elsewhere (7), and need not be repeated here. The details of the procedures and findings will be published at a later date. In the meantime, it is sufficient to present the final classification of the amino acids. This is shown in table 1.

As will be observed, the amino acids which are required by adult man for the maintenance of

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nitrogen equilibrium are identical with those previously found necessary for the maximum growth of the rat, except in the case of histidine and arginine. The findings with respect to arginine are not surprising inasmuch as the adult rat (8) and the adult dog (9) have been shown to be capable of manufacturing this amino acid at rates commensurate with its use for maintenance. On the other hand, the observation regarding histidine was quite unexpected. This amino acid is required by all higher animals thus far tested. Despite this fact, its exclusion from the diets of the human subjects induced no change whatsoever in the state of the nitrogen balance. At first, the results were viewed with skepticism. It was suspected that histidine had found its way into the food despite the intensive purification

TABLE 1 CLASSIFICATION OF AMINO ACIDS WITH RESPECT TO THEIR ROLE IN MAINTENANCE OF NITROGEN EQUILIBRIUM IN NORMAL ADULT MAN

ESSENTIAL	NON ESSENTIAL
Valine	Glycine
Leucine	Alanine
Isoleucine	Serine
Threonine	Cystine
Methionine	Tyrosine
Lysine	Aspartic acid
Phenylalanine	Glutamic acid
Tryptophan	Proline
	Hydroxyproline
	Histidine
	Arginine
	Citrulline

of the amino acids. Careful tests excluded this explanation. Furthermore, repetition of the feeding trials with other subjects led to identical conclusions. To date, 42 individuals have been kept in nitrogen balance upon diets which were devoid of this amino acid. Evidently, histidine, like arginine, is not an essential dietary component for the maintenance of nitrogen equilibrium in human adults. Whether the growing child, like the growing animal, requires histidine, arginine, or both of these amino acids, must await the results of further investigations. Moreover, one cannot yet exclude the possibility that certain amino acids which are not necessary for nitrogen equilibrium in normal subjects under ordinary circumstances may become indispensable during disease, or for special body functions such as detoxication, reproduction, or lactation.

Having established *qualitatively* the amino acid requirements of normal man, *quantitative* studies were next undertaken. The objective in the latter was to discover the minimum intake of each of the 8 essentials which is capable of permitting nitrogen balance when the diet furnished sufficient quantities of the other 7, and an adequate supply of nitrogen for the synthesis of the non-essentials. In the following pages attention is directed particularly to this aspect of the problem.

Before proceeding further, the reader should be appraised of certain limitations inherent in nitrogen balance studies, particularly when applied to the establishment of minimum requirements. The first of these is associated with the daily fluctuations in nitrogen output encountered in investigations of this sort. Regardless of how accurately one may control the nitrogen intake, and how diligently he may prescribe the routine physical activities of the subjects, moderate day to day variations in the excretion of nitrogen are observed almost invariably. This fact is not limited to experiments involving amino acids. It is equally true of subjects upon protein diets. Perhaps the explanation is to be found in slight alterations in the daily rate of metabolism or of excretion, or possibly in the degree of muscular tonus. In any event, the factors responsible appear to be beyond the present range of experimental control. Certainly, the fluctuations to which we refer are not to be accounted for by errors in the collection of the excreta. In order to minimize alterations in nitrogen loss via the perspiration, all of our tests were terminated before the onset of hot weather.

These variations due to unknown factors are of little consequence when one is making a qualitative experiment. The effects exerted by the exclusion of an essential amino acid from the food are so profound that they are unmistakable. However, after a moderate change in the intake of an indispensable dietary component, the alteration in the nitrogen balance is usually correspondingly small. Therefore, in all of our quantitative experiments the policy has been adopted of inducing a distinctly negative balance at some stage of the test, and then raising the intake of the amino acid under investigation until a consistently positive balance, as measured by the average for a period of several days, was attained. In this fashion, daily fluctuations are leveled, and the results are exceedingly clear-cut. In most

instances, no attempt has been made to establish the minimum level more accurately than within 0.1 gm. of the L amino acid

Another observation which has proved to be somewhat disconcerting is the difference in the minimum amount of an amino acid which is required by different individuals under comparable experimental conditions. Originally, we had hoped that two quantitative experiments with each amino acid would suffice to establish the minimum requirements of man. This assumption was soon found to be incorrect. With certain amino acids, the results are quite consistent from one subject to another. With other amino acids, the quantities found necessary vary as much as 100 per cent in different individuals. Moreover, no correlation appears to exist between the requirement on the one hand and the body weight, body surface, or creatinine output on the other. Whether such data indicate that certain essential amino acids can be synthesized in part, but with varying success by different subjects, or whether they merely reflect the extent to which alimentary microorganisms participate in the syntheses, cannot be stated at the present time. Experiments in rats appear to demonstrate that alimentary microorganisms play a much less dominant role in the formation of amino acids than they do in the synthesis of vitamins (10). Regardless of the explanation, the variations in individual requirements serve to demonstrate the tentative nature of the quantitative findings. This fact has always been emphasized when reference has been made by us to any part of these studies. Furthermore, when several subjects show different requirements for a given amino acid, the highest level must be taken as the minimum. Any 'recommended intakes' must make further allowances for the possibility that certain individuals may need still larger quantities than the subjects thus far tested. This aspect of the problem will be considered below.

Perhaps the most disturbing, and certainly the most surprising, feature of experiments involving the use of diets containing mixtures of amino acids relates to the caloric requirements of the subjects. It has been our experience that with such diets the caloric intakes must be increased above the expected levels in order to maintain nitrogen balance. This is true even when the amino acids furnish 7 or more grams of nitrogen daily. We had assumed that intakes of 40 calories per kilo of body weight per day would be

sufficient. This, however, proved not to be the case. It seemed necessary, therefore, to compare the caloric needs of individuals, under otherwise identical conditions, when they were ingesting diets containing respectively casein, hydrolyzed casein, and mixtures of pure amino acids as sources of nitrogen. One of 3 such experiments is summarized in table 2.

Throughout the test the daily nitrogen intake was kept within a range of 10.03 and 10.08 gm., and the ratio of calories from carbohydrates to calories from fats was maintained at 2.6. The diets were similar in composition to those employed throughout the quantitative investigations. The acid-hydrolyzed casein was supplemented with L-tryptophan. The mixture of 9 amino acids was composed of the 8 essentials with sufficient added glycine and urea² to raise the

TABLE 2 CALORIC REQUIREMENTS ON DIETS CONTAINING PROTEINS OR AMINO ACIDS

Period averages Subject W A S, 71.7 kilos

SOURCE OF DIETARY N	DURATION OF PERIOD	DAILY INTAKE OF CALORIES	AVERAGE DAILY N BALANCE
	days	per k b w	gm
Whole casein	6	45	+0.48
	7	35	+0.14
Acid-hydrolyzed casein	8	35	-0.29
	6	45	+0.50
9 Amino acids	5	45	+0.33
	6	35	-0.91
20 Amino acids	6	35	-0.93
Whole casein	5	35	+0.46
Enzymatically hydrolyzed casein	6	35	-0.09

nitrogen content to the desired level. Hence, the only variables were the sources of nitrogen and the fuel value of the rations.

The subject came into positive balance promptly upon the casein diet when the latter furnished 45 calories per kilo of body weight per day, and continued in positive balance during the 7 days in which the fuel value of the food was reduced to 35 calories per kilo of body weight.

²As pointed out later in this paper, extensive investigations in this laboratory have shown that glycine and urea can serve, in the growing rat, as sources of nitrogen for the synthesis of the non essential amino acids. This is true even to a greater extent of ammonium citrate.

However, when the whole casein was replaced by acid-hydrolyzed casein supplemented with L-tryptophan, negative balance ensued until the caloric intake was raised to the higher level. Like results were observed with the mixtures of pure amino acids. With the 35 calorie intake, neither the mixture of 9 amino acids (the 8 essentials plus glycine and urea) nor the mixture of all known protein components including citrulline (but without urea) was capable of inducing nitrogen equilibrium. Furthermore, no difference could be detected in the nutritive quality of the two mixtures for maintenance purposes. Several months later, the same subject was used in a comparison of whole casein and enzymatically hydrolyzed casein, as recorded in the last two lines of table 2. As will be observed, he was brought into strong positive balance upon the casein diet with a fuel intake of 35 calories per kilo of body weight. Replacement of the whole casein by the enzymatically hydrolyzed product led to a slight negative balance. Apparently, even the latter may be somewhat inferior to the whole protein for maintenance purposes.

No explanation is apparent for the above observations. Whether proteins contain, or carry as a contaminant, some component which is involved in the efficient utilization of food energy or amino acids and is destroyed by hydrolysis, or whether our findings are attributable to the more rapid absorption of free amino acids, as contrasted with native proteins which are slowly digested in the alimentary tract, is not clear. If the rate of absorption were the critical factor, one might anticipate a considerable loss of amino acids in the urine. Our experience does not warrant that conclusion. Possibly the casein, which was a commercial 'vitamin-free' product, may have carried an adsorbed agent which was not present in the liver concentrate used as a source of the less well-defined vitamins. Tests will be undertaken shortly to determine whether vitamin B₁₂ influences the caloric requirements of our subjects.³

Since the above comparisons were made, the practice has been followed in the quantitative experiments of administering 55 calories per kilo of body weight per day. Lower intakes might have sufficed in some instances. However, the

higher values avoided prolonged adjustment periods. Generally, balance was established within three or four days. Occasionally, somewhat larger intakes were found necessary. This was true of tall, slender individuals who were underweight for their height. One subject who was receiving 55 calories per kilo complained of hunger, and was permitted to increase the fuel value of his ration to 58 calories per kilo.

One may be warranted in asking whether the high energy content of the diets influenced the outcome of the investigations. To this question a categorical answer cannot be given. The necessity of using diets of higher than normal caloric content is demonstrated by the data in table 2, and by additional data of a similar sort not included in the present paper. Furthermore, any criticism of the techniques used in the present studies applies, with equal force, to like tests made with completely hydrolyzed proteins. As has been shown, the latter are not superior to mixtures of purified amino acids as regards the required fuel value of the diets, and have the disadvantage that one cannot be sure, in most instances, of the quantitative removal of the amino acid under investigation. Thus, one is forced to employ high caloric rations if studies of this general nature are to be pursued successfully. Despite the well known protein-sparing action of carbohydrates, it seems unlikely that the minimum requirements of the organism for individual amino acids could have been lowered significantly by the imposed experimental conditions. Even if one assumes that native proteins ordinarily carry an 'accessory factor' the function of which is to promote physiological economy in the utilization of food energy or amino acids, the absence of this factor would be expected to lead to a higher rather than a lower minimum requirement for a dietary essential. It does not seem reasonable to postulate that the absence from the ration of so important a substance, if it exists, would actually lessen the need of the body for something which it cannot manufacture.

Finally, attention should be called to the fact that the experience of others with low protein diets, as contrasted with diets containing amino acids, has led to the use of relatively high caloric intakes. In describing an experiment in which egg protein furnished approximately 95 per cent of the nitrogen, Murlin, *et al.* (11) state that the total energy value at the beginning of the test was placed at 45 calories per kilo of body

³There are reasons for suspecting that in the preparation of our liver concentrates from Wilson's 'liver powder 1-20' much of the vitamin B₁₂ may have been lost.

weight, "but when in individual cases it became apparent that this allowance was not sustaining body weight it was raised to 48 cal per kilo or sometimes higher, by addition principally of carbohydrate." Comparable energy intakes were employed by Bricker, *et al* (12) in a recent study of the protein requirements of young women. One of the 10 subjects needed 50 calories per kilo to maintain body weight. Our young men were encouraged to avoid unusual physical exertion during the course of the tests. However, as graduate students they found it necessary to work long hours in the laboratory, for the most part in a standing position. Also, they walked to and from their living quarters which frequently were located more than a mile from the campus. In the light of these facts, and the strange increase in fuel requirements associated with amino acid diets, the caloric intakes herein employed were not so excessive as first impressions might lead one to believe.

The intricacies involved in a study of the minimum amino acid requirements have been dealt with at some length in order that the reader may be fully advised as to the exact conditions under which the findings described below were obtained. The nitrogen balance technique has its limitations, but, at the present time, it is the only procedure which can be applied to adult man for the purposes in question. Possibly, the preceding pages have over-emphasized the difficulties and imperfections. If so, this is to be preferred, in our judgment, to unwarranted simplification of a problem which is beset by many meagerly understood variables. As Mary Swartz Rose and MacLeod (13) remarked many years ago, "the search for an absolute biological constant in protein metabolism is a vain one, any so-called biological minimum is such only for a given set of conditions."

Despite what has gone before, the values obtained in the present studies are believed to approximate very closely the true minimum needs of the human species. Under the conditions specified, remarkably definite conclusions can be reached regarding the requirements of a given individual for any essential amino acid. A valid criticism of an investigation of this nature is the comparatively small number of tests that can be made in a reasonable time. Thirty-one quantitative experiments have now been completed. These represent a tremendous undertaking, extending over a period of almost four years. The data are presented with complete

confidence, but must be viewed as strictly tentative until experience demonstrates their general applicability to a much larger number of individuals.

The procedures followed in the conduct of the quantitative experiments were almost identical with those used in the qualitative tests. One difference is to be noted. In determining which amino acids are necessary dietary components, the removal of one from the food was compensated for by raising all others sufficiently to maintain the nitrogen intake at a constant level. In demonstrating the minimum requirement of the organism for a given component, all amino acids were kept at constant amounts throughout the test except the one under investigation. The latter was progressively lowered, and the nitrogen content of the ration was maintained by the addition of glycine, urea, or both. Growth studies in this laboratory (14) have shown that glycine and urea can be utilized by rats as sources of nitrogen for the manufacture of the non-essential amino acids. The two compounds were included in the human diets for a like purpose. Throughout the entire investigation, the rations furnished approximately 10 gm of nitrogen daily except during the first two or three tests of the series. In these, the food supplied 7 gm of nitrogen daily. Thus, an abundance of nitrogen was always available to the cells for synthetic uses. This is an important point, for it does not follow that nitrogen balance could be maintained with the minimum levels of the 8 essentials alone. Under the latter circumstances, the manufacture of the non-essentials by the tissues would be impossible.

One other detail of the procedure should be described. After the minimum requirement for each amino acid had been determined, twice this value was selected as the intake of the subjects in all subsequent experiments involving other amino acids. This may be rendered more intelligible by a specific illustration. The first amino acid to be investigated was tryptophan. The diet employed at the beginning of these tests supplied 1.85 gm of L-tryptophan daily. By progressively reducing the intake, the minimum level compatible with consistent positive balance was found to be 0.15 gm daily in each of two subjects. Inasmuch as the two individuals yielded identical results, it was assumed tentatively, and in spite of the remarkable low figure, that 0.15 gm represented the minimum requirement. Later events have necessitated an up-

ward revision of this value, as will be explained later

In the meantime, the question arose as to the quantity of L-tryptophan which should be included in the food in measuring the requirements of man for other amino acids. It seemed likely that twice the minimum would be a safe intake. Thus, 0.3 gm of L-tryptophan per day has been the standard in subsequent quantitative experiments, although in some tests 0.2 gm was employed successfully. A similar procedure has been followed with each amino acid as its minimum was established. Consequently, the quantitative data have been supplemented by the accumulation of evidence, in a considerable number of individuals, that twice the minima are satisfactory intakes. The latter are arbitrarily being designated as the 'recommended intakes'.

Returning for a moment to the question of the tryptophan requirement, attention is called to the fact that 31 individuals have been kept in positive nitrogen balance upon daily intakes of 0.3 gm or less. On the other hand, a recent subject has shown a minimum requirement of 0.25 gm daily. This is decidedly the highest requirement for this amino acid we have ever observed. Eleven other subjects have been kept in balance with intakes of 0.2 gm daily, although no attempt was made to establish their minimum requirements. Because of the single high value, it seems necessary to regard 0.5 gm (twice the highest minimum) as the recommended daily intake until and unless later events demonstrate that this amount is unnecessarily large.

In table 3 are summarized the minimum daily requirements and the 'recommended daily intakes' of the 8 amino acids which are necessary dietary components for the maintenance of nitrogen equilibrium in normal human subjects. Both groups of values must be regarded as strictly tentative. In the column to the extreme right is listed the number of subjects in whom each recommended intake has been shown to be satisfactory. As will be observed, the numbers are smallest for leucine and isoleucine, since these amino acids were the last for which minimum values were established.

Many tests have been made to determine the availability in the human organism of the D amino acids. The nitrogen balance technique is not sufficiently delicate to enable one to exclude the possibility that certain D amino acids may be utilized in trace amounts which are insufficient to affect the balance. On the other hand, one

can readily decide whether the D amino acids possess any practical significance in metabolism. Our data show that D- and L-methionine are equally effective in the maintenance of nitrogen equilibrium in man, and that D-phenylalanine is partially utilized although the human organism appears to be limited as to the amount of this amino acid which it can invert. Contrary to its behavior in the rat, D-tryptophan exerts no detectable influence upon nitrogen balance in man. In our experience, this is true also of acetyl-D-tryptophan despite the report to the contrary in the literature (15). The D modifications of valine, leucine, isoleucine, threonine, and lysine appear not to be available at all in human subjects.

TABLE 3 MINIMUM AND RECOMMENDED INTAKES FOR NORMAL MAN WHEN DIET FURNISHES SUFFICIENT NITROGEN FOR SYNTHESIS OF NON-ESSENTIALS (STRICTLY TENTATIVE VALUES)

AMINO ACID	MINIMUM DAILY REQUIRE- MENT	RECOM- MENDED DAILY INTAKE	SUBJECTS TESTED
	gm	gm	no
L-Tryptophan	0.25	0.5	31 ¹
L-Phenylalanine	1.10	2.2	22
L-Lysine	0.80	1.6	27
L-Threonine	0.50	1.0	19
L-Valine	0.80	1.6	23
L-Methionine	1.10	2.2	13
L-Leucine	1.10	2.2	8
L-Isoleucine	0.70	1.4	8

¹ All of these subjects have been kept in balance on 0.3 gm or less.

Finally, the reader is reminded that in the formulation of diets of low amino acid content the so-called recommended intakes, rather than the minima, should now be employed. The purpose of the former is to make allowance for any further possible variation upward in the minimum requirements of individual subjects. Such diets, to be successful, must furnish most certainly sufficient nitrogen for the synthesis of the non-essentials. This point is emphasized repeatedly in this paper because of its utmost importance. No attempt has yet been made to ascertain the minimum total nitrogen content of a diet, carrying the recommended intakes of the eight essentials, which will sustain nitrogen equilibrium. Experiments of this nature will be undertaken at an early date. The results should provide an

approximate measure of the extent to which synthetic reactions involving nitrogen occur *in vivo*. It is contemplated also in the near future to investigate the possible replacement of phenylalanine by tyrosine, and of methionine by cystine and/or choline.

As to the applications of the above findings, it may be stated that the values summarized in table 3, if typical of male adults as we believe they are, should render it practicable, for the first time, to evaluate proteins in terms of their ability to meet human needs. Hitherto, all such appraisals of proteins have been based upon animal tests. Qualitative and quantitative differences are now recognized in the amino acid requirements of man and animals. Consequently, it does not follow necessarily that estimates of nutritive quality based upon animal tests are applicable to human subjects. It should be possible, by reference to the values now made available, to predict with reasonable accuracy how much of a given protein, if its composition is known, will be required to maintain nitrogen equilibrium in the human organism, provided a sufficient supply of nitrogen to cover the synthetic needs of the cells is also included in the food. It is to be hoped that studies of this nature will be undertaken in many laboratories. In this way only can it be ascertained whether the recommended intakes herein proposed possess general applicability. Obviously, tests of this character must take into account the digestibility of the protein under investigation.

Another application relates to preparations for parenteral use. Much interest has been manifested in recent years concerning the possibility of successful intravenous alimentation in patients who temporarily are unable to consume food in the usual way. The practicability of such a procedure was suggested by the author almost 15 years ago (16), and since then has been explored in many laboratories. Much of the enthusiasm previously displayed has now vanished. This has been due in part to the adverse clinical reactions which sometimes follow the adoption of this therapeutic measure, and in part to the fact that a relatively large quantity of a protein hydrolysate must be administered in order to approach nitrogen balance. In this connection, it

should be realized that the most appropriate mixture for clinical purposes has not yet been prepared, since the prerequisite information respecting the amino acid requirements of man has not hitherto been available. With the presentation of the facts recorded in this paper it should be feasible to formulate a mixture the composition of which is adjusted to the needs of the body for each component. Such a material may prove to be the most efficient source of nitrogen yet devised for parenteral use. One disadvantage which cannot be overcome immediately, and which may have contributed to the discouraging results observed in certain quarters, is the excessive caloric requirement manifested by subjects receiving mixtures of amino acids in place of proteins. Further studies may reveal a method, or a chemical substance, which is capable of counteracting this peculiar feature of amino acid mixtures.

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CHEMICAL MEASUREMENTS IN RELATION TO PHYSICAL EVIDENCE OF MALNUTRITION

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As the science of nutrition has progressed, numerous methods have been evolved for the appraisal of human nutritional status. Some years ago, recognition of malnutrition depended on simple observations, such as gross evidence of loss of weight or dehydration. The demonstration that certain physical stigmata appeared when sufficient quantities of particular chemical substances were not available to the organism, led to refinement and greater specificity of diagnosis. Advances in biochemistry made possible measurement of the concentration of essential nutritive factors and their metabolites in bodily tissues and fluids. Recently, attempts have been made to delineate chemical and physical findings at various levels of nutriture in order to detect incipient malnutrition and to evaluate nutritional status.

In discussing the relationships of chemical tests to physical signs of malnutrition, it is pertinent to emphasize certain concepts of the development of nutritional deficiency. Good nutrition is associated with an abundant supply of all essential nutrients. As malnutrition develops, bodily stores of these factors are depleted, smaller quantities are available to the tissues, urinary excretion diminishes and concentration in the blood falls. Finally a critical level is reached below which metabolic defects occur and impairment of function becomes evident. As the end stage of malnutrition, cellular structure is modified and gross evidence of deficiency disease is apparent. Incipient malnutrition exists when a decrease in reserve functional capacity occurs. Chemical procedures are particularly useful in detecting early abnormalities but are applicable in all zones of nutriture. Physical stigmata occur in chronic advanced malnutrition and may persist as scars long after tissue repletion has been accomplished.

The manifestations of acute and chronic nutritive failure are somewhat different. Acute depletion causes sudden, profound biochemical and physiologic abnormalities, chronic depletion grad-

ual chemical and structural changes, with good compensation for long periods of time. Accurate diagnosis is difficult. Physical signs of malnutrition are non-specific, absence of any one of several factors may give rise to the same structural alteration, while similar tissue change may result from disease unrelated to nutritive failure. A number of the physical signs used as indices of malnutrition rest on a very tenuous foundation. Interpretation should be cautious, since normal variability is great and the question of what constitutes a departure from normal has not been settled.

The concentration of essential nutrients in blood, urine, or other bodily fluids, reflects, in general, recent dietary intake, but pathologic conditions not primarily related to nutrient supply may influence the findings. Load tests, in which a large quantity of a nutrient is administered and changes in the concentration in the blood and urine are measured, are useful in determining the degree of saturation or depletion of the tissues. Saturation, in the sense of maximal tissue concentration, indicates at least adequate nutrition, while unsaturation is a requisite in the diagnosis of malnutrition, although not synonymous with it. Findings in load tests may be misleading in the presence of extensive breakdown of tissue since release of certain nutrients may result in an increase in excretion compatible with saturation. In persons who have suffered long periods of depletion, recent access to nutrients may elevate the concentration in the blood or tissues, without restoring function or reversing pathologic changes. Few chemical or physiologic tests are available for detection of metabolic abnormalities or decreased functional reserve capacity, yet it is here that the early and readily reversible types of malnutrition should be diagnosed. Tests of metabolic derangement are often non-specific and similar abnormalities may occur from causes unrelated to nutrient supply.

The above concepts of the pathogenesis and complexities of malnutrition make it obvious that correlation between chemical measurements and physical findings will not necessarily be observed. Such lack of correlation is common, as will be pointed out in the subsequent discussion of the relationship of chemical to physical findings in specific types of malnutrition.

CALORIC DEFICIT

Caloric deficit is manifested chiefly by physical findings. In childhood, growth failure occurs, in adults, loss of weight, wasting of tissues, weakness and fatigability are the usual findings. Prolonged, severe starvation is manifested by apathy, lethargy, syncope, dry staring hair, dullness of eyes, bradycardia, hypotension, amenorrhea, sterility, and dry inelastic skin with areas of pigmentation (1). Protein deficiency is usually an associated finding and, in any event, the nitrogen balance is negative. Anemia is common, and a low-fasting blood sugar with a diminished tolerance for glucose may be present. A decrease in the basal metabolic rate occurs. Diagnosis of profound caloric deficiency is relatively simple and the severity may be judged by the percentage loss in body weight, the rapidity of such loss, and associated findings of starvation. Detection of mild caloric undernutrition is difficult. There are no adequate standards for weight in relation to height, age, bodily build and genetic potentiality. Simple procedures which will detect the extent of depletion of vital bodily tissues, as compared to fat stores, are not available.

ANEMIA

Anemia is a useful index of malnutrition in spite of its nonspecificity, and determination of the hemoglobin concentration in the blood is widely used in evaluating nutritional status. Anemia may be due to a deficiency of protein, iron or of vitamins of the B complex, notably folic acid and vitamin B₁₂. It is likewise caused by many conditions of non-nutritional origin, such as acute or chronic blood loss, infections and neoplasia. Physical evidence of chronic anemia is minimal in the early stages and diagnosis is dependent on chemical examination. Determination of the size of the red cells (mean corpuscular volume) and of the concentration of hemoglobin in the cells (mean corpuscular hemoglobin concentration) will permit differentiation of the macrocytic anemias, which are the rule in defi-

ciency of protein, folic acid and vitamin B₁₂, from the normocytic or microcytic, hypochromic anemias of iron deficiency. Physical examination aids in detecting non-nutritional causes of anemia and assists in evaluation of its severity. In profound anemia of long standing clinical signs include pallor, glossitis, dyspnea, palpitation, tachycardia, weakness, dizziness, syncope and edema.

Determination of the level of serum iron is a useful procedure in detecting anemia due to iron deficiency. In normal persons on adequate diets, the concentration of serum iron is usually greater than 70 μg per cent (2-4). Although low levels may occur in acute and chronic infections (5, 6) a concentration of less than 60 μg per cent is suggestive of iron deficiency (7). Glossitis occurs in iron deficiency anemia (8, 9) as it does in anemia due to deficiency of vitamins of the B complex, and cheilosis has been reported. These physical findings do not correlate with the type or degree of anemia but are more common in anemia of considerable chronicity.

PROTEIN DEFICIENCY

Manifestations of protein deficiency include cessation or slowing of growth, loss of weight, muscle wasting, and edema. Nitrogen balance is negative, serum proteins decrease, particularly serum albumin, and anemia may occur. The concentration of plasma proteins may remain normal for long periods of time in the presence of protein deficiency. This is due in part to hemoconcentration (10) and if the volume of the circulating plasma is determined, protein deficiency may be detected at an earlier stage. Investigations of Elman and others (11) which indicate that a decrease of one gram in total circulating serum protein represents a loss of 30 gm of tissue protein, suggest that protein deficiency is advanced before a significant drop in the concentration of serum protein occurs.

Correlation has not always been demonstrated between clinical edema and the concentration of albumin or total proteins in the serum. This is not surprising in view of the many factors which influence edema formation. During World War II mild hypoproteinemia was common in certain famine areas (12) and concentration camps (13) but close correlation between edema and the level of serum proteins was not observed. Keys *et al* failed to obtain such a correlation in studies of 34 men maintained on a European type of famine diet. Govaerts (14), who studied famine edema

in Brussels, found serum proteins to be less than normal in the majority of patients, the mean reduction being 29 per cent. The mean osmotic pressure of the serums was reduced to an even greater extent, namely, 41 per cent. Petrides (15) reported low serum protein values in children with hunger edema in Athens. Denz (16) found a significantly lower concentration of plasma proteins in German civilian internees than in normal British subjects. The degree of edema appeared to be proportional to the reduction in plasma protein concentration but considerable variation occurred. Values for plasma albumin were low (2.58 to 3.81 gm per 100 cc of plasma), but were not correlated with the degree of edema. Worsham (17) studied the relationship of hypoproteinemia to edema in women during pregnancy. Edema was present in 8 per cent of women when the serum protein level was above 6.0 gm per 100 cc, in 21 per cent when the level was between 5.5 to 6.0 gm, and in 72 per cent when the level was less than 5.5 gm.

Further studies of protein nutrition may include measurement of the excretion of individual amino acids. This may be informative as to the biologic value of dietary protein (18), as well as uncovering specific amino acid deficiency.

VITAMIN A

Chemical tests for evaluation of vitamin A nutrition include determination of the levels of vitamin A and carotene in the blood, tests of absorption of vitamin A from the intestinal tract and estimation of the vitamin A content of the liver in specimens obtained by biopsy. Physical findings in vitamin A deficiency are dryness and scaling of the skin, follicular hyperkeratosis, xerophthalmia, Bitots spots, and night blindness or lesser degrees of abnormal dark adaptation.

The normal range of values for vitamin A and carotene in the plasma has not been entirely settled. It has been suggested that the lower limit of normal be placed between 50 and 100 μ g per cent for carotene, and between 40 and 75 International Units (19-22) or 20 to 30 μ g per 100 cc (23, 24) for vitamin A. Levels of vitamin A in the blood increase with age and seasonal variations have been noted (25). When the intake of vitamin A is reduced, the concentration of carotene in the blood falls rapidly, while that of vitamin A decreases slowly. In Hartzler's experiments (26), it took 140 days of a diet low in vitamin A before a reduction in plasma level was observed. When diets high in vitamin A are administered, concen-

tration in the blood may rise before signs of deficiency disappear (27). Hsu (28) found the mean level of vitamin A in the blood to be 56 I U in healthy Chinese, 33 I U in hospital patients without clinical signs of vitamin A deficiency, and 6 I U in patients with ocular or skin lesions attributable to lack of this vitamin. Mean carotene values in the blood in these same groups were 140, 65, and 24 μ g respectively. Van Veen and Postmus (29) found very low levels of blood vitamin A in the Netherlands East Indies, with a high incidence of poor dark adaptation, xerophthalmia and blindness. In several areas mean levels varied from 27 to 77 I U in children, and from 49 to 100 I U in adults. Xerosis conjunctivae was usually apparent when values of less than 40 I U per 100 cc were encountered. J. H. Jonxis (30) found low to absent vitamin A in the blood of children in Holland. The first clinical finding observed was follicular keratosis while keratomalacia did not occur. In surveys in Tennessee and North Carolina, Anderson and Milam (31) found no correlation between the plasma level of vitamin A and clinical signs considered to be evidence of vitamin A deficiency. Harris and co-workers (32) found lower values for carotene and vitamin A in clinic patients than in normal subjects but the former had no signs of deficiency. Adamson *et al* (33) in a survey in Newfoundland, found a significant correlation between serum vitamin A levels and excess tissue in the conjunctivae in children aged 6 to 10 years, but not in other age groups. No correlation was observed between other physical signs and the concentration of vitamin A in the serum. Numerous other studies have failed to show any correlation between the level of vitamin A in the blood and thickening of the conjunctivae (34-36).

Krause *et al* (37) found no difference in the concentration of vitamin A in the blood in children with and without follicular keratosis but carotene values were lower when folliculosis was present.

A lack of correlation between levels of vitamin A in the serum and follicular keratosis was reported in a survey in Newfoundland (36). Nor El Din (38) found an average concentration of vitamin A in the blood of 40 μ g per cent in ordinary hospital patients, 20 μ g per cent in patients with cirrhosis of the liver or with diabetes, 15 μ g per cent in patients with pellagra, and traces only in 27 persons with catarrhal or obstructive jaundice. Night blindness was present in $\frac{1}{4}$ of the patients who had cirrhosis, diabetes or jaundice, in $\frac{1}{10}$ of those with pellagra. Other workers have

shown that plasma vitamin A values are decreased in many types of disease (39, 40). It has been suggested that the levels of carotene and vitamin A in the blood are an expression of liver function (41). In cirrhosis, a low concentration of vitamin A in the blood and in the liver has been reported and the rise in blood values after oral administration of vitamin A is subnormal (42-44). Clinical evidence of deficiency was not always associated with these findings. In sprue, low values for plasma vitamin A and carotene are often obtained, tolerance tests indicate poor absorption (45) and physical signs of deficiency are frequently noted.

Impairment of dark adaptation has been produced in experimental vitamin A deficiency (46, 47) and several studies of the relationship between abnormalities of dark adaptation, the concentration of vitamin A in the blood, and clinical findings have been reported. In a nutrition survey in Tennessee (48) blood values and findings in visual tests indicated a degree of deficiency comparable to that suggested by dietary intake but few signs of vitamin A deficiency were present. Hasson and Khanna (49) noted that abnormal dark adaptation occurred only when plasma vitamin A was low, but not all subjects with low values had defective adaptation. Caveness, Satterfield, and Dann (50) found only slight correlation between biophotometer readings and vitamin A levels in the blood. Bodansky, Lewis and Haig (51), in a study of infants, found that low levels of vitamin A in the plasma were associated with low stores in the liver and poor dark adaptation. They suggested that plasma levels may be a more sensitive index of deficiency than tests of dark adaptation. Pett and LePage (52) observed a relationship between visual tests and blood levels in vitamin A deficiency. In other studies, no correlation has been demonstrated between dark adaptation response and the concentration of vitamin A in the plasma (53, 54).

The above findings indicate that while some relationship exists between levels of plasma vitamin A and carotene, dark adaptation tests, and physical evidence of vitamin A deficiency, wide individual variations occur. The many diseases which affect vitamin A metabolism make evaluation of vitamin A nutrition a difficult problem.

VITAMIN B COMPLEX

Chemical measurements for a number of the vitamins of the B complex have been developed, but only for thiamine, niacin and riboflavin is

there sufficient data relating chemical findings to physical evidence of deficiency to justify discussion at this time.

Early signs of endemic vitamin B complex deficiency, and also of experimentally induced deficiency, include non-specific complaints such as decreased appetite, easy fatigability, lack of ambition and nervousness as well as personality disturbances, including irritability, moodiness, depression, vague fears, lassitude and quarrelsomeness (55). In acute experimental deficiency, outstanding manifestations are disinclination to spontaneous activity, and personality alterations of psychoneurotic type, i.e., depression, hypochondriasis and hysteria. Long before these symptoms appear, the urinary excretion of the B vitamins has fallen to low levels (56). In one study (57), the development of mental and physical changes correlated well in the individual case with a rise in pyruvate and lactate in the blood, especially in samples taken after exercise.

Nutrition surveys in Newfoundland have always shown a low intake of the B vitamins and, until recently, a high incidence of beriberi. In 1944, members of two survey groups were impressed by the apathy and lack of spontaneity of the adults, the docility and absence of play among the children (33, 58). In re-surveys in 1948, these findings had disappeared, adults were alert, children were curious, played spontaneously and required monitoring during examination (36, 59). In the interval between 1944 and 1948 the dietary supply of the B vitamins had increased. The urinary excretion of thiamine and riboflavin was much higher in 1948, than in 1944, corroborating dietary and clinical findings.

THIAMINE

Chemical tests used in evaluating thiamine nutrition have included measurement of thiamine excretion in the urine during fasting, for 24-hour periods or for several hours after the administration of an oral or parenteral test dose of this vitamin, determination of thiamine in the blood or in tissues obtained by biopsy, estimation of cocarboxylase in the blood, determination of blood pyruvic acid and of the lactate:pyruvate ratio during fasting, after exercise or following the administration of glucose.

Clinical evidence of thiamine deficiency includes polyneuritis (dry beriberi), cardiovascular disturbances and edema. In severe deficiency acute cardiac failure may occur. Anorexia is common, while neurasthenic symptoms, digestive

disturbances and constipation have been reported as early manifestations

The urinary excretion of thiamine falls rapidly when the dietary intake of this vitamin is decreased (60-67) and approaches zero when the intake is about 0.07 mg per day. Mickelsen, Caster and Keys (68) have shown that thiamine excretion is characteristic of the individual as well as being linearly related to the intake, and that excretion of pyrimin, a pyrimidine-like component of the thiamine molecule, is valuable in assaying thiamine nutrition when the dietary supply of this vitamin is low.

The urinary excretion of thiamine during fasting has been found to correlate with the average dietary intake (64, 69, 70, 71) and with the 24-hour excretion (71, 72). This determination has been suggested as a useful procedure in detecting the presence, although not the degree, of thiamine deficiency. It has been suggested that an output of zero to 4 μg in one hour during fasting is indicative of inadequate thiamine intake or chemical unsaturation (71, 73, 74). Melnick and Field found a lower fasting excretion in subjects with clinical signs of thiamine deficiency than in normal persons. Salcedo and co-workers (75), found that the fasting one-hour urinary excretion of thiamine in beriberi varied from 0 to 8.51 μg . Mean excretion was 2.85, 2.82, and 2.75 μg in frank, suggestive and doubtful cases respectively. In apparently normal subjects, excretion ranged from 0.7 to 20 μg with a mean of 5.72 ± 3.72 . The presence of symptoms and signs of deficiency, together with an excretion of 0 to 2 μg of thiamine, was considered helpful in the diagnosis of beriberi. Hou (76) found no thiamine in the urine in acute or prolonged, chronic beriberi, a low excretion (14 μg in 24 hours) in subacute or chronic beriberi of short duration. Excretion in normal persons varied from 17 to 176 μg in 24 hours.

A number of studies of experimental thiamine deficiency have been carried out. Elsom and co-workers (77) reported that 6 subjects developed clinical signs of thiamine deficiency on a dietary intake of 0.35 mg/1000 calories and excreted less than 42 μg of thiamine in the urine in 24 hours, 3 subjects on intakes above this level were without signs of deficiency and excreted 40 to 60 μg . Objective signs of deficiency included muscle tenderness and disturbance of vibratory sense. Foresight and judgment, as measured by maze tests, were affected deleteriously. One subject developed edema after 98 days on a deficient diet.

The urinary excretion was proportional to the intake and did not change with the appearance of symptoms of deficiency.

Najjar and Holt (62) gradually reduced thiamine intake to 0.128 mg/1000 calories. Urinary excretion had been at minimal levels for many months before this final reduction in diet and no further decrease in excretion occurred when signs of deficiency, i.e., neuritis and edema, appeared. Thiamine load tests, in which the 4-hour urinary excretion was measured after the intravenous injection of 1 mg, showed a decrease preceding and associated with the development of physical evidence of lack of thiamine. Symptoms never occurred when the excretion was more than 60 μg in 4 hours but were nearly always present when excretion was less than 50 μg in 4 hours.

Williams and his collaborators (61) found the first chemical evidence of thiamine deficiency to be a decrease in the 24-hour urinary excretion, followed by a decrease in excretion after an intramuscular load test of 1 mg. Almost simultaneously with the drop in test dose excretion, values for pyruvic acid in the blood, after dextrose administration, became elevated, and symptoms of fatigue, listlessness and anorexia appeared. Symptoms progressed to apathy, nausea, epigastric pain, prostration, mental confusion and paresthesias and were associated with a progressive decrease in test dose excretion to less than 100 μg and a rise in pyruvic acid in the blood. When definite evidence of polyneuropathy appeared, the output following the test dose was less than 50 μg . The load test appeared to be valid for assessment of tissue stores while the level of pyruvic acid in the blood was valid for detecting the biochemical defect of thiamine deficiency, provided other causes of abnormal elevation could be excluded. The biochemical defect developed rapidly when the intake was 0.17 mg/1000 calories, more slowly when dietary thiamine was increased to 0.22 mg/1000 calories, while with an intake of 45 mg/1000 calories, 4 of 5 subjects developed mild metabolic abnormalities and 3 of 5 subjects depletion of tissue stores.

Keys (78, 79) studied healthy adult males on various dietary levels of thiamine and found no change in work output, or in chemical tests related to carbohydrate metabolism, after 10 to 12 weeks on an intake of 0.23 mg/1000 calories, although the urinary excretion of thiamine and the test dose returns were low. When dietary thiamine was reduced to 0.185 mg/1000 calories,

the only evidence of deficiency was an elevation in the resting and post-exercise level of blood pyruvate

Foltz and associates (68) found a decrease in appetite within 3 weeks on a diet containing 0.33 to 0.38 mg of thiamine per 1000 calories. Within 4 weeks after the intake was reduced to 0.17 to 0.21 mg/1000 calories, a decrease in work output, an increase in muscle tenderness and pain, fatigue and desire for sleep, and deterioration in mental attitude, nervous stability and alertness occurred. Urinary thiamine excretion ranged from 5 to 20 μ g in 24 hours. No change occurred in blood pyruvic acid values.

Melnick (72) studied normal subjects and persons with clinical symptoms of thiamine deficiency by means of 4 different tests: 24-hour basal excretion, fasting 4-hour excretion, response to the oral administration of 5 mg of thiamine, and 4-hour excretion after parenteral administration of 350 μ g of thiamine per square meter of surface area. Good correlation was obtained between the tests. With the parenteral test dose, all the normal but none of the deficient subjects excreted more than 50 μ g of thiamine in the subsequent 4-hour period. With this same test, Blanchaer and Cameron (80) found low excretions in debilitated subjects regardless of the etiology of their condition, and larger excretions during convalescence or in subjects with minor illness. The incidence of low excretion increased with age.

Ruffin *et al* (22) reported a significant difference in the excretion of thiamine by normal persons and patients with clinical findings of mild deficiency of the B vitamins, when a test dose of 1 mg was given intramuscularly and excretion measured for 4 hours thereafter.

Johnson (73) using a 5 mg oral load test, concluded that an output of less than 20 μ g in 4 hours was subnormal as judged by nutrition surveys in troops. Goldsmith and Sarett (66), using a similar test, found that normal subjects on adequate diets excreted more than 100 μ g in 4 hours, while persons on diets containing 0.2 to 0.3 mg/1000 calories excreted an average of 58 μ g. In patients with clinical evidence of thiamine deficiency, namely, peripheral neuritis or cardiovascular disturbances with edema, the excretion was less than 80 μ g in the 4-hour period.

The thiamine content of blood has been studied by several investigators. Most of the thiamine is found in the cellular elements (81). Foltz (68) reported that the thiamine concentration tends to correspond to the character of the diet, while

Greenberg and Rinehart (82) found it to be relatively static and not easily affected by thiamine intake. Thiamine content of the leucocytes has been reported to be decreased in thiamine deficiency (83). Estimation of cocarboxylase in whole blood has been considered an unreliable method of detecting thiamine deficiency (84), but further investigation of this test seems warranted. Oldham *et al* (64) found a decrease in blood thiamine when the dietary intake was 14 mg/1000 calories. The urinary excretion during fasting and in 24 hours was low, as was the excretion after an oral test dose of thiamine. No definite signs of deficiency occurred after 59 days on this diet but 4 of 9 subjects noted severe constipation. When the intake was raised to 0.2 mg/1000 calories and maintained at this level for 47 days, blood values returned to those found prior to the experiment, as did values for excretion during fasting. However, test dose returns were further reduced, constipation increased, and certain psychologic changes developed, namely, difficulty in grasping ideas and in the ability to concentrate. These symptoms improved when the thiamine intake was raised to 0.36 mg/1000 calories, while urinary excretion increased slightly and test dose returns remained low.

Hulse (85) reported a decrease in the yeast stimulating activity of plasma and in the thiamine content of muscle in subjects maintained for 18 days on a diet containing 0.3 mg of thiamine per 1000 calories. The only clinical finding was a vague loss in 'sense of well being'. The relationship of blood levels of thiamine to definite clinical signs of deficiency (beriberi) has not been reported. Blanchaer and Cameron (80) found a significant negative correlation between blood cellular thiamine and urinary excretion of less than 90 μ g following a load test of 350 μ g per square meter of body surface. Defective renal clearance could produce such findings, which suggests caution in classifying subjects as normal or deficient solely on the basis of response to load tests.

As noted above, elevation of pyruvic acid in the blood occurs in thiamine deficiency. Since numerous other conditions can lead to a similar rise in pyruvic acid, Allibone (86) has suggested that a decrease in the level of pyruvic acid after administration of thiamine may serve as a test of 'subclinical' deficiency. Investigation by Stotz and Bessey (87) indicated that determination of the lactate-pyruvate ratio might be of assistance

in evaluating thiamine nutrition Goldsmith (88) found a change in this ratio in patients with polyneuropathy and heart disease due to thiamine deficiency, as well as in persons with physical signs suggesting deficiency of other vitamins of the B complex. Horwitt (89) has suggested that simultaneous measurement of lactic acid, pyruvic acid and glucose in the blood, after mild exercise and glucose ingestion, may prove to be a valuable procedure in detecting early thiamine deficiency. When thiamine intake was 0.2 mg daily, an apparently pathologic level was observed with this test one to four months before 'incontrovertible clinical signs of thiamine deficiency appeared.'

From the many studies of thiamine nutrition discussed above, it seems clear that 2 chemical procedures have particular merit in detecting thiamine deficiency: 1) the test suggested by Horwitt (89) in which the levels of glucose, pyruvic acid and lactic acid in the blood are measured after a metabolic load is placed on the organism, and 2) measurement of the urinary excretion after an oral or parenteral test dose of thiamine, which permits estimation of the degree of saturation or depletion of the tissues with this vitamin.

RIBOFLAVIN

Chemical measurements suggested for evaluation of riboflavin nutrition are estimation of the quantity in the blood, either in the serum or cellular elements, and determination of the urinary excretion in 24 hours, in one hour during fasting and for several hours after the oral or parenteral administration of a test dose. Clinical signs ascribed to riboflavin deficiency include corneal vascularization, seborrheic dermatitis, glossitis, cheilosis, angular stomatitis, and lesions of the vulva and scrotum. Each of these signs has been noted in conditions other than riboflavin deficiency and the specificity of the syndrome has been questioned (90). Slight changes in corneal vascularity occur in normal subjects, do not correlate with riboflavin intake and fail to respond to therapy with riboflavin (91). Although vascularizing keratitis may be due to lack of riboflavin, this sign alone is not diagnostically significant. Cheilosis, angular stomatitis and glossitis may occur in deficiency of any one of several essential nutrients and in states unrelated to inadequate nutrition. The uncertainties in clinical diagnosis render interpretation of the relationship of chemical to physical findings most difficult.

Many studies have shown that the urinary excretion of riboflavin parallels dietary intake until high levels are reached (92, 93). Excretion may be dependent in part on the protein content (94) of the diet and a high excretion may occur after several days of fasting (95). In subjects maintained on diets restricted in riboflavin, basal excretion is low as in the response to an oral or parenterally administered test dose (61, 66, 93, 96, 97). In most instances, evidence of tissue depletion has not been associated with clinical findings considered to be characteristic of riboflavin deficiency. Horwitt (89) concluded that attempts to correlate riboflavin excretion with nutritional state were of little value except as indicative of dietary intake. Recent studies have shown that the riboflavin requirement is related to body size rather than to caloric intake (98) which may explain apparently divergent conclusions in metabolic balance experiments.

Sebrell *et al* (99), who produced cheilosis, angular fissuring and slight seborrheic lesions in patients on a diet containing 0.5 mg of riboflavin per day, found a mean excretion in 24 hours of 77 μ g with no decrease in output in the presence of objective signs of deficiency. Keys' (100) subjects who received 99 mg per day showed no clinical evidence of deficiency and no change in work performance after 52 days on this regime. The urinary excretion in the last few months was about 13 per cent of the intake while the test dose excretion, which was reduced after the first week, remained fairly constant, with perhaps slight reduction at the end of the experiment. Williams (101) maintained subjects on 0.7 mg of riboflavin per day for 288 days. No clinical signs of deficiency developed but the 24-hour excretion of riboflavin was low (14% of the intake) and there was a progressive decrease in the excretion of an intraperitoneal test dose of 2 mg of sodium riboflavin. Williams' (101) subjects received a greater daily intake of riboflavin than Sebrell's and had been fortified with riboflavin prior to the experimental period. Davis and associates (93) maintained subjects on 54 mg of riboflavin daily for over 100 days. No clear evidence of riboflavin deficiency developed. Urinary excretion averaged about 80 μ g in 24 hours and 8 μ g in one hour during fasting. Only 4 per cent of a test dose of 0.2 mg per kilogram of body weight was recovered in the urine while 20 per cent was recovered in the pre-experimental period. It seems likely that prolonged depletion is necessary for signs of deficiency to become

evident in persons previously well supplied with this vitamin

No given level of riboflavin excretion can be suggested as indicative of clinical deficiency but when the dietary intake is about 0.5 mg per day, the level at which deficiency has been demonstrated, from 50 to 100 μ g is the usual output in 24 hours. This level of excretion may be present with or without physical evidence of deficiency, and no further change occurs when lesions develop.

Kark and Bean (102) found urinary riboflavin excretion, in the post-absorptive state, lower in Indian and Occidental troops and reported a higher incidence of folliculosis, corneal invasion and cheilosis in the Indian group. Hou and Dju (103) reported a daily riboflavin excretion of 100 to 200 μ g in normal Chinese, an excretion of 39 μ g in subjects with glossitis and of 27 μ g in patients with ocular lesions of riboflavin deficiency. Anderson *et al* (104), in studies in Mexico found the mean riboflavin excretion to be 0.398 ± 0.052 mg per liter in the population as a whole. Persons with angular stomatitis excreted, on an average, 0.186 ± 0.023 mg per liter.

In studies in Newfoundland, no correlation was demonstrated between clinical signs of riboflavin deficiency and urinary excretion of this vitamin either in random specimens or after a test dose had been administered (33, 36). However the mean excretion following a test dose was lower in subjects in Newfoundland than in apparently normal persons in this country (36). Goldsmith and Sarett (105) found no significant difference between normal subjects and patients with signs of vitamin B complex deficiency in 4-hour urinary excretion after an oral test dose of riboflavin. Occasionally, high excretions occurred which could not be adequately explained.

Najjar and Holt (106) placed infants on a diet devoid of riboflavin and reported a 24-hour urinary excretion of 40–50 μ g, a serum riboflavin level of 0, and a reduction in the riboflavin content of the cellular elements of blood. When 0.4 to 0.45 mg of riboflavin was given daily, the vitamin reappeared in the serum. Burch *et al* (107) showed that total riboflavin concentration in the serum decreased by 8 to 25 per cent in 4 subjects whose dietary intake of riboflavin was reduced to 0.4 mg daily for 4 days. Axelrod (108) found essentially the same riboflavin values in the blood in persons with cheilosis and conjunctivitis as in normal subjects. In a survey in Newfoundland, no correlation was demonstrated between

the level of riboflavin in the serum and glossitis, cheilosis or circumcorneal injection (36).

It is obvious that the state of saturation or depletion of the bodily tissues can be determined by chemical tests for riboflavin, but that functional and structural impairment can not be diagnosed by these measures.

NIACIN

Chemical tests for evaluation of niacin nutrition have not been very satisfactory. Niacin is excreted in the urine in several forms, and although reliable procedures are available for measuring acid-hydrolyzable niacin and N¹-methylnicotinamide, it has not been possible, until recently, to account for more than 30 to 35 per cent of the quantity of niacin administered. In 1946 Knox and Grossman (109) isolated a compound from urine, N¹-methyl-6-pyridone-3-carboxylamide, which appears to be the major excretory product of niacin. Determination of the excretion of this pyridone should assist in appraising human niacin nutrition. Unfortunately great difficulty has been encountered in developing an accurate and simple procedure for this determination and only 2 reports have been published dealing with this subject (110, 111). Other chemical tests used in studying niacin nutrition are determination of niacin and of pyridine nucleotides in the blood, particularly in the cellular elements, and of pyridine nucleotides in the tissues (112). There is a paucity of data concerning the concentration of pyridine nucleotides at various levels of nutrition. The few studies available suggest that neither the measurement of coenzymes or of niacin in the blood offers information of diagnostic importance in niacin deficiency (113–116). Nevertheless, further studies along this line may prove valuable.

The clinical diagnosis of mild niacin deficiency also presents certain difficulties. The signs which appear prior to the development of frank pellagra, with characteristic dermatitis, are glossitis, diarrhea, and certain mild psychic disturbances, all of which are non-specific. Diagnostic reliance has been placed largely on lingual abnormalities but deficiency of other B vitamins and conditions unrelated to malnutrition can produce indistinguishable lesions.

Investigation of the urinary excretion of niacin derivatives has shown that the output of freely hydrolyzable niacin varies little with changes in diet and only a slight decrease occurs in pellagra. Early work indicated a decrease in 'trigonelline'

excretion in niacin deficiency, both in a 24-hour period and for several hours after the administration of a test dose of niacinamide, but there was overlapping of findings in normal and deficient subjects (117-119). Subsequently it was shown that the excretory product of niacin was N^1 -methylnicotinamide rather than trigonelline, and several procedures were developed for determination of this metabolite. Until a procedure is developed which can be used to measure the excretion of N^1 -methyl-6-pyridone-3-carboxylamide the only biochemical measurement which can be applied in assessment of niacin nutrition is estimation of N^1 -methylnicotinamide in the urine in 24 hours, during fasting or after a test dose of nicotinamide has been administered. The relationship of findings in these tests to physical evidence of niacin deficiency has been studied in several laboratories. The intricacies of niacin metabolism make interpretation of findings extremely difficult. There is marked individual variation in the percentage of various metabolites of niacin excreted in the urine. The availability of methyl groups in the body may be a factor in this variation. Of perhaps greater importance, is the relationship between tryptophan and niacin metabolism which has been demonstrated in man as well as in experimental animals (120-121). The excretion of N^1 -methylnicotinamide (N^1 Me) appears related to the tryptophan as well as the niacin content of the diet. The administration of tryptophan to persons on normal or restricted diets leads to an increase in the excretion of N^1 Me and of a niacin compound obtained by hydrolysis with strong acid. Goldsmith and Sarett (122) have shown abnormalities in the urinary excretion of derivatives of both niacin and tryptophan in pellagra. Furthermore, when patients with pellagra were maintained on diets low in niacin and protein and were given tryptophan, improvement in mental symptoms, glossitis and dermatitis resulted. The relationship of diets high in corn to the pathogenesis of pellagra has not been completely elucidated. The low tryptophan content of corn may be the explanation but the presence of some inhibitory substance has not been ruled out.

Studies of niacin nutrition in the future must include consideration of the dietary intake of tryptophan as well as of niacin and measurement of excretion of this amino acid and its metabolites, as well as of the excretion of niacin, N^1 -methylnicotinamide and of N^1 -methyl-6-pyridone-3-carboxylamide.

The status of chemical tests used currently in determining niacin malnutrition may be briefly summarized. The excretion of N^1 Me in 24 hours usually falls to low levels in patients with pellagra and in subjects maintained on diets low in niacin and protein (120, 123, 124). Reports of normal excretion of N^1 Me on diets containing minimal quantities of niacin (125) may perhaps be explained by the tryptophan content of the diets. A low or zero excretion of N^1 Me in the urine during fasting is indicative of low niacin stores in most instances. Normal persons, however, occasionally excrete no N^1 Me in the post-absorptive state (126). A high output of N^1 Me has been reported in prolonged fasting and in diseases associated with severe muscle wasting (126, 127).

Normal subjects usually show a prompt rise in N^1 Me excretion after a test dose of nicotinamide (66, 69, 73, 95, 128). A similar rise occurs in persons who have been maintained on diets low in niacin but supplemented with tryptophan (120). Johnson (73) suggested that an excretion of less than 0.5 mg of N^1 Me, in 4 hours after an oral test dose of 50 mg of niacinamide, indicated chemical deficiency. With a similar oral test, Goldsmith and Sarett (66, 105) found the mean excretion of N^1 Me to be lower in patients with signs of vitamin B complex deficiency than in normal subjects. Perlzweig *et al* (95) administered a single dose of 100 to 500 mg of niacinamide and noted that subjects with signs suggestive of deficiency of the B vitamins excreted only about half as much N^1 Me as did normal subjects. Ruffin *et al* (22) reported that the urinary excretion of N^1 Me after a test dose of niacinamide was lower in patients with glossitis, papillary atrophy, cheilosis and peripheral neuritis than in normal individuals. The significance of these data is greatly decreased in that many persons who are hospitalized show a low excretion after a test dose of niacinamide and yet have no lesions of deficiency. More precise evaluation of niacin nutrition awaits further investigation.

VITAMIN C

Chemical tests for assessment of vitamin C nutrition include determination of the concentration of ascorbic acid in the plasma, white cell-platelet layer or whole blood, estimation of the urinary excretion during fasting and in a 24-hour period, and load tests in which changes in blood levels and/or urinary excretion are measured for varying periods of time after administration of a dose of vitamin C. Certain changes in these

chemical tests occur before the appearance of physical evidence of scurvy. In an individual whose bodily tissues are saturated with vitamin C, the 24-hour urinary excretion reflects any increase in the amount of this vitamin ingested. The excretion falls when the dietary intake is reduced but is a poor index of deficiency since in the unsaturated person most of the ascorbic acid reaching the kidney is reabsorbed (129). Although 24-hour urine excretion is not a measure of vitamin C deficiency, there is no ascorbic acid in the urine in clinical scurvy.

The plasma concentration of ascorbic acid reflects dietary intake. When 75 to 100 mg of vitamin C are included in the diet, plasma levels of 1 to 1.4 mg per 100 cc are observed, when only 15 to 25 mg are ingested, values range from 0.1 to 0.3 mg (130). When vitamin C is removed from the diet, the plasma concentration falls fairly rapidly to zero without any physical evidence of scurvy. The ascorbic acid of the white cell-platelet layer decreases more gradually and is a better index of total body concentration (131, 132). Plasma ascorbic acid levels of zero have been reported in scurvy (133-135), and also in persons without clinical signs of vitamin C deficiency. A number of pathologic states, notably infections, are associated with reduction in plasma ascorbic acid concentration.

Load tests measure the degree of saturation of the tissues with ascorbic acid. Rall and Sherry (129) found that normal subjects excreted 50 per cent of a dose of 100 mg given intravenously, while scorbutic subjects excreted less than 5 per cent.

Kadji and co workers (136) concluded that a correlation existed between the level of plasma ascorbic acid 4 hours after the intramuscular injection of 200 mg of vitamin C and clinical signs of ascorbic acid deficiency in infants. Values below 0.2 mg per cent were found in scurvy, and below 0.6 mg per cent when bodily stores were seriously depleted. Clinical evidences of scurvy were swollen, bluish or bleeding gums, sharp costochondral junctions, swollen and tender extremities, and in most instances typical x-ray changes. Numerous other load tests have been described (137-139) in which vitamin C is given orally or parenterally and the urinary excretion or blood concentration determined at intervals thereafter. Oral tests are influenced by rate of absorption from the intestinal tract, intravenous tests by the ability of the kidney to compete with other tissues when the vascular system is flooded with ascorbic

acid. A procedure suggested recently for evaluating tissue depletion is administration of an amount of ascorbic acid larger than the expected deficit (1-3 gm), orally in divided doses throughout the day, with collection of all urine for 24 hours. The quantity of the test dose retained is estimated with allowance for 25 per cent destruction. An absolute figure for tissue depletion is thus obtained (140).

Studies of induced vitamin C deficiency have been made by several groups of investigators. Crandon, Lund and Dill (141) found that plasma ascorbic acid concentration reached zero after 41 days of a vitamin C deficient diet. The white-cell-platelet layer reached zero after 121 days. The first clinical signs of scurvy, perifollicular hyperkeratotic papules over the buttocks and posterior aspects of the thighs, appeared in 132 days. Perifollicular hemorrhages and petechiae on the lower legs were noted after 161 days. No gross changes in the gums occurred, but x-ray films of the teeth showed interruptions of the lamina dura. Capillary fragility tests remained negative. In total ascorbic acid deficiency, experimental wounds healed poorly. At this time, when 1 gm of ascorbic acid was injected intravenously, the plasma level fell to zero in 5 hours.

In Farmer's (138) experiments, a diet containing 0 to 10 mg of vitamin C was administered. Plasma ascorbic acid fell to zero in 70 days while the white cell-platelet level did not reach zero until the end of the fifth month. The physical signs of ascorbic acid deficiency were hyperkeratotic papules surrounding hair follicles, poor healing of experimental wounds, and a measurable decrease in work output. No spontaneous bleeding occurred but petechiae were noted in areas of surgical manipulation. No oral pathology was noted grossly, on biomicroscopic or on x-ray examination. Depletion must apparently be more protracted for characteristic changes in the gums or atrophy of alveolar bone with loosening of the teeth to occur. A saturation test, which consisted of measuring the blood concentration and urinary excretion of ascorbic acid at hourly intervals for 5 hours after the administration of 15 mg of ascorbic acid per kilogram of body weight, proved to be a reliable index of tissue depletion.

Peters *et al* (142) studied 10 subjects on a diet low in vitamin C, hyperkeratosis occurred in 17 to 21 weeks, exacerbation of acne in 22 weeks, perifollicular hemorrhages and tiny hemorrhages in the tips of the interdental papillae in 26 to 34 weeks, and poor wound healing in 36 weeks. The

gums became grossly abnormal (purplish, swollen and spongy) in 2 subjects. These two were the only ones showing gingivitis and periodontal disease before the experiment. No changes occurred in the capillary resistance test. Plasma ascorbic acid remained below 0.05 mg per cent after 5 weeks and the concentration in the white cells dropped below 1 mg/100 gm after 16 weeks. A leukocyte concentration of less than 2 mg/100 gm, on repeated analysis, was suggested as supporting the diagnosis of scurvy.

Johnson *et al* (143) found no detectable change in physical vigor in manual workers after 2 months on a diet containing no vitamin C although minimal gum changes were observed. Severe desaturation of tissues was shown by chemical measurements, including blood levels, urinary excretion and load tests.

In surveys of nutritional status, attempts have been made to determine the relationship of changes in the gums, capillary fragility, purpura or petechiae, and follicular hyperkeratosis, to vitamin C nutrition. In a number of studies (144-149) little or no correlation has been demonstrated between changes in the gums and the amount of ascorbic acid in the plasma or the degree of saturation of the tissues with vitamin C. Leeson (148) administered supplements of ascorbic acid to patients with low plasma ascorbic acid levels (mean 0.07 mg per cent) and obtained no change in hyperkeratosis, petechial hemorrhages, joint signs or gingivitis, although a marked rise in plasma ascorbic acid occurred. In a few investigations, the incidence of gingivitis appeared related to the intake of ascorbic acid or to the levels of the vitamin in the plasma (134, 150, 151, 152). Langhorne's (150) careful study showed that ascorbic acid intake influenced significantly the incidence of gum lesions where local treatment of gingivitis preceded the test period. Adamson *et al* (33) found a definite correlation between serum ascorbic acid and gum changes, particularly acute hyperemia, swelling, pain and bleeding on pressure, the correlation being marked in persons under 20 years of age. It may be noted that in experimental deficiency, changes in the gums do not occur before other evidences of scurvy.

The capillary fragility test is of no value in diagnosing vitamin C deficiency (129). No correlation was demonstrated between capillary fragility and saturation tests by Holland and co-workers (153). Likewise, no correlation was noted between follicular hyperkeratosis and the degree of unsaturation of the tissues with ascorbic acid

by Durham (154) or by Goldsmith and associates (36). In a survey in Newfoundland only a slight correlation, of questionable statistical significance, was observed between gum changes (marginal redness, swelling, recession, or atrophy of the interdental papillae) and values for serum ascorbic acid (136).

The studies noted above, and many others have shown a correlation between dietary intake of vitamin C and the concentration in the plasma or the excretion in the urine. Load tests are indicative of the degree of saturation or depletion of the tissues with respect to this vitamin. The concentration of ascorbic acid in the white cell-platelet layer of the blood is an index of tissue stores and is found to be zero prior to the appearance of physical evidence of scurvy. These findings are most useful in the diagnosis of vitamin C malnutrition but it should be emphasized that the presence of infection, burns, or the use of certain drugs, may influence ascorbic acid nutrition including the findings in chemical tests.

VITAMIN D

The chemical evaluation of vitamin D nutrition is dependent on measurement of the levels of alkaline phosphatase, of calcium and of phosphorus in the blood. There are no methods for estimation of vitamin D in materials of low potency. Normal values for alkaline serum phosphatase range from 5 to 15 Bodansky units in infants and young children and from 3 to 5 units in adults (155, 156). It has been suggested that 20 units is the upper limit of normal in premature infants (157). Phosphatase activity increases early in rickets, the rise is related to the severity of the rachitic process, and activity returns to normal when healing occurs (156-158). In mild rickets values of 20 to 30 units have been noted, in severe rickets values as high as 60 units (159) have been observed. In the age group in which rickets is prevalent and easily detected, other causes of an increase in serum phosphatase are uncommon. Klemmer (156) found a parallelism between the severity of clinical signs of rickets, particularly cranio tabes and swelling of the epiphyses, and serum phosphatase activity. Only rarely was there clinical and x-ray evidence of rickets without elevation of serum phosphatase. Determination of phosphorus in the serum is a less reliable measure of vitamin D deficiency, a reduction being noted only in severe rickets, and at times in the absence of rickets. Barnes and Carpenter (158) reported that roentgen examination and estimation of serum phosphorus fulfilled

to detect early rickets in a large percentage of cases, while good agreement was obtained between the degree of phosphatase activity and the development of clinical rickets or the rate and progress of healing. Sydow (160) found elevated phosphatase values in association with roentgenographic evidence of rickets. The level of serum calcium was unrelated to these findings. Determination of serum calcium is valuable primarily in the differential diagnosis of tetany.

The significance of elevated alkaline phosphatase values in children over 5 years of age has been questioned (161). Bessey (162) found a slight increase in certain groups of school children and suggested that determination of phosphatase activity might be useful in nutrition surveys. In view of the histological evidence of rickets reported by Follis *et al.* (163) in 46 per cent of children who died between the ages of 2 and 14, further studies of serum phosphatase in older children seem indicated. Elevation of serum phosphatase has been reported in osteomalacia.

These and other data indicate that phosphatase activity is the most sensitive index of active rickets, while skeletal abnormalities and roentgenographic changes are also useful in the diagnosis of avitaminosis D.

VITAMIN K

The detection of vitamin K deficiency depends on indirect measurements to determine the amount of prothrombin in the blood. In the absence of vitamin K, hypoprothrombinemia occurs and the blood-clotting time may be greatly prolonged. The clinical manifestation of vitamin K deficiency is hemorrhage. Vitamin K is synthesized by micro-organisms in the intestinal tract and dietary deficiency is extremely rare. Since bile salts are necessary for the absorption of this fat soluble vitamin, deficiency occurs when bile is absent from the gastrointestinal tract. It is also observed in the new born, in steatorrheas, and after the administration of certain drugs. Hypoprothrombinemia occurs in severe liver disease but in this instance is not due to vitamin K deficiency.

ciency but to disturbed liver function (164). Various methods (165-167) of determining prothrombin in the blood have been proposed. A number of variables other than prothrombin influence findings with all these procedures. Consequently, the exact concentration of prothrombin at which hemorrhagic phenomena occur can not be stated. When prothrombin activity exceeds 20 per cent of normal, hemorrhage is rarely due to deficiency of prothrombin. Studies in persons receiving therapy with dicumarol indicate that bleeding seldom occurs unless prothrombin activity is less than 10 per cent of normal (168, 169). Alexander (170) has reported that a prothrombin concentration of between 5 and 10 per cent of normal, determined by the method of Rosenfield and Tuft (171), is a reasonably safe level.

In the above discussion only the chemical measurements and physical signs commonly used in the appraisal of human nutritional status have been considered. Problems of mineral nutrition and electrolyte balance were of necessity omitted from this brief resume. Chemical tests for some of the newer vitamins have not been sufficiently correlated with physical evidence of malnutrition to be included.

Research up to the present time has shown that chemical tests are useful in reflecting recent dietary intake of a number of nutrients, in determining adequacy of body stores of several dietary factors, and in detecting early functional derangement of a few of these essential substances. The presence of certain physical signs, in conjunction with chemical findings, will permit diagnosis of specific types of malnutrition in many instances. Accurate diagnosis in nutrition, as in other fields of medicine, will always be dependent on careful evaluation of data from many sources and marked specificity of findings can not be anticipated. Future study of chemical and physical changes in various zones of nutriture should provide methods for more precise diagnosis of malnutrition, both in regard to nutrients now known to be important, and to new factors whose role in bodily economy has not yet been clarified.

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ANEMIAS CAUSED PRIMARILY BY MALNUTRITION

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I SHALL not attempt to review the older literature on this subject since I am sure it is familiar to most of this audience. Instead, I shall devote my time to a discussion of current research and the important problems which loom in the immediate future.

Advances in this field have been so rapid that I am no longer entirely sure what my title actually embraces. Research on anemia is constantly dragging nearly all anemias closer to nutrition. This is not too surprising if we think of blood as a body tissue which is constantly growing and being replaced at a rapid rate, also that it is a highly organized tissue of great complexity involved in many intricate and rapid chemical reactions. It therefore *must* be bound intimately with the nutritive factors which it must both transport and utilize.

Unfortunately, the word anemia has been very loosely used to mean a variety of defects.

Blood, being so complex, can be varied in a number of different ways. It is necessary to consider not only alterations in the number of the red blood cells, but also variations in their size, in the content of hemoglobin and in the total quantity of blood. In considering etiology, it is necessary to determine whether the disturbance is due to interference with or cessation of production, a block in maturation, increased destruction, or blood loss.

Without attempting to classify the anemias of malnutrition, they may conveniently be discussed in two groups: 1) those microcytic or normocytic anemias which accompany nutritional deficiencies, 2) the macrocytic anemias.

In the category of microcytic or normocytic anemias which accompany vitamin deficiencies, the most important are those associated with the vitamin B complex.

There is no need to discuss each of the B vitamins individually in relation to anemia. Biotin, P-amino benzoic acid and choline seem to be little concerned directly with erythropoiesis, nor

does thiamine appear to be of great importance. Riboflavin, pyridoxine, pantothenic acid and niacin are of sufficient interest to warrant some attention.

In view of the enormously complicated chemistry of the blood, it is not surprising that a number of different substances are intimately concerned in its formation and normal function, or that complex interrelationships exist. This is a field in which we are obviously just beginning to learn the fundamentals. As with other symptoms of nutritional difficulty, anemia is a non-specific end result which may occur when any one of many essential substances for formation is missing.

RIBOFLAVIN

There is much evidence of the importance of riboflavin in erythropoiesis in several species of animals. Several observers have reported anemia in dogs on riboflavin deficient diets (1-3) and have observed favorable responses to treatment with riboflavin (4). It also has been observed in rats (5, 6), swine (7), and monkeys (8, 9). The added strain of hemorrhage has helped to bring out the effect of riboflavin deficiency on erythropoiesis (3, 6). In some instances the anemia seen in riboflavin deficient animals has not responded to the administration of riboflavin (1, 10), suggesting a complex relationship with other factors or the presence of some other unrecognized deficiency. A similar situation was observed by Moore, Minnich, Vilter and Spies (11), who found that the anemia in their patients with riboflavin deficiency responded to iron alone. This usually would be regarded as proof of a concomitant iron deficiency, but the observation of Darby (12, 13) that some patients with angular stomatitis similar to that of riboflavin deficiency respond to iron therapy suggests the possibility of an iron-riboflavin relationship in erythropoiesis.

Riboflavin has, of course, been tried in pernicious anemia without effect (14). It is rather

surprising that in spite of the obvious importance of riboflavin in experimental anemias in animals there is such little evidence of any effect in man

PYRIDOXINE

Here, as in the case of riboflavin, there is much proof of an effect of pyridoxine on erythropoiesis in experimental animals. It has been observed in dogs (15-17), rats (18, 19), monkeys (20), swine (21), chicks (22), and ducks (23). In rats the added strain of hemorrhages shows the effect more clearly (19), as is the case with riboflavin. One of the very interesting observations that runs through the pyridoxine anemia literature is that once it is produced, complete remission cannot be obtained by pyridoxine unless a liver concentrate is also used (24, 20). This does not appear to be due to folic acid (20) and further strengthens the view, as with riboflavin, that there are complex interrelationships in these anemias. The work of Cartwright, Wintrobe and Humphreys (25) with swine has shown that a combined deficiency of iron and pyridoxine results in a more severe anemia than with deficiency of either alone, and that serum iron is unusually high in pyridoxine deficiency anemia. The administration of pyridoxine results in a rapid fall in plasma iron and a rapid rise in red cell count, indicating that the animal continued to absorb iron although unable to utilize it. This, I am sure, will suggest interesting possibilities for a role of pyridoxine to many members of this audience.

As complex as this possible interrelationship may seem, it is relatively simple compared to the real possibilities. Thus we see that a deficiency of pyridoxine results in a disturbed tryptophane metabolism as indicated by increased excretion of xanthurenic acid and kynurenine (26, 27). This introduces the possibility of a relationship between amino acids and anemia, as well as a role in the well known tryptophane-niacin effect which also is concerned in erythropoiesis, as will be discussed later in this paper.

In spite of all the evidence on animals, just as with riboflavin, there is no proved effect of pyridoxine on erythropoiesis in man (28, 11, 29, 30).

PANTOTHENIC ACID

In the case of pantothenic acid deficiency, as with pyridoxine, there is much evidence of an effect on erythropoiesis in experimental animals, but no good direct evidence that it is concerned in anemia in man.

Anemia in pantothenic acid-deficient animals has been observed with dogs (31), swine (32), monkeys (20), and rats (33). There is also evidence here, as with pyridoxine, that the problem is not a simple one, in that in some instances complete recovery does not follow the administration of pantothenic acid. Daft *et al.* (33) have obtained a partial explanation for this. Working with pantothenic acid-deficient rats, they found that the pantothenic acid deficiency resulted in a secondary folic acid deficiency. This was not the complete explanation, however, and there is still the likelihood of another unidentified factor being concerned.

The important point is that with pantothenic acid deficiency, as with pyridoxine deficiency, the failure to obtain a complete therapeutic result with the synthetic vitamin after the deficiency has been produced may be interpreted as indicating a probable interaction or metabolic relationship with other factors essential in erythropoiesis.

NIACIN

The complexities in the possible role of niacin in erythropoiesis make this substance especially interesting. In the case of niacin, effects have been observed both in man (34) and in experimental animals (35-37). However, the observations in man are so complicated by multiple deficiencies that no clearcut human anemia due to niacin can be recognized at this time (38). In the case of swine, the observation of Petri *et al.* (39) is of special interest. These workers found that removal of the fundus of the stomach of swine led to a loss of the anti-anemia substance from the liver, which could be prevented by nicotinic acid. This observation is of such importance, if true, that the work should be confirmed and extended.

Wintrobe *et al.* (40) have found that niacin deficiency in swine does not develop unless the diet is also poor in protein. The anemia can be corrected either by protein or niacin.

The work with niacin deficiency anemia in dogs is of especial interest (41). Handler and Featherston (42) observed anemia in dogs on three different diets deficient in niacin. They postulate that the effect of niacin deficiency is through deficiency in cozymase since niacin functions as a constituent of pyridine nucleotides.

One interesting aspect of niacin deficiency anemia in dogs is based on the observation of Rhoads

et al (43) 11 years ago, that on the Goldberger diet dogs were more sensitive to an anemia produced by feeding indole than were dogs on normal diets, and that this extra hemolytic effect of indole under these conditions could be abolished by liver extract. Sebrell and Hundley (44) have recently reopened this subject with some interesting results.

We have been able to show that dogs on the Goldberger diet number 123 given 500 mg of indole daily develop a severe anemia which can be corrected by the administration of 100 mg

lapse. Daily 5 mg of folic acid was then added with a third reticulocyte response to 32.8 per cent, but no rise in hematocrit or hemoglobin. The animal died shortly thereafter, apparently due to the large dose of indole (1 gm daily) (fig 2).

The second dog showed no response after 7 days of purified liver extract and had only a questionable rise in reticulocytes to 4.1 per cent. With niacin in addition to the 'Reticulogen' there was a slow response, with a reticulocyte peak of 6.9 per cent and an eventual hematocrit of 39 after 21 days.

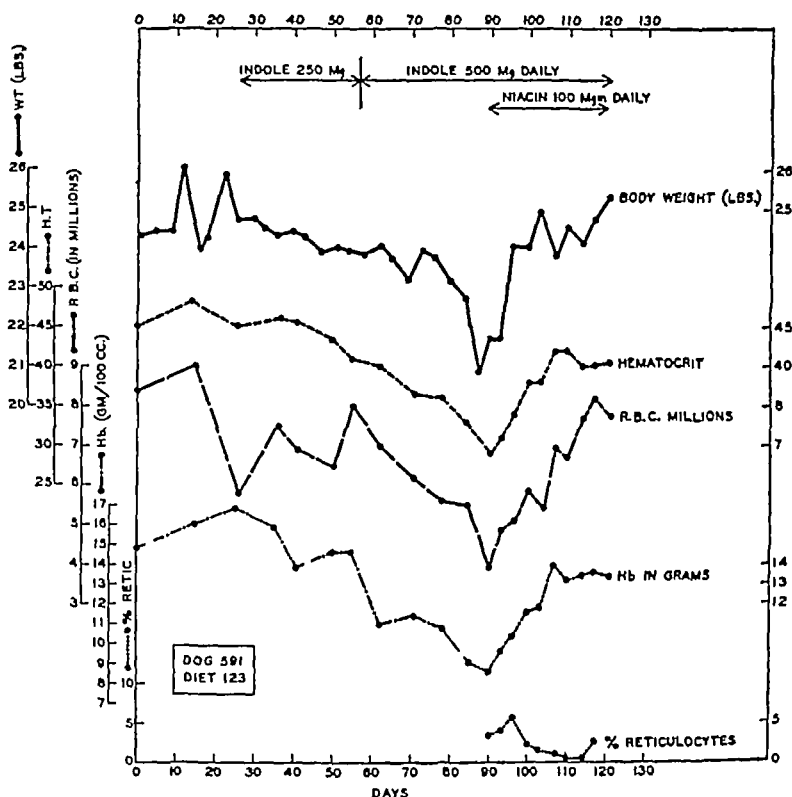


FIG 1 EFFECT OF NIACIN on blood changes produced by indole given to a dog on Goldberger Diet No 123

of niacinamide daily, in spite of continued administration of indole (fig 1).

Although the primary factor required for response appears to be niacin, there are indications that the condition may be much more complex. Thus, there is no characteristic reticulocyte response to niacin. But 2 dogs made anemic with this technique were treated with a purified liver extract (Reticulogen) furnishing 20 units of anti-pernicious anemia factor and less than 5 mg of niacin daily. One dog showed a definite blood response with 16 per cent reticulocytes in 4 days, but relapsed. The addition of 100 mg of niacin daily gave a second response with 24.9 per cent reticulocytes, followed again by a re-

A third dog treated with 100 mg of niacinamide showed no significant response after 14 days, but reached a hematocrit of 41 in 71 days after being given 20 units of 'Reticulogen' daily while the niacin was continued.

Thus a factor present in purified liver extract containing practically no niacin appears to be involved in some of these dogs.

The possible role of tryptophane is again suggested by the fact that tryptophane is the only naturally occurring amino acid which is an indole derivative. Since there is much experimental evidence that tryptophane can be converted into niacin, 2 of the anemic dogs were treated with

tryptophane in doses of 1 gm and 2 gm of DL-tryptophane daily Neither responded favorably

Thus it appears that the indole effect is not an interference with normal tryptophane metabolism or conversion of tryptophane to niacin The

direct antagonism of indole by niacin The many questions involved offer interesting possibilities

You have no doubt noted how tryptophane has appeared in this discussion, first in connection with the anemia of pyridoxine deficiency,

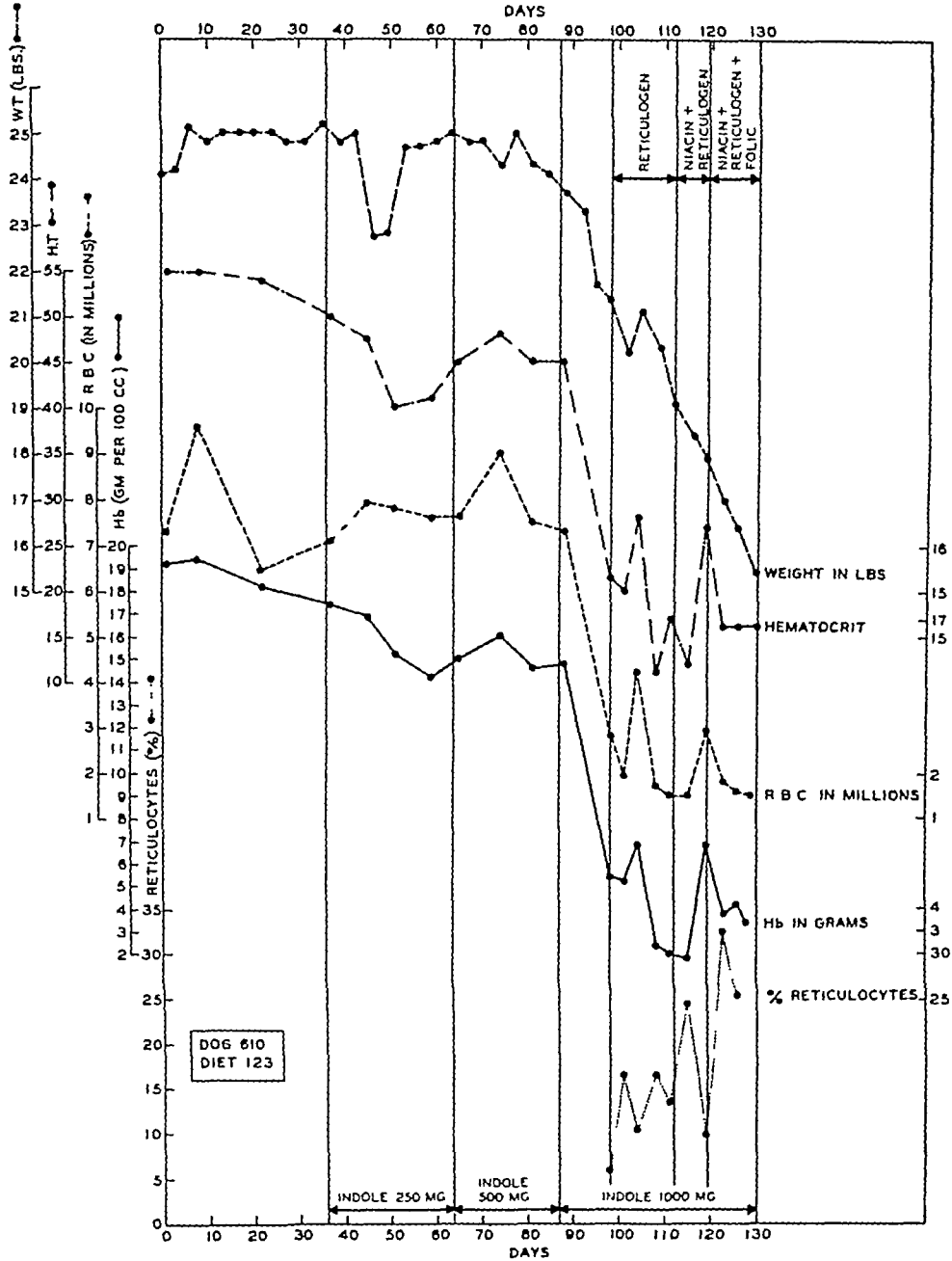


FIG 2 EFFECT OF 'RETICULOGEN,' niacin and folic acid on blood changes produced by indole given to a dog on Goldberger Diet No 123

mechanism of the response to niacin is not clear Niacin may be an essential element in blood formation, and when indole given with a niacin deficient diet results in hemolytic anemia, niacin may become a limiting factor in erythrocyte formation There is also the possibility of a

and again here in connection with niacin deficiency This will be mentioned further in the discussion of amino acids

AMINO ACIDS

The production of anemia on diets low in protein is too well known to warrant review here

This really is to be expected if it is remembered that globin, a sulfur containing protein, constitutes more than 90 per cent of hemoglobin (27). The physiological importance of this is indicated by the production of blood from body protein in the anemic dog during protein deprivation (45). In spite of its importance, the complexity of the problems involved still leaves us very much in the dark as to the specific roles of the various amino acids, the presence of unidentified factors, or the metabolic interrelationship with other factors. The evidence suggests all these mechanisms.

Daft (46) found that rats on a 4 per cent casein diet failed to grow and became anemic, leukopenic and granulocytopenic. Such animals showed a leucocyte and granulocyte response following treatment either with folic acid, vitamin B₁₂, or with at least one other factor in liver. He obtained a complete response following treatment with extra protein or the essential amino acids. The deficiency condition could be prevented either by increasing the protein in the diet or by giving a mixture of methionine, threonine and tryptophane.

These observations suggest several important possible interactions, and the mechanism by which the low protein diet results in a blood dyscrasia accompanied by a deficiency or depletion of folic acid and B₁₂ is not clear. Daft's data support the view that these vitamins may be synthesized from the amino acids in the animal and that this relation between amino acids and vitamins may account for the findings. His data show that although methionine, tryptophane, and threonine have preventive action, they alone are of little value in treatment. The simultaneous administration of folic acid, PABA or of isoleucine, valine, and histidine corrected the dyscrasia in a large proportion of the experimental animals. It appears that folic acid may be synthesized in animal tissue under these conditions.

Wintrobe *et al* (27) have shown in swine that a diet apparently deficient only in protein resulted in anemia which responded to folic acid. Shehata and Johnson (47) have fed rats a low protein diet containing sulfathalidine and have shown that the incidence of anemia under these conditions depends on the level of protein in the diet.

Daft (46) has suggested the possibility that amino acid imbalance or protein deficiency may be concerned in some human blood dyscrasias.

There are old clinical observations suggesting that tyrosine metabolism is concerned in human pernicious anemia (48-50). Recent work by Swendsen *et al* (51) based on the excretion of phenols in this disease supports this viewpoint. The fact that folic acid, pyridoxine, and ascorbic acid deficiencies all affect tyrosine metabolism (52-54) and are all concerned in experimental anemias is also suggestive.

Other work of considerable interest in this connection is that of Pontes and Thivolle (55), who have observed therapeutic benefit in some cases of pernicious anemia from the administration of histidine and tryptophane. Although Robschert-Robbins *et al* (56) have shown that no one amino acid is the essential one in erythropoiesis, it does not necessarily follow that they are all of equal importance. We have recently obtained some interesting results in this field and the data which I shall now present has not been previously reported.

Young albino rats were fed a protein free basal ration for 5 days while being subjected to severe hemorrhage by a technique previously described (57). On the fifth day the protein free ration is replaced by a test ration differing only in that mixtures of amino acids replace an equal weight of dextrose. The mixture of amino acids used was based on the 10 essential ones for rat growth (58, 59). One diet contained all 10 amino acids. The other diets differed only in the omission of the indicated amino acid. Results in table 1 are given on 10 rats in each group which survived 10 days on the diet. Although only the results on hematocrit values are given in the table, hemoglobin and red cell determinations gave values which agree with the hematocrit figures. These observations with those on serum protein will be reported elsewhere in more detail. The findings are most interesting in that they indicate that absence from the diet of any of the amino acids essential for growth except arginine causes some interference with blood regeneration under these conditions. However, the variation in the effect of the omission of the various amino acids is of greater interest. It is apparent that the omission of valine or histidine most seriously handicaps blood regeneration. On the valine free diet there was great difficulty in securing 10-day survivors, and we were able to include only 9 animals in this particular test. The results with the histidine deficient diet indicate the great importance of this amino acid in hemoglobin and erythrocyte production. This is to be

expected in view of the known high histidine content of hemoglobin (60, 61). It was rather surprising that dietary methionine and tryptophane deficiency resulted in such slight interference with blood regeneration. However, the important points I wish to make are that there are apparent variations in the importance of amino acids in blood formation, that valine and histidine are especially concerned, and that a dietary deficiency of any one of the 10 essential amino acids for growth, with the exception of arginine, causes some interference with blood regeneration following hemorrhage in rats on a protein free diet.

TABLE 1 AVERAGE CHANGES IN HEMATOCRIT ON AMINO ACID DIETS FOLLOWING HEMORRHAGE

DIET	AMINO ACID OMITTED	HEMATOCRIT				T
		Days		Increase in 10 days		
		0 Vol %	10 Vol %	= S D Vol %		
1305		14 2	43 8	29 6	= 2 4	
1337	Valine	15 0	29 1	14 1	= 1 3	5 0
1356	Leucine	14 1	34 6	20 5	= 2 5	2 2
1407	Tryptophane	14 9	37 7	22 8	= 2 0	1 8
1408	Histidine	14 5	22 8	8 3	= 2 7	5 2
1409	Isoleucine	14 0	39 4	25 4	= 2 0	2 2
1410	Threonine	14 4	35 9	21 5	= 1 7	2 4
1411	Methionine	14 2	39 1	24 9	= 1 8	1 3
1412	Phenylalanine	14 4	34 4	20 0	= 1 7	2 8
1413	Arginine	14 6	42 7	28 1	= 1 0	3
1414	Lysine	14 6	32 6	18 0	= 2 2	3 2

^t values of 4.0 indicate significant results 999 times in 1000 trials

VITAMIN C

The only other vitamin except those of the B complex which appears to be intimately concerned with erythropoiesis is vitamin C. Although anemia has been found in experimental animals with scurvy (62), opinion has been divided as to whether a true anemia occurs with human vitamin C deficiency (63, 64). The weight of evidence is much in favor of a role for vitamin C in erythropoiesis, which, however, may be a complex one as is the case with the other vitamins. This is indicated by the work of Dyke *et al.* (65) who observed cases of pernicious anemia which failed to respond satisfactorily to adequate doses of potent liver extract unless vitamin C was administered. These cases probably were re-

ceiving an insufficient amount of vitamin C in their diets.

Vilter has recently reported (66) observations on 26 cases of uncomplicated scurvy of which only 2 had no anemia. He concludes that vitamin C is essential for normal hematopoiesis. Some of the characteristics of the anemia suggest to him that hemolysis may be a factor and that there is also a depression of bone marrow activity.

The question of the relation of vitamin C to erythropoiesis is further complicated by the observation that a macrocytic anemia sometimes occurs in patients with vitamin C deficiency and responds to the administration of vitamin C (67). The entire problem of the mechanism of action of vitamin C in erythropoiesis seriously needs further study. This is especially suggested by the report of Woodruff and Darby (54) that tyrosine metabolism in the scorbutic guinea pig can be favorably influenced by either vitamin C or pteroylglutamic acid.

MACROCYTIC ANEMIAS

Many new opportunities for research on the macrocytic anemias have occurred as a result of the discovery of the pteroylglutamates and vitamin B₁₂. There is such intense activity and rapid progress in this field that much of what I say today may be made out of date by work reported during this meeting.

The literature on the pteroylglutamates is already enormous and would require a monograph in itself. I shall not even attempt a superficial review of such an extensive and important mass of material, but rather shall select just a few points of special current interest. The truly brilliant research involved in the discovery, isolation and synthesis of folic acid, together with the steadily growing body of knowledge concerning its physiology, has been covered in previous reviews (68-70).

It is now clear that the pteroylglutamates are essential for a variety of animals and are found widely in plants. In man they are effective therapeutic agents in sprue, in nutritional macrocytic anemia, in the macrocytic anemia of pregnancy, in the megaloblastic anemia of infancy, and in securing a hematopoietic response in pernicious anemia (71-73).

The clinical activity of the pteroylglutamates overlaps that of vitamin B₁₂, although the 2 substances are quite obviously entirely different chemically. The most important clinical differ-

ence is that the pteroylglutamates have no beneficial effect on the central nervous system lesions of pernicious anemia (74-76), although these lesions are adequately controlled by vitamin B₁₂ (77).

In spite of the demonstrated therapeutic effect of the pteroylglutamates on human macrocytic anemias, there is as yet no proof that added folic acid is needed by normal persons. Although estimates of the quantity needed have been made on the basis of excretion studies (70, 78), such estimates do not tell us much about the normal human requirement or the controlling conditions. The solution of such problems must await better knowledge of the mechanism of action and relation to other substances essential in erythropoiesis.

I have already mentioned Daft's evidence suggesting that folic acid may be synthesized by the rat. If it can be synthesized by other mammals, this observation may be of great significance. There is great research interest in the relation of folic acid to other nutrients as well as to its mechanism of action, and it has been suggested that it is concerned in the metabolism of purines and pyrimidines (79, 80).

Elvehjem and his associates have studied the relation between folic acid and niacin deficiency and anemia in the dog (81). There are many implications of importance in their observations. The recent paper of Ruegamer *et al* (82) reports on young dogs fed a niacin deficient diet plus sulfasuxidine. Niacin therapy was only partially successful. Folic acid brought about a better response to niacin, but had no effect on a macrocytic anemia which developed progressively. One USP unit of 'Reticulogen' brought about complete recovery. Other liver extracts were only partially effective unless given in combination with folic acid. There was no need for folic acid if high levels of protein were used. One of the important points in this work was that several of the dogs developed a flaccid paralysis, suggesting that these workers may be opening the way to study of the neurological changes found with some macrocytic anemias for which we now have no explanation.

One of the most interesting developments in connection with the pteroylglutamates is the synthesis and action of inhibitors or antagonists. Jukes (83) has described seven pteroylglutamic acid antagonists synthesized by different workers. Some of these compounds have proven especially

useful in acute leukemia of children, although they are toxic and must be handled with care (84).

These compounds offer important research possibilities, and, according to the metabolite-antimetabolite theory of inhibitors, offer a chance to find further substances. This also suggests the possibility of inhibitors for vitamin B₁₂, which no doubt would assist in clarifying the action of that substance.

In this connection, I would like especially to call attention to the possibilities offered by study of the macrocytic anemia due to infestation by the fish tape worm. This anemia has been reported to respond to treatment with anti-pernicious anemia liver extract. This, therefore, offers another line of approach to the etiological factors involved in the macrocytic anemias, and I am surprised that this unusual anemia has not attracted more special study of a fundamental nature. It seems quite possible that the fish tape worm may elaborate an inhibitor or antagonist, although von Bonsdorff (85) failed to obtain any evidence of such action with dried tape worm.

Cartwright *et al* (86, 87) have produced a macrocytic anemia in swine using a pteroylglutamic acid antagonist (methyl folic acid) in the presence of one USP unit of purified liver extract. There was rapid response to pteroylglutamic acid, but response to vitamin B₁₂ or purified liver extract was less than with pteroylglutamic acid. Thymine and xanthopterin had little activity and tyrosine, adenine, and uracil were inactive. Finally, we came to one of the greatest advances in the field of erythropoiesis. This was the isolation from liver of vitamin B₁₂ in 1948 by Rickes *et al* (88) and by Smith (89). This discovery has already produced a voluminous literature and stimulated research on anemia to a degree never before seen.

Vitamin B₁₂ is reported to be a red crystalline compound with a molecular weight probably between 1500-1600, containing cobalt, phosphorus, and nitrogen (90, 91). This material is now the most potent known therapeutic substance. West (92) has obtained a maximal reticulocyte response on pernicious anemia with one microgram daily, and 25 mcg once a week has resulted in normal blood counts and improvement in spinal cord lesions. Cobaltous ion in doses of 150 mg was ineffective, and 5.4 mg of thymidine was practically without effect (93).

This observation is of particular interest be-

cause of the results of bacterial studies Stokes (79) has found that with certain bacteria thymine (5-methyl uracil) may substitute for pteroylglutamic acid Prusoff, Tepley and King (94), using a medium partially deficient in pteroylglutamic acid but otherwise satisfactory for *Lactobacillus casei*, obtained a lower content of desoxyribonucleic acid without any change in the content of ribonucleic acid An excess of thymine caused a moderate rise in both desoxyribonucleic acid and ribonucleic acid

Shive *et al* (95) have shown that thymidine (thymine desoxyriboside) may substitute for liver extracts with antipernicious anemia activity and presumably the substitution was for vitamin B₁₂.

Wright (96) has found that with some lactic acid bacteria desoxyribonucleic acid, but not ribonucleic acid, may substitute for vitamin B₁₂, and has postulated that vitamin B₁₂ functions in the conversion of thymine to thymidine

Frommeyer and Spies (97) have shown a hematopoietic effect in human macrocytic anemia from large doses of thymine, and Spies *et al* (98) report successfully treating macrocytic anemias in relapse first with thymine, on second relapse with folic acid, and on third relapse with vitamin B₁₂, thus indicating the overlapping of these widely different substances therapeutically, but in very greatly differing doses On the basis of the massive doses of thymine used by Spies, it is quite possible that the failure of thymidine in West's hands may have been due to the relatively small dose It will be interesting to see the results when larger amounts can be tested

There is considerable evidence that vitamin B₁₂ is identical with at least one animal protein factor (99-101) Further work will be required to determine whether the essential chick growth factor concentrated from fish products by Pensack, Bethke and Kennard (102) is identical with vitamin B₁₂

Although vitamin B₁₂ is effective in controlling pernicious anemia, nutritional macrocytic anemia and sprue (98), Bethell *et al* (103) have reported a case of macrocytic anemia of pregnancy which did not respond to vitamin B₁₂ but did respond to intramuscular pteroylglutamic acid It is not yet clear whether this is an unusual case similar to the few cases of sprue and pernicious anemia which fail to respond to pteroylglutamic acid, or whether vitamin B₁₂ will prove to be ineffective in this type of macrocytic anemia Lubby (104),

studying the megaloblastic anemia of infancy which responds to pteroylglutamic acid, failed to secure a response with vitamin B₁₂ alone and suggests that both vitamin B₁₂ and folic acid are normally involved in the protein metabolism of blood formation.

Bethell *et al* (105) have made the interesting observation that the feces of normal persons and of pernicious anemia patients in relapse contains a substance which promotes the growth of test organisms for vitamin B₁₂ in approximately the same amount Furthermore, he has produced a significant response in patients with pernicious anemia by injecting an extract of feces from patients with untreated pernicious anemia These findings suggest that one of the factors in pernicious anemia may be an inability to absorb vitamin B₁₂, or that vitamin B₁₂ is produced by bacterial action in the intestine and is unavailable for absorption

Castle (106) reports that gastric juice potentiates the effect of oral vitamin B₁₂, but the activity is not as great as when vitamin B₁₂ is given intramuscularly without gastric juice Vitamin B₁₂ and extrinsic factor may be identical, and the action of the intrinsic factor may be to promote absorption of vitamin B₁₂ rather than to act upon the extrinsic factor in food

Thus the availability of vitamin B₁₂ has, by moving us ahead, presented a multitude of opportunities to solve new problems It is leading rapidly to further studies of the metabolism of nucleic acids

It is a tribute to the alertness of the planners of the Federation Meetings that a symposium on the chemistry and metabolism of nucleic acids and their constituents was held at the 1947 meeting (107) when the possible importance of this subject in the anemias could not be clearly foreseen

The activity of ventriculin still must be explained, and finally, there is the possibility of additional new factors—such as the vitamin B₁₄ which Norris and Majnarch (108) have obtained from human urine and which appears to be exceedingly potent in accelerating bone marrow proliferation This substance does not contain cobalt Hays (109) has obtained evidence of another factor in liver affecting *in vitro* red blood cell maturation which he believes is not vitamin B₁₂.

It is clear that the field is broad, progress rapid and that much remains to be explained.

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American Association of Immunologists

IVORY PAWN IN THE IVORY TOWER¹

MICHAEL HEIDELBERGER

*From the College of Physicians and Surgeons, Columbia University
and the Presbyterian Hospital*

NEW YORK CITY

UNTIL recently it was the privilege of the scientist to hold himself aloof from the mundane cares and problems of the business man, the politician, the diplomat and the soldier, and to pursue his butterfly, his gene, his diplococcus, or his macromolecule relatively undisturbed by the rumblings and creakings of the outside world. But two global wars in two generations have made this isolation dangerous, if not impossible. Many of his kind have been engaged in applying his very science to more and more efficient, less and less selective methods of mass destruction. Others have brought the far ends of the earth closer by air than are our most distant cities by train, so that all the peoples of the globe are neighbors. The economic dislocations of two wars and the unrest born of them have reached into the hospital, the university, and into the laboratory of our hitherto aloof scientist. Where will the money come from for the life-blood of his work, where can he publish his data, and will they actually be speeded to the far corners of the earth, now only a few air-hours away? How much individualism, if any, shall we give up in our domestic planning?—shall there be a National Science Foundation?—how much sovereignty or international individualism shall we give up in order to avoid the unspeakable catastrophe of another war and attempt to ensure an era of fertile peace?

These problems crowd upon our scientist, for he must protect and preserve those he loves even as he must strive for the continuity of his work, so seriously threatened. I shall leave to others consideration of our internal political and economic problems and confine myself entirely to international relations, which determine whether we are to be at peace with our neighbors and can

work on in peace, or whether we are to be sucked into another war which will surely make an end to our aspirations. What do you and I know about the foreign policy of our nation or of other nations, what can you and I do to know more about these international relations, and what can you and I do to exert our influence for fair play, for justice and for peace?

You and I, as scientists, interest ourselves in our foreign policy not only as a matter of self-preservation, but also because of our duty to do so by virtue of our special training. We are accustomed to reason clearly, to balance cause and effect, to bring every resource to bear upon the elusive detail which clears up the whole mystery. Equally important, through years of practice and discipline, we have formed the habit of self-criticism, and knowing our own weaknesses and limitations, we can the more readily see those of others. Added to these attributes of maturity is an enduring patience, the knowledge of when and how to watch and listen. What better equipment could there be for a critical examination of our foreign relations?

Last September, in Paris, I spent 10 days listening to the General Assembly of the United Nations and its Political and its Social and Economic Committees, where were gathered the diplomats, the politicians, the experts and the soldiers of the nations of the world. Only rarely did I hear evidence of those qualities of the human mind which we, as scientists, admire and honor in the practice. Were we to abandon these, science could not progress and, I submit, the construction of a durable peace will be equally impossible if emotions are appealed to rather than reason, and if partisanship, the emphasis on prestige, and the unwillingness to admit fallibility are not abandoned. It is in this area that our special qualifications can make our influence felt, and

¹ Presidential Address, Detroit, April 19, 1949

there are several organizations and activities which can help us be directly useful

How many of you are members of the American Association for the United Nations? How many are members of the Foreign Policy Association? How many belong to foreign policy study groups of the League of Women Voters? These organizations publish bulletins and hold meetings which will keep us informed and provide opportunities and occasions for our active participation in the search for the quiet atmosphere of peace without which our scientific effort will be wasted and doomed. But, you say, what can you and I do, except to read books and pamphlets and answer questionnaires, even within these groups?

While the United Nations Organization is one of governments, the American Association for the United Nations represents the people of the United States in their aspirations for the United Nations. The American Association for the United Nations is a member of the World Federation of United Nations Associations, which meets every year, and where delegates from some forty national associations for the UN discuss common problems of building an alert public opinion on the UN and plan the measures they would like to see the UN take. The Federation has consultative status A, as it is called, which means that the Economic and Social Council of the United Nations and its Commissions must receive and consider its recommendations. I have been a delegate from our Association these last two years, and in this way, some of you, too, could make the influence of your scientific judgment felt. This year there were delegations from such central European countries as Czechoslovakia, Poland, Hungary, Bulgaria, and Roumania present among the others, and although some of their members were belligerent and antagonistic at the start, all of our resolutions and recommendations were, in the end, unanimously adopted in the various working commissions and at the final plenary session. Clearly, *people* of divergent views can often agree even when their *governments* fail to find a common basis for action. Two of our 1947 resolutions were unanimously adopted, with only slight modification, by the General Assembly of the United Nations when it convened in New York in the winter of 1947-8, and until one of these was considered, the General Assembly had been unable to agree upon anything. As stated at the time by Dr. Evatt, of Australia, this changed the whole atmosphere of the meeting for the better. The General Assembly, as you see,

is quick to respond to constructive popular suggestions—their implementation, which so far has rested with the individual governments, is another matter.

Now that the more moderate portion of the World Federalists has decided to work for the evolution of the United Nations into a world government there should be large areas in which cooperation of the World Federalists and the American Association for the United Nations should be feasible, including participation in the work of the World Federation of United Nations Associations.

Another international activity in which you and I can participate is the United Nations Appeal for Children, which will continue under the United Nations International Children's Emergency Fund, or UNICEF. This work was organized by Mr. Aake Ordning, of Norway, so that everyone could take part by giving food or a day's work or its equivalent, and so strive actively in a world-wide cooperative effort for the relief of suffering. We can also give aid of books, materials or funds to the Pestalozzi Village in Trogen, Switzerland, where orphans from the devastated countries, often demoralized and brutalized by their experiences, are given a quiet family life in the cultural atmospheres of their own nations while attempts are made to solve their psychological problems. The French have a similar *Ecole Médico Pédagogique* at Nivillers, Oise, for orphans of the Résistance, and this effort is desperately in need of aid.

As you see, it is not only by our membership in international scientific societies that we can promote exchanges among peoples and can help to lessen the international tensions and rivalries that have hitherto led to wars, but must be allowed to do so no longer. You and I, as scientists, have a special fitness and a special aptitude, born of long practice and tradition, for this work, and we cannot hold aloof from it without inviting forfeiture of the rights and privileges of science and our privileges as scientists. These rights and privileges are in grave danger. As immunologists we have always preferred active intervention to passive immunization. I have suggested several available vaccines—you may grasp at these or reach for others before the onset of chills and fever. Bring on the hypodermics! The time is short in which to bestir ourselves as active, thoughtful, informed, critical, yes, patriotic citizens.

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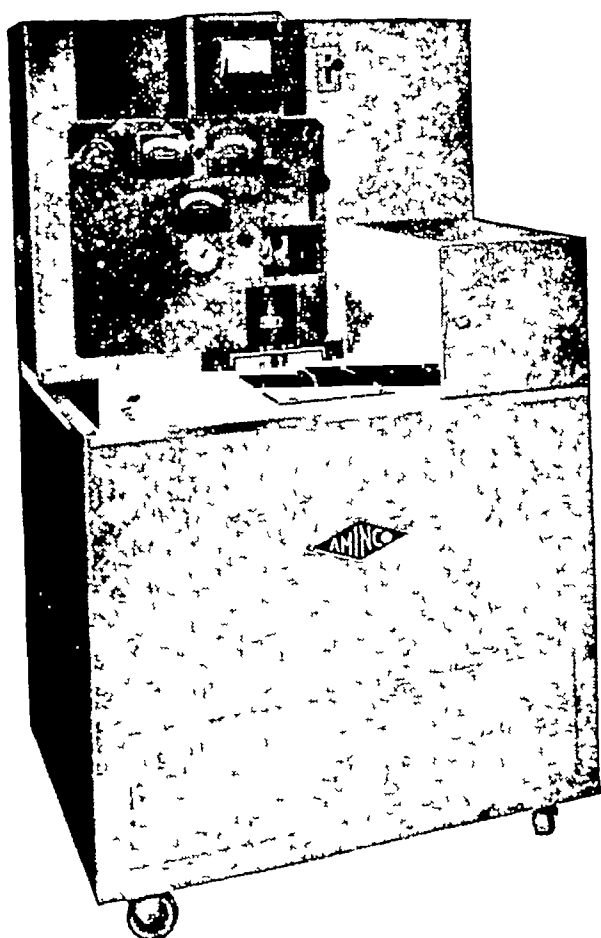


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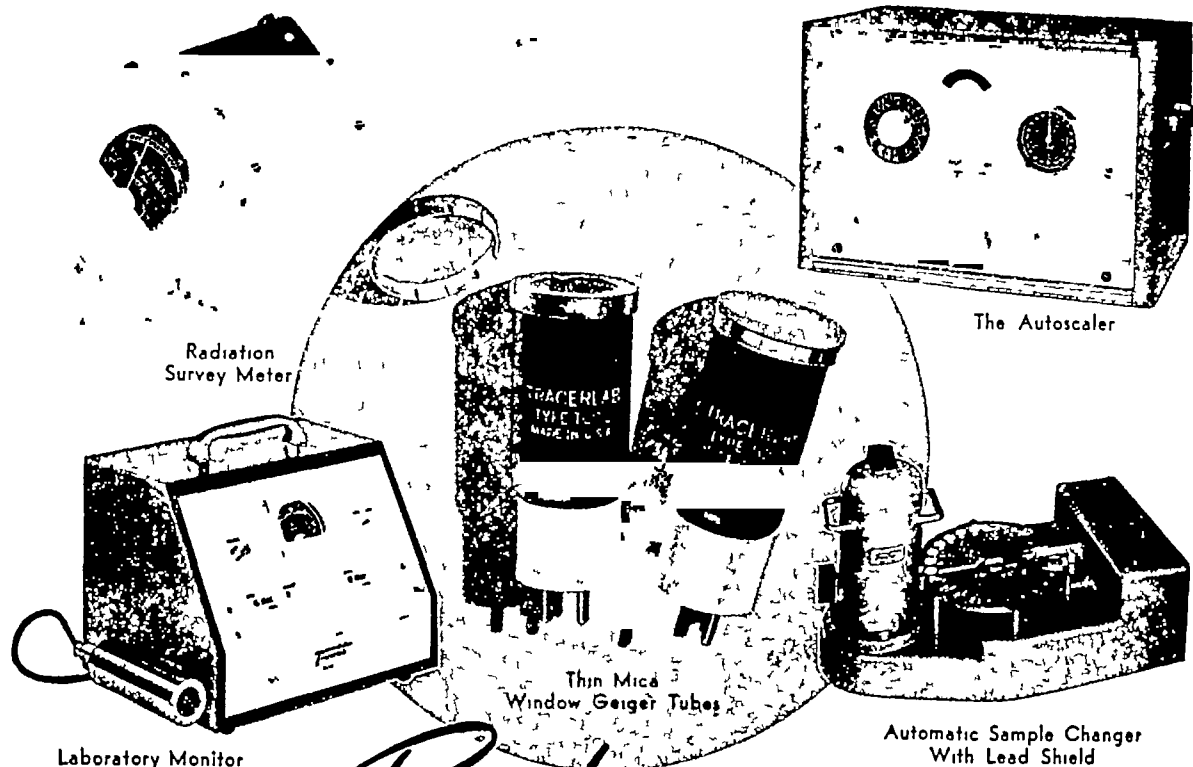


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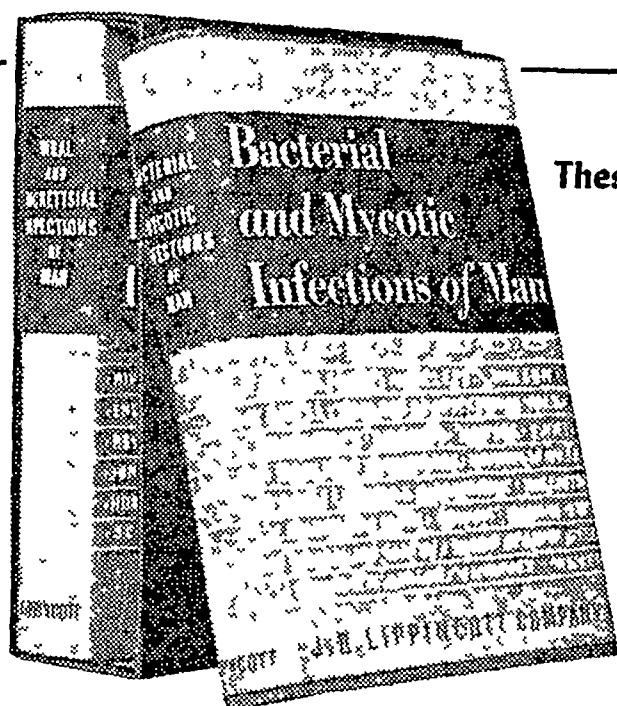
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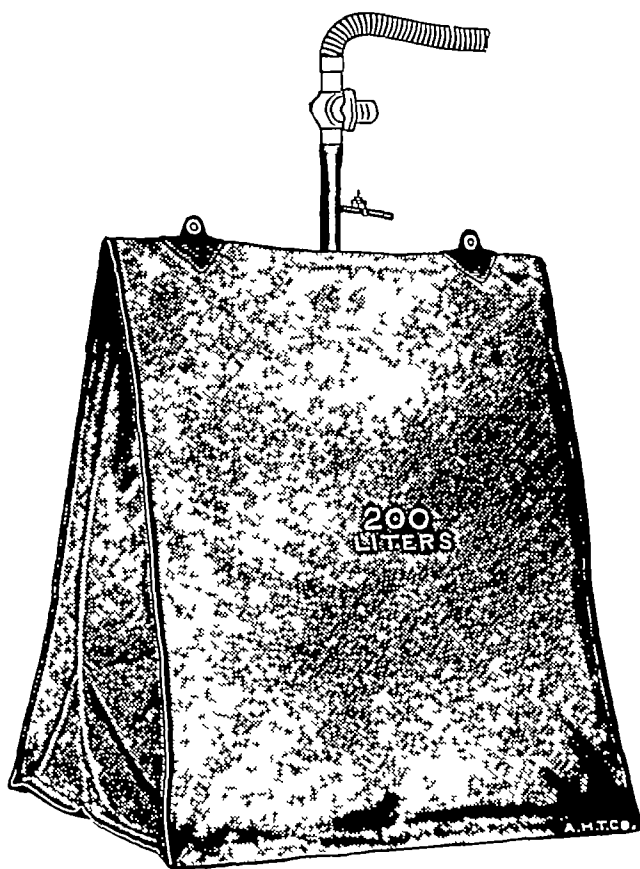
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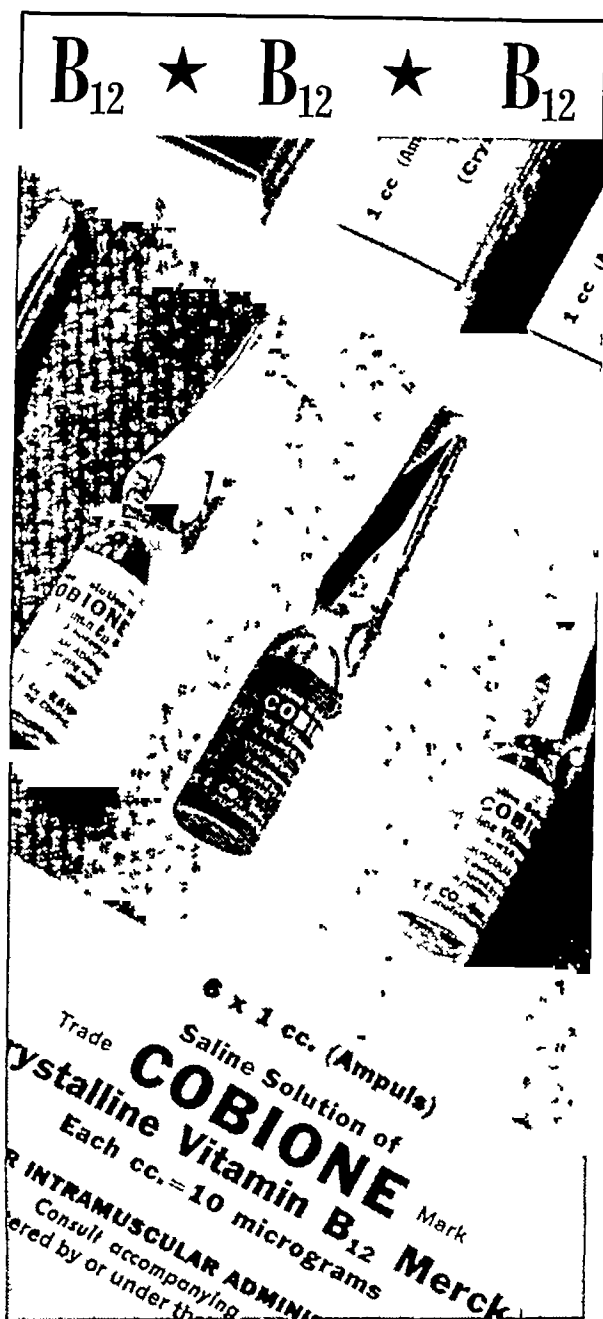
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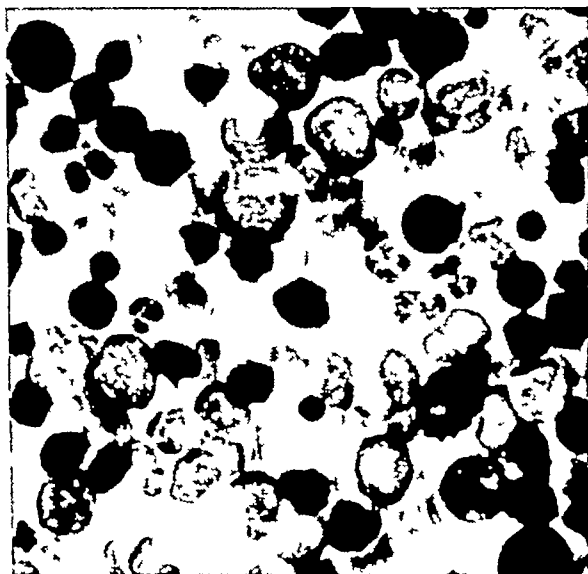
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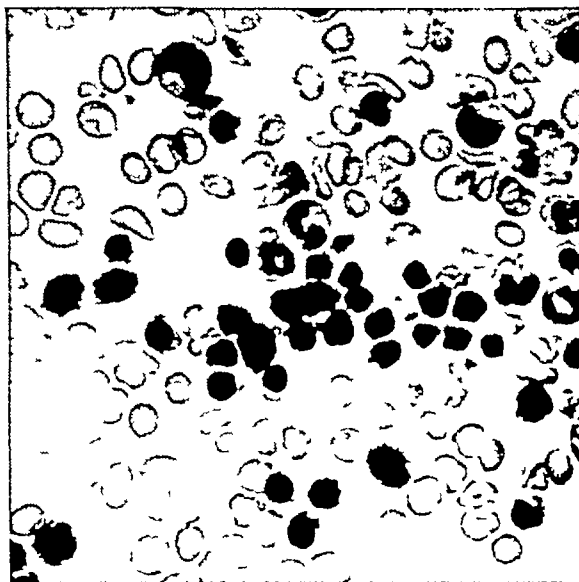
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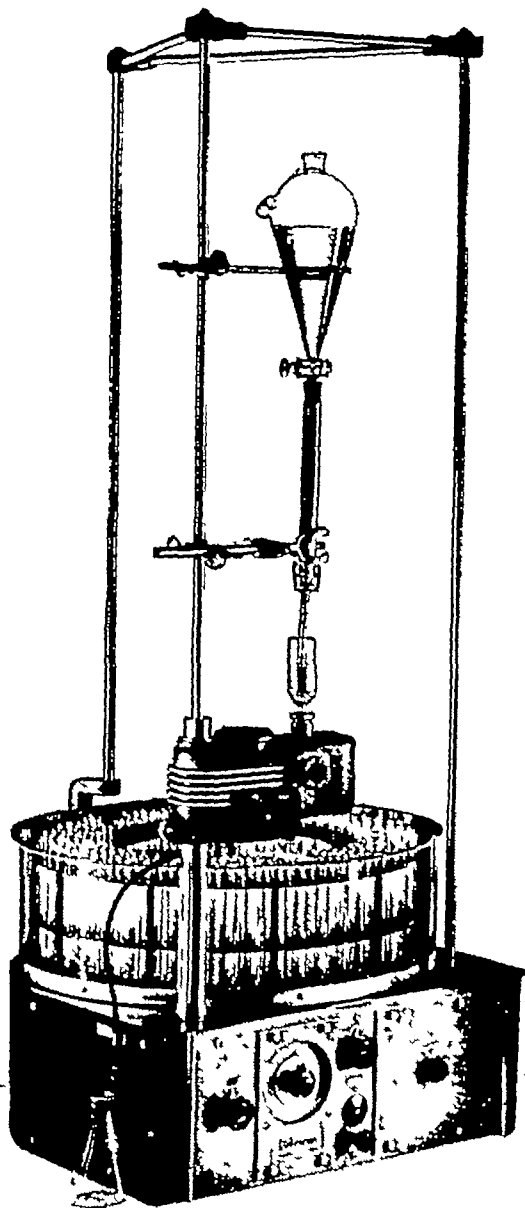
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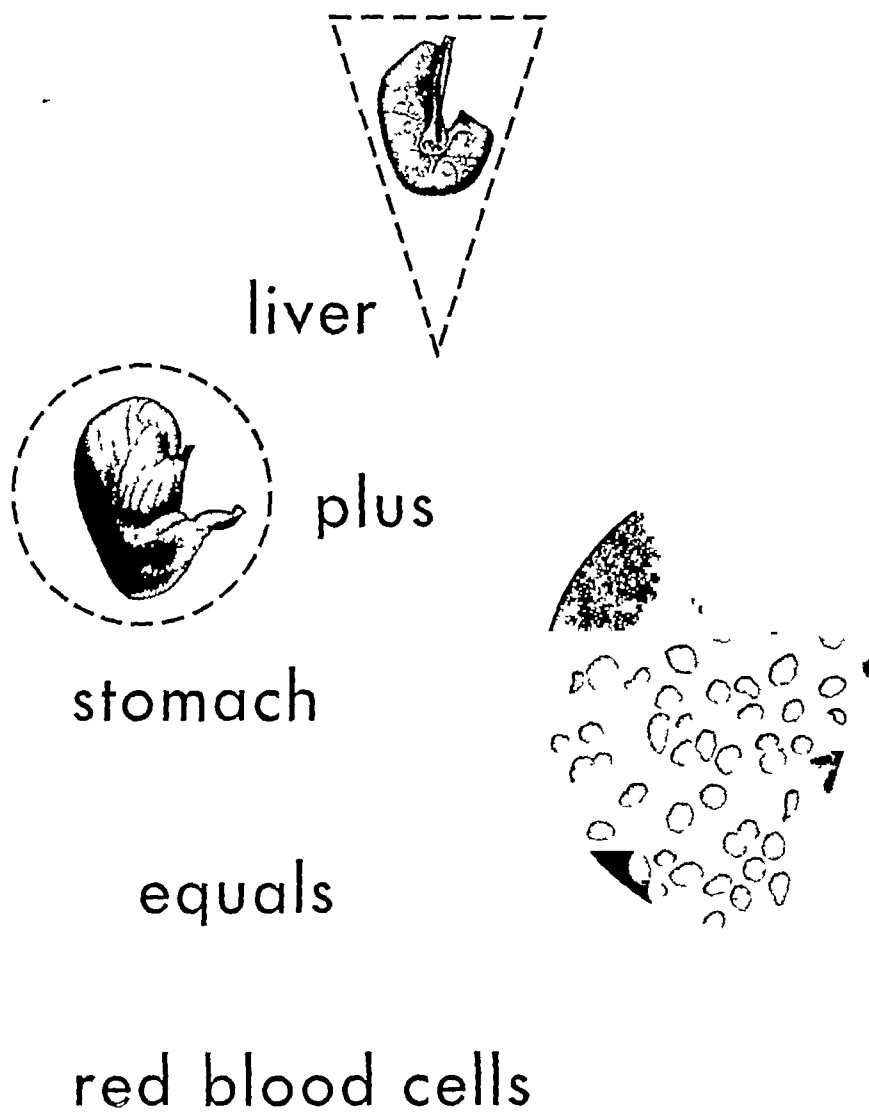
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Fifty-eighth Annual Meeting

DETROIT, MICHIGAN, APRIL 18-22, 1949

(For possible corrections in any of the following abstracts see the June issue)

Experimental mitral insufficiency W E ADAMS, E B BAY and M M NEWMAN (introduced by Lester R. Dragstedt) *Dept of Medicine and Surgery, Univ of Chicago, Chicago, Ill* Recent revival of interest in surgical treatment of valvular heart disease has prompted study of the effects of pure mitral insufficiency. Thirty dogs were operated by transthoracic approach and the anterior mitral leaflet was cut with a hooked knife introduced through the left auricular appendage. Each dog was studied 4 to 5 times preoperatively at intervals of 1 to 2 months, determinations being made of cardiac output (Fick) and right ventricular and pulmonary artery pressures by right heart catheterization. Tracings were also made on a low-frequency, critically damped ballistocardiograph. Nineteen animals survived the immediate operative period, and 10 have survived 8 to 18 months to provide the present studies. All dogs have loud systolic murmurs and the heart size has increased appreciably on fluoroscopy, but no gross evidences of heart failure or obvious limitation of physical activity have developed. Cardiac output and systemic blood pressure have remained essentially unchanged. Right ventricular pressure has been elevated in most dogs to 30-40 mm Hg systolic. In 4 of the 10 dogs, diastolic pressure has risen to 5-10 mm Hg. Left auricular pressure was palpably increased at time of operation, but could not be measured in the intact animal. Contrary to expectations, the ballistocardiograph waves have shown no change in contour, and the correlation with direct Fick outputs showed no constant change. It would appear that those animals which survived operation by 2 weeks or more show no abnormality at the present time other than moderate pulmonary hypertension.

Adrenalectomy and renin proteinuria in the rat T ADDIS and ROBERT I BOYD (by invitation) *Dept of Medicine, Stanford Univ Medical School, San Francisco, Calif* In the rat the intraperitoneal

injection of 4 mgm of renin, with the pressor value of 1 U/mg, induces a variable but pronounced increase in the protein content of the urine. This increase quickly rises to a maximum and in a few hours is gone. A unique feature of this proteinuria is the excretion of fibrinogen, for in short time urine collections made soon after an injection there may form a fine network of what seems to be fibrin. Immediately after adrenalectomy, however, no proteinuria is induced by renin, and for 10 days after adrenalectomy rats maintained on sodium chloride and glucose with added B vitamins give no definite increase in protein excretion after renin. Attempts to reestablish the renin effect in adrenalectomized rats by the injection of massive doses of cortical extracts and of desoxycorticosterone acetate gave only uncertain and equivocal results. Several adrenalectomized rats responded with slight augmentation of protein excretion after renin, but these performances were not confirmed by group averages. Intermittent injection intraperitoneally of adrenalin in maximum tolerated dosage at short intervals failed to reestablish the renin effect in adrenalectomized animals. In 2 normal rats the administration of 2,4-methylpiperadyl benzodioxane (Fournand's 993) prior to the renin did not prevent nor appreciably alter the proteinuria response to renin.

Pulsatile evaporative rates from small skin areas as measured by an infra-red gas analyzer R E ALBERT and E D PALMES (introduced by A D KELLER) *Medical Dept Field Research Lab, Fort Knox, Ky* In the course of thermal balance studies, the question arose as to why the sweat rate, as measured from the whole man by an infrared gas analyzer, was pulsatile. As an approach to the problem, the original method (Palmes *Rev Sci Instr* 19 711, 1948) was modified so that measurements could be taken from relatively small skin areas. This was accomplished

by passing dry oxygen through a cup placed on the skin and then conducting the gas to the analyzer, where the concentration of the water vapor in the oxygen was determined. In this manner, continuous quantitative measurements of the evaporative rate were obtained from skin areas measuring about 20 cm². It was found that at low sweat rates, the pulses appear in bursts. At moderate rates, they occur continuously with an average frequency of 6-7 per minute, and at very high evaporative levels, both the frequency and amplitude of the pulsations are damped. The fluctuations in the evaporative rate, as determined by this method, are dependent on the variations in the amount of skin area that is wet. The 90% response time of the instrument, which is 8 seconds, limits the maximum frequency of change that can be detected. Therefore, higher frequencies of change of rate may exist. Using duplicate sets of equipment, the measurement of dissimilar, actively sweating skin areas demonstrates that the rate variations occur simultaneously. This indicates that most of the active glands are discharged at the same time, possibly by a common stimulus arising centrally.

Serum prothrombic activity and prothrombin conversion accelerator in hemophilia and thrombocytopenia BENJAMIN ALEXANDER and GRETA LANDWEHR (introduced by HERRMAN L. BLUMGART) *Medical Research Lab, Beth Israel Hospital and Dept of Medicine, Harvard Medical School, Boston, Mass.* The prothrombic activity (1-stage) of normal human serum averages 6%. Serum and plasma mixtures exhibit more activity (faster prothrombin conversion) than the sum of the components. This is referable to a conversion accelerator (spca) which evolves during coagulation, and which can be adsorbed with BaSO₄ and eluted with citrate. Sera from thrombocytopenic and hemophilic blood, or blood clotted slowly in silicone, show abnormally high prothrombic activity, in hemophilia often exceeding that of the parent plasma. Also, they cannot accelerate thrombin evolution from added prothrombin. Nevertheless, adsorption and elution of such sera yields fractions poorer in prothrombin and capable of accelerating prothrombin conversion of added prothrombin preparations. The agent can thus, in the one stage method, accelerate thrombin formation from unconsumed prothrombin in these sera, interpretable as a much higher prothrombin concentration than actually obtains. That these sera cannot accelerate conversion of additional prothrombin supplied as plasma or prothrombin fractions suggests that spca is linked to unconsumed prothrombin. By the 2 stage method, which does not measure velocity, it would have no effect. This may explain the discrepant results obtained on hemophilic serum with both procedures. Furthermore, prothrombin consumption in the coagu-

lation of hemophilic or thrombocytopenic blood computed from differences between plasma and serum prothrombic activities (1-stage) are erroneous. Spca can be separated in a fraction containing 20 mg protein from 100 cc of serum. Many physiological and biochemical properties have been delineated. It appears different from serum Agglobulin since the latter is said to be remarkably unstable in human serum whereas spca is relatively stable.

Femoral arterial pulse in aortic insufficiency ROBERT S. ALEXANDER *Dept of Physiology, Western Reserve Univ Medical School, Cleveland, Ohio.* Aortic insufficiency was produced acutely in anesthetized dogs while recording pressure pulses from the femoral artery with optical manometers. When these recordings were analyzed and compared with similar recordings obtained under other experimental conditions it was found that, 1) the high peak of systolic pressure in the femoral artery represents an augmentation of the large central aortic pulse pressure that is relatively no greater than that observed normally. 2) The sharp drop from this systolic peak does not represent aortic regurgitation, but is simply a consequence of the high systolic level attained, relatively the fall in pressure is slower than normal. 3) The only abnormal change in pulse form produced by aortic insufficiency is the disappearance of the dicrotic waves. The latter is attributed to the elimination of the aortic standing wave, primarily because the creation of an opening at the central end of the aorta during diastole destroys the resonant properties of this system. It is therefore concluded that the Corrigan pulse observed in aortic insufficiency can be explained simply in terms of present concepts of pulse dynamics without the necessity of any additional assumptions.

Changes in the clotting mechanism produced by protamine sulfate and heparin J. GARROTT ALLEN and WILLADENE EGNER (by invitation) *Dept of Surgery, Univ of Chicago, Chicago, Ill.* Changes produced in the whole blood clotting time of the normal dog are fairly standard when 0.5 mg/kg body weight of heparin is administered intravenously. The normal clotting time is spontaneously returned to normal 90 to 120 minutes after heparin injection. The whole blood clotting time was returned to normal within 3 minutes when 0.5 mg/kg body weight of protamine sulfate was administered 10 minutes after the initial heparin injection. The clotting time, in the similarly heparinized dog, can be returned to near normal with 0.25 mg/kg of protamine sulfate, although it did not reach pre-injection level for 30 or 40 minutes. There was no effect with the injection of 0.22 or less mg/kg body weight of protamine sulfate. When 1.0 mg/kg body weight of protamine sulfate was administered intravenously in normal

dogs, it protected against the subsequent injection of 0.5 mg/kg of heparin. The extent of the protection began to diminish when heparin injection was made 30 minutes or longer after the protamine injection. No protection occurred when heparin was given 90 minutes after the protamine. The effect of 0.5 mg/kg of heparin was also inhibited by the injection of 1.0 mg/kg of gelatin given 10 minutes after heparin. Gelatin given prior to heparin showed some anti-heparin action. However, when larger amounts of gelatin (1.0 gm/kg) were injected there was no immediate protection although definite protection was observed if there was a time lapse of 4 hours before injection of heparin.

Reaction of T-1824 anion and T-1824 albumin with liver slices THOMAS H. ALLEN and PETER D. ORAHOVATS (by invitation) *Dept. of Physiology, College of Physicians and Surgeons, Columbia Univ., New York, N. Y.* The affinities of liver cells for T-1824 anion and T-1824 albumin were measured in an attempt to find the mechanisms by which this blue dye escapes from the blood stream. Rat liver slices were rapidly prepared by freehand section, rinsed twice and suspended in 2.7 ml of Ringer-phosphate (pH 7.3) of low CaCl_2 concentration (0.00024 M) contained in reaction vessels of Warburg respirometers. After equilibration at 37.5°C with oxygen, 0.3 ml of 0.000176 M T-1824 was added from the sidebulb either as T-1824 anion or T-1824 albumin (dye in 2% bovine serum albumin). For controls 0.3 ml of either Ringer or albumin-Ringer was used. The initial QO_2 was 9.3 and throughout was not changed by the addition of T-1824 although the slices became visibly blue in color. At various intervals after mixing with dye the slices were removed, and the suspension fluid was centrifuged for 5 minutes. The concentration of T-1824 in the fluid was determined using a König-Martens visual spectrophotometer. The maximum optical density always remained at 605 μ for T-1824 anion and at 625 μ for T-1824 albumin. After 200 minutes in the presence of liver slices equivalent to 10 mg dry weight, the relative amount of added dye which leaves the suspension fluid is 36% for T-1824 anion and 18% for T-1824 albumin. Graphic analysis suggests that the dyeing process follows a second order reaction in which dye is sorbed thrice as rapidly from T-1824 anion as from T-1824 albumin.

Effect of extirpating the prefrontal suppressor area 8s on correct olfactory conditioned differential responses in dogs WILLIAM F. ALLEN, *Dept. of Anatomy, Univ. of Oregon Medical School, Portland, Ore.* It was reported previously that prefrontal lobectomy abolished olfactory conditioned differential foreleg responses for hundreds of tests. The purpose of this study is to localize the area. The positive and negative conditioned stimuli con-

sisted of inhalation of clove oil and asafetida. Extirpation of the cortex was accomplished by suction. It included the caudal portion of the proreus gyri and the praesylvian (orbitalis) gyri to the level of proreus sulci. In the median line the lesion extended about 5 mm rostral of the cruciate sulci and was about 3 or 4 mm wide in front of the left and right praesylvian sulci. The forelegs did not show motor symptoms following the operations and there was no injury to the lateral olfactory tracts. After the extirpations, previously acquired positive conditioned reflexes from olfactory, auditory and cutaneous stimuli, required a varying number of tests before they appeared. Correct conditioned differential responses of the foreleg from olfactory stimuli were not obtainable during 4500 to 5000 tests, which were continuous over periods of 6 months. On the other hand, correct conditioned differential responses from auditory and cutaneous stimuli were always obtainable. The chief effect of the lesion was inability to withhold foreleg flexion during a negative conditioned test or lack of correct inhibition, which occurred when area 8s was intact. A smaller ablation involving only the caudal part of the proreus gyri produced no effect on the olfactory conditioned differential responses, but like the more extensive lesion required a number of trials before the positive conditioned reflexes could be elicited.

Effect of rest intervals on maintenance of previously induced high altitude acclimatization PAUL D. ALTLAND (introduced by HEINZ SPECHT) *Laby. of Physical Biology, Exptl. Biology and Medicine Inst., National Inst. of Health, Bethesda, Md.* This investigation was made as a part of a series of studies analyzing the mechanism of acclimatization. Maximum hematocrit values (74.0–80.0%), which have been associated with increased altitude tolerance by other investigators, were induced in Sprague-Dawley rats by 4-hour daily exposures to 18,000 and 25,000 feet simulated altitudes between ages of 14 and 100 days. Following this, cessation of exposures restored normal hematocrit values in 28–35 days. When 92 hours' rest was allowed between repeated exposures normal values were attained in 45 days. With 68 hours' rest between exposures at 18,000 feet hematocrit values leveled off at approximately 56.0% in 40 days, and at 25,000 feet they leveled off at approximately 66.0% in 20 days. With 44 hours' rest between exposures at 18,000 feet, the hematocrit values leveled off at approximately 66.0% at 20 days, and at 25,000 feet at approximately 73.0% in 20 days. When daily exposures were continued maximum levels persisted throughout 25 weeks of test. Retarded growth of male rats was observed at both altitudes (approximately 30.0% at 25,000 feet, 15.0% at 18,000 feet). Control body weight levels were

regained only in the rats of the 92-hour rest group and in those in which exposures were stopped. These experiments show that relatively high hematocrit levels may be maintained by short exposures to 18,000 or 25,000 feet at 2 or 3-day intervals, but that such acclimatization is lost completely by exposure at greater intervals.

The Rorschach test as an aid to electroencephalographic analysis MARIE D AMOROSO (introduced by T C BARNES) *Dept of Electroencephalography, Hahnemann Medical College and Hospital of Philadelphia, Penna* According to Darrow (*Psychol Rev* 54 157, 1947) electroencephalography has contributed little to psychology. This is a result of the crude clinical electroencephalographic routine now prevalent in America where thousands of patients are rushed through the EEG lab by the neurologists. Physiological factors (blood sugar, vital capacity, skin temperature) are also completely ignored (*J Psychol* 22 67, 1946, *Federation Proc* 5 5, 1946, *Federation Proc* 6 74, 1947). Personality can be related to EEG (Barnes, *Federation Proc* 6 75, 1947, Wheeler, *Am Psychologist* 3 278, 1948). In patients receiving glutamic acid Wagner (*Federation Proc* 6 220, 1947) found improved Rorschach responses correlated with disappearance of abnormalities in the EEG. We have found that blocking and repetition in the Rorschach occurs with delta activity. Of 189 patients 25 had 'good' Rorschach, 8 had normal EEGs, 11 borderline, 6 abnormal EEGs, 81 'average' Rorschach had 34 normal, 22 borderline, 24 abnormal EEGs, and 83 'poor' Rorschach had 21 normal, 13 borderline, 49 abnormal EEGs. There is a tendency for 'poor' Rorschach scores to occur with abnormal EEGs, therefore the Rorschach serves as an aid in analysis when doubtful significance is found in the electrical picture.

Carbohydrate metabolism in the decerebrated rat EVELYN ANDERSON and WEBB HAYMAKER (by invitation) *National Inst of Health, Bethesda, Maryland, and Army Inst of Pathology, Washington, D C* It has been demonstrated by Claude Bernard and numerous others since his time, that piqure of the floor of the IVth ventricle or decerebration at the pontile level in rabbits and cats gives rise in less than an hour to hyperglycemia and glycosuria and that the increase in sugar is usually maintained for 3-4 hours, but may last as long as 9 hours. The decerebrated animals generally survived not more than a day or two. This communication describes the effects of decerebration on carbohydrate metabolism in rats which survived the operation 1-3 weeks. Decerebration was done at pontile and midbrain levels. Immediately following operation, blood sugar levels were determined, and a few days to 3 weeks post-operatively, glucose tolerance, insulin sensitivity, and liver and muscle

glycogen studies were carried out. At autopsy the brains were fixed in 10% formalin, and the site of the lesions determined by microscopic examination. During the first few hours after pontile decerebration there was marked hyperglycemia, following which the glucose tolerance curve became markedly elevated. The animals showed a normal sensitivity to insulin, and their capacity to store fed glucose as liver and muscle glycogen also appeared normal.

Effect of aging on the oxygen consumption of non-nucleated erythrocytes LUIS ANGELONE (by invitation) and CLIFFORD A ANGERER *Dept of Physiology, Ohio State Univ, Columbus, Ohio* Preparatory to further studies (Gonzalez and Angerer, 1947) on the respiratory metabolism of enucleated erythrocytes, it becomes necessary to study the effect of aging on isolated erythrocytes. These cells, obtained from 32-43 normal male white rats (100-150 gm) by means of cardiac punctures, were pooled, washed twice, centrifuged to constant volume, resuspended in an equal volume of Krebs' solution (pH 7.4), and stored at 2°-8°C. Suspensions were returned to room temperature (19°-25°C) for oxygenation and withdrawal of 2.5 ml into each of 3-6 respirometers. Five different blood pools were studied from the first day to varying periods of time (12-22 days). Simultaneous determinations were made of bacteria, erythrocyte counts and oxygen consumption (Warburg technique). Since the general nature of all curves are identical, save for time, data from the last blood pool are presented as typical. Beginning the day after isolation the QO_2 (dry wt) shows a progressive decrement from -0.16 mm^3 by 14, 31, 44 and 44% on 2, 4, 7 and 13 days respectively, after which a 700% increase occurs at 21 days. An unexpectedly linear decrement from 4,820,000 to 1,180,000 erythrocytes/ mm^3 occurs at 13 days. Between 7 and 13 days a relative increase in bacteria occurs and the leveling of the curve is interpreted as due, at least in great part, to this contamination.

Dietary protein and cholic acid synthesis in dogs J H ANNENEGERS, and F FRIEND (introduced by JOHN S GRAY) *Dept of Physiology, Northwestern Univ Medical School, Chicago, Ill* This study was undertaken in order to identify the factor(s) present in protein foods which is responsible for their stimulatory effect on cholic acid output. Nine cholecystostomized dogs were used, bile-urine mixtures were analyzed spectrophotometrically for cholic acid (Irvin, Johnston, and Kopola, *J Biol Chem*, 153 439, 1944). The animals were given a basal daily diet of one half can of 'Pard' alone, then 25 and 50 gm daily of crude casein, vitamin-free casein, casein hydrolysate ('Amigen'), zein, or gelatin were added. Each diet was fed for 7 days and cholic acid output determined the last 5 days. Cholic acid output in-

creased in linear fashion with the quantity of casein or zein added to the basal diet. Vitamin-free casein increased cholic acid output to a slight but significant extent. Neither casein hydrolysate nor gelatin significantly altered cholic acid excretion when added to the basal diet. These findings suggest that some factor(s) other than amino acids is necessary for the increase in cholic acid synthesis which follows feeding of protein foods.

Mechanism of the biosynthesis of propionic acid. D. S. ANTHONY, S. F. CARSON, M. KUNA, and E. F. PHARES (introduced by ALEXANDER HOLLAENDER). *Biology Division, Oak Ridge National Lab., Oak Ridge, Tenn.* Experiments on the biosynthesis of propionic acid (by *Propionibacterium pentosaceum*) from 3-carbon substrates have indicated that C_2 and C_4 compounds are intermediate. The following C^{14} labeled compounds were used as tracers: $C^{14}H_3$, $COOH$, CH_3 , $C^{14}OOH$, CH_3 , $C^{14}O$, $COOH$ and $C^{14}OOH$, CH_2 , CH_2 , $C^{14}OOH$. Three pathways from a 3-carbon substrate to propionate were indicated, namely, 1) direct conversion without breakdown of the carbon skeleton, 2) conversion through acetate and succinate, and 3) conversion through oxalacetate and succinate. Since this is an anaerobic process, the amount of propionate formed from each of the several mechanisms depends to a large extent upon the redox state of the substrate. Determination of the extent of each of the several mechanisms was calculated from the relative specific activities of the individual carbon fragments after separation and isotopic degradation of the end-products.

Effect of vagotomy on gastric secretion and emptying time in the dog. F. ANTIA (by invitation) and A. C. IYR. *Dept. of Clinical Science, Univ. of Illinois, College of Medicine, Chicago, Ill.* Secretory studies were made on 8 dogs with gastric fistulas before and after vagotomy. After collecting a 30-minute basal sample 0.1 mg/kg histamine dihydrochloride was injected subcutaneously and samples were collected every 30 minutes for 2 hours. About 10 experiments were made before vagotomy and 10 after vagotomy in each dog. The average HCl output in response to histamine after vagotomy expressed as percentage of the average prevagotomy output was 103, 77, 69, 53, 51, 47, 33, and 26 with an average of 57%.

Six of the same dogs were studied in the same fashion before and after vagotomy using 1 mg of urecholine as the secretory stimulus. The response to urecholine varied considerably both before and after vagotomy. After vagotomy the average response to urecholine expressed as a percentage of the average prevagotomy output was 140, 105, 36, 36, 34 and 12 with an average of 61%. After vagotomy no gastric secretory response occurred in any of the dogs when insulin was given intravenously (12 mg/kg). The response to dimethyl-

allyl-5-ethyl-barbituric acid was also abolished by vagotomy. The time required for 80% emptying of a test meal of 250 cc of boiled milk or 200 cc boiled milk plus 50 cc of corn oil was studied in 4 dogs. The gastric emptying time for either meal was not significantly altered by vagotomy, thus the inhibitory effect of fat was not changed by vagotomy.

Oxygen consumption of the descending aortae of adrenalectomized rats in presence of various substrates. PHYLLIS M. ARSCOTT (by invitation) and CLIFFORD A. ANGERER. *Dept. of Physiology, Ohio State Univ., Columbus, Ohio.* Young, male, white rats, weighing between 75 and 135 gm, were used in the following experiments which treat of isolated aortae and urinary bladders. The O_2 consumption (QO_2) was studied by the Fenn technique. When aortae from adrenalectomized rats were compared with those from normal rats, there was found a decrease of 28% in the QO_2 values for the former group of rats. This figure is statistically significant. There was no observable change under the same conditions for the QO_2 values for urinary bladders of the same groups of rats. The addition, respectively, of various substrates to the aortae of normal rats gave the following percentage differences in QO_2 when compared to the aortae of normal rats without substrate: glucose -9%, lactate +18%, pyruvate -21%, succinate +214%, while under the same conditions but for aortae of the adrenalectomized rats the percentage differences were as follows: glucose -11%, lactate +126%, pyruvate +19%, and succinate +171%. The net changes between the respective pairs of results were: glucose +2%, lactate +108%, pyruvate +40%, and succinate -43%. Since the urinary bladder is composed of connective and smooth muscle tissue which simulates the histology to a certain extent of the tunicae externa and media, the change of respiratory metabolism of the aorta following adrenalectomy is related to the endothelium (tunica intima) and yellow elastic tissue, or both. If this is true, then it appears that metabolism and certain well-known permeability changes of the capillary may be correlated.

Reflex respiratory and circulatory action of veratridine on cardiac, pulmonary and carotid receptors. DOMINGO M. AVIADO, JR. and ROBERT G. PONTIUS (introduced by CARL F. SCHMIDT). *Lab. of Pharmacology, Univ. of Pennsylvania, Philadelphia, Penna.* Intravenous injection of the veratrum alkaloids has been repeatedly shown to cause reflex apnea, bradycardia and hypotension by acting on receptors in the chest. Almost all previous attempts at localization of the receptors were on open-chest preparations and the observations were on the circulatory reflexes. Catheterization of the different parts of the cardio pulmonary circulation of anesthetized dogs (morphine and chloralose) has enabled the localization of the receptors responsible

for the reflex apnea. Injection of veratridine in doses of 1 microgm/kg into the left or right pulmonary artery or into the artery far out in the substance of the lungs was followed by an immediate apnea, and a hypotension and bradycardia about 5 seconds later. The apnea is due to the drug action on pulmonary receptors because cold block of the ipsilateral cervical vago sympathetic trunk eliminated the reflex apnea without noticeably changing the circulatory response. Subsequent warming was followed by the return of the apneic response to the drug. The cardiac receptors were stimulated as long as a sufficient concentration of the drug could reach the coronary artery. Although their response was predominantly circulatory, they slightly inhibit respiration. There are receptors in the carotid region, exclusive of the carotid sinus pressoreceptors, that respond to veratridine with hyperpnea, bradycardia and hypotension. The chemoreceptors sensitive to cyanide can explain the hyperpnea but not the circulatory effects.

Hyperplasia of chromaffin material in the sympathetic paraganglia of adrenalectomized rats. H BACCHUS (by invitation), A DURY, and D B YOUNG (by invitation) *Depts of Physiology, and Zoology, George Washington Univ., Washington, D C*. Histological sections of paraganglia of the coeliac and inferior cervical plexi of bilaterally adrenalectomized and sham adrenalectomized rats 3, 4, 5, 6, 7 and 9 weeks post-operatively were examined for possible alteration and compensatory changes in their chromaffin content. An intense chromaffin reaction and hyperplasia was found in the paraganglia of the adrenalectomized rats. This was not observed in the paraganglia of the sham-adrenalectomized or normal controls. The chromaffin material was diffusely distributed and showed evidence of hyperplasia and hypertrophy in the paraganglia removed from rats which were 3 and 4 weeks post-adrenalectomized. The hyperplasia assumed a concentrated and nodular character in the paraganglia of rats which were 5 through 9 weeks post adrenalectomy. These nodular areas in the paraganglia resembled adrenal medullary tissue. Such changes were not observed in the sham-adrenalectomized rats. This alteration in the chromaffin content of the paraganglia of the coeliac and cervical plexi suggest a compensatory reaction which might also be a site for the elaboration of adrenalin in adrenalectomized rats.

The effect of the bulbar facilitatory and inhibitory systems on spinal reflex activity. L M N BACH. *Dept of Physiology, School of Medicine, Tulane Univ., New Orleans*. Because of the extreme importance of the bulbar reticular formation in facilitation and inhibition of both reflexly and cortically induced movement, it seemed of interest to determine the effect of this system on various facets

of spinal reflex activity. Animals were decerebrated under ether by either the ischemic method or by a new discrete method to be described elsewhere. Kymograph recordings were made of the reflex contractions of the tibialis anticus and the gastrocnemius muscles in response to stimulation of the ipsilateral plantar and popliteal nerves and to the contralateral popliteal nerve. Coincident stimulation of the bulbar inhibitory system (which alone had no obvious effect) caused a diminution in the degree of both reflex contraction of the tibialis muscle and reflex inhibition of the gastrocnemius muscle. The amount of rebound inhibition and contraction respectively in these two muscles was considerably increased. Bulbar facilitation causes an increase in the reciprocal innervation phenomenon by enhancing the contraction of the tibialis and the inhibition of the gastrocnemius following stimulation of the contralateral popliteal nerve. Reflex after-discharge is increased also as a result of coincident bulbar facilitation. The unexpected effects of the bulbar systems on reflex inhibition may indicate that two different processes of inhibition may obtain at the motoneuron. Studies are being carried out on the effects of these systems on occlusion and fractionation and their significance in the production of spinal shock.

The effect of local cooling and heating of the finger and wrist during exposure to high ambient temperature. MORTIMER E BADER and JERE MEAD (introduced by H S BELDING). *The Quartermaster Corps, Climatic Research Lab., Lawrence, Massachusetts*. Utilizing plethysmograph cups containing salt solution bath, surrounded by a thermoregulated air stream, with thermocouples on the skin beneath the cups as well as in the bath, measurements of blood flow through finger and wrist (a 1" segment approximately 1" above the radial prominence) were made in a warm environment (32°C). Control observations were made on one arm while local cooling and heating of the two regions were carried out on the other arm.

With a bath temperature maintained at 0°C the finger skin temperature fell to and remained approximately at 7°C, but blood flow through the cooled finger did not differ significantly from base line values prior to cooling, or from the control values in the uncooled finger. Stimuli such as pain, startle and deep inspiration produced sudden diminution in blood flow through both fingers, the decrease was transient, blood flow rising rapidly to normal levels within 2 minutes. The cooled finger, however, responded with much greater diminution than did the control. Local warming (bath 44°C skin 43°C), of the finger produced no significant increase in blood flow. In the wrist, local cooling (bath 0°C skin 12°C) did produce a decrease in blood flow, warming increased the flow above base line levels.

In an individual exposed to 32°C, local thermal stimuli do not appreciably affect finger blood flow, except that cooling increases the sensitivity to some general vasoconstrictive stimuli. In the wrist, the flow is directly responsive to local temperature changes. The presence of arteriovenous shunts in the fingers may be a factor in this difference.

Renal and hormonal mechanisms of cold diuresis RICHARD A. BADER, JOHAN W. ELIOT, and DAVID E. BASS (introduced by H. S. BELDING) *The Quartermaster Corps, Climatic Research Lab, Lawrence, Massachusetts*. The mechanisms of cold diuresis have been studied by exposing men at 15°C, reclining nude except for shorts, and comparing with results of exposure at 24°–27°C, covered by blankets. Endogenous "creatinine" clearances in 9 experiments showed no significant change in glomerular filtration, and in 16 experiments there was no significant change in either endogenous "creatinine" or para-aminohippuric acid clearance, during cold diuresis. In 12 experiments there was a substantial reduction in cold diuresis during standing, as compared with reclining. In 12 experiments cold diuresis was decidedly inhibited following 15 minutes of walking.

During cold diuresis the specific gravity and chloride concentration of the urine fell as the volume rose, and each change appeared to be correlated with the previous state of hydration and chloride content of the diet. Use of an index of chloride reabsorption showed that chloride tended to be reabsorbed to a greater degree than water.

In 27 experiments three men received small intramuscular doses of either 0.15, 0.3, 0.6 or 1.2 units per kg. of pitressin at the beginning of each exposure, either cold or warm. Inhibition of cold diuresis was proportionately longer lasting following the larger doses, but even the smallest dose reduced urine flow for 1½ hours to the same small output seen in the warm exposures, with or without pitressin. The strong similarity shown between water and cold diuresis and the extreme sensitivity to pitressin constitute suggestive evidence that cold diuresis is controlled by the posterior pituitary gland.

Hepato-renal factors in circulatory homeostasis XXIII The antidiuretic action of crystalline VDM (ferritin, apoferritin) SILVIO BAEZ (by invitation), ABRAHAM MAZUR and EPHRAIM SHORR *Dept. of Medicine, Cornell Univ. Medical College, and The New York Hospital, New York City*. A previous study from this laboratory established the antidiuretic action in animals (dogs, rabbits) of partially purified hepatic VDM concentrates. The subsequent identification of hepatic VDM, as ferritin (Mazur and Shorr, *J. Biol. Chem.*, 176, 771, 1948) has made it possible to determine whether the antidiuretic effect was due to VDM or to other unidentified components of the crude concentrates. Crystalline ferritin was prepared from horse spleen

and dog liver. Experiments were carried out on rabbits and dogs. A priming dose of ferritin (500–600 gamma ferritin nitrogen) was given intravenously 5 minutes before oral hydration with 35–40 cc/kg. of water. Then, a total of 200–300 gamma/kg. of ferritin nitrogen was infused intravenously in 50 cc. of saline over 30 minutes. In control experiments, the peak of diuresis was reached in 60–90 minutes and the ingested water excreted in 180–270 minutes. With ferritin, the peak of diuresis occurred at 170–270 minutes, excretion was complete in 300–360 minutes. Doses of 50–180 gamma ferritin nitrogen were ineffective. Antidiuresis was also observed in dogs given dog liver ferritin, this excluded the possibility of non-specific effects of heterologous protein. Crystalline horse spleen apoferritin, which has identical vasodepressor effects with ferritin, exerts comparable antidiuretic action. Rabbits immunized against horse spleen ferritin failed to exhibit antidiuresis following intravenous horse spleen ferritin while antibody to ferritin was detectable in blood. The relation of the posterior pituitary to the antidiuretic action of ferritin and apoferritin will be reported.

Urinary excretion of calcium during intravenous infusion STANLEY M. BALL (by invitation) and A. V. WOLF *Dept. of Physiology and Pharmacology, Albany Medical College, Albany, N. Y.* Female dogs received continuous intravenous infusions of calcium chloride or gluconate solution (concentrations ranging from 0 to more than 20 mEq Ca per liter) at ca. 4 cc/min for 5 hours. The diuretic effects of these solutions were not significantly different from those of 5½% glucose solution (water). The plasma Ca level is not effectively regulated by renal activity alone, there being relative retention of Ca to water even when solutions less concentrated than plasma Ca were administered. Thus Ca has no clear threshold of retention and its limiting isorrheic concentration under these conditions is smaller than the normal plasma concentration of Ca. No prominent specific effects of intravenous Ca were observed on the urinary excretion of sulfate, phosphate, bicarbonate, potassium, magnesium, hydrogen ion, sodium, or chloride, although some increased urinary excretion of the latter two have been observed with toxic doses of Ca. Loads of Ca in excess of about 24 mEq in a 15 kg. dog, created within 5 hours of steady infusion, lead to vomiting. The velocity constant of excretion for Ca (minute rate of excretion per unit load) is of the order of 1×10^{-4} to $1 \times 10^{-3} \text{ min}^{-1}$, somewhat less than previously found values for chloride from sodium chloride.

Comparative evaluation of several methods for determining alveolar gas tensions in man EARL S. BARKER (by invitation), ROBERT G. PONTIUS (by invitation), DOMINGO M. AVIADO, JR. (by invitation) and CHRISTIAN J. LAMBERTSEN *Laby of*

Pharmacology, Univ of Pennsylvania, Philadelphia, Penna Simultaneous measurements of alveolar gas tensions by 4 methods and of arterial blood gas tensions by one method were made in 11 human subjects in an attempt to establish comparative values and to determine the possible existence of a direct alveolar gas sampling method which could, in the absence of a direct standard of reference, be used to provide values comparable to those obtained by indirect calculation of mean alveolar gas tensions. Blood tensions were determined by a method having a standard deviation of ± 2.0 mm Hg. Alveolar tensions were determined by the end-expiratory Haldane-Priestley technique, the automatic end-normal expiratory method, indirect calculation of effective gas tensions and sampling of the last part of each normal expiration from the tracheal bifurcation using a catheter inserted through the cricothyroid membrane.

	$p\text{CO}_2$	$p\text{O}_2$
Arterial blood	40.5	98.0
Alveolar gas		
Haldane-Priestley End Expiratory	40.7	97.9
End-normal Expiratory (tracheal)	40.5	99.7
'Effective'	40.5	100.3
End normal Expiratory (beyond mouth)	38.6	103.1

Statistical analysis revealed no significant differences among a) $p\text{CO}_2$ of blood, Haldane-Priestley and tracheal samples, b) $p\text{O}_2$ of blood and Haldane-Priestley samples, c) the alveolar $p\text{O}_2$ obtained by the 'effective', the Haldane-Priestley and the tracheal methods. All other relationships showed statistically significant differences.

Evaluation of potential thyroxine inhibitors S B BARKER and (by invitation) C E KIELY, JR, S WAWZONEK and S C WANG *Depts of Physiology and Chemistry, State Univ of Iowa, Iowa City, Iowa*. It is desirable to have procedures available for determining whether various chemical compounds are capable of interfering with the peripheral action of thyroxine in the mammalian organism. Because of the interest of this laboratory in energy metabolism, one test has centered around O_2 -consumption studies on groups of trained albino rats. Such a method enables the measurement of the effect of appropriate dosages of potential thyroxine antagonist on endogenous as well as exogenous thyroxine. Another technique takes advantage of the increased sensitivity of the animal without thyroid to small doses of estrogen. This heightened sensitivity can be depressed by injection of thyroxine. Presumably, any inhibitor of thyroxine action should restore the greater sensitivity to estrogen. Since the vaginal smear changes can be followed without damage to the animals, sacrifice is unnecessary, and the same operated rats can be used for several tests, with an adequate interval of rest. Several compounds structurally related to thyroxine have been tried, among which are the following

3,5-dibromotyrosine, 3,5-diiodotyrosine, 3,5-diiodo-4-hydroxybenzoic acid, 4-(p-methoxyphenoxy)-3,5-dibromocinnamic acid, 4-(p-methoxyphenoxy)-3,5-diiodocinnamic acid, and (furnished by Dr M J Schiffrin of Hoffmann LaRoche, Inc) N-(4-hydroxy-3,5-diiodobenzoyl)-3-(4-hydroxy-3,5-diiodophenyl)-alanine. None of these has been found by either test to show significant evidence of antagonistic effect. Some exhibit a weak thyroxine-like activity.

Quantitative method of estimating the physiological effects of deep-breathing on the electroencephalogram T C BARNES and MARIE D AMOROSO *Dept of Electroencephalography, Hahnemann Medical College and Hospital of Philadelphia, Penna*. Methods of scoring the electroencephalogram in apnea (*J Psychol* 22:67, 1946; *Federation Proc* 6:74, 1947; *J Gerontology* 3: Supplement to No. 4, p. 4, 1948) have a greater diagnostic value than crude clinical electroencephalographic procedure. Scores for abnormal waves range from -15 (most abnormal) to -2 (normal). In 276 patients these were: 15 to 19 years -7.7, 20 to 24 -5.5, 25 to 29 -4.2, 30 to 34 -5.0, 35 to 39 -3.5, 40 to 44 -2.0, 45 to 49 -2.8, 50 to 54 +0.12, 55 and over -1.7. When age and other factors are scored the index is as follows: 15 to 19 +3.0, 20 to 24 +2.2, 25 to 29 +1.9, 30 to 34 -1.4, 35 to 39 -0.76, 40 to 44 -0.77, 45 to 49 -0.33, 50 to 54 -2.0, over 55 -1.6. It is concluded that age together with physiological factors play an important part in the effect of deep breathing on the electroencephalogram and should be taken into consideration when a final analysis is made.

CO_2 pressure gradient across the placenta DONALD H BARRON *Laby of Physiology, Yale Univ School of Medicine, New Haven, Conn*. The carbon dioxide pressure gradient between the fetal and maternal sheep blood separated by the syndesmochorial (5 layer) placenta has been estimated in animals in the last third of the gestation period. The gas content of samples drawn (with the usual precautions and with animal under a spinal anesthesia) from the uterine artery, uterine vein, umbilical artery, umbilical vein, have been referred to CO_2 dissociation curves for the estimation of pressures. The results indicate that the CO_2 pressure in the uterine artery varies between 12 and 18 mm Hg whereas the pressure in the umbilical vein fluctuates between 32 and 40 mm Hg, the average pressure difference between the fetal and maternal blood appears to be about 20 mm Hg. Further, the pH of the fetal blood leaving the placenta appears to be fairly constant at 7.35-7.42, and points to the possibility that the fetal circulation through the placenta may be adjusted to regulate the pH of the blood reaching the fetal brain.

Limitations of the polarographic method of determining the oxygen tension in whole blood J

PERCY BAUMBERGER, GABOR MARKUS (by invitation) and KATHLEEN BARDWELL (by invitation) *Physiology Dept., Stanford Univ. School of Medicine, Stanford, Calif.* In 1938 Baumberger measured the O_2 tension of dilute blood polarographically in order to determine the oxygen dissociation curve of oxyhemoglobin. In 1942 Berggren (*Acta Physiol Scand.*) determined the O_2 tension of arterial blood using separated plasma for his polarographic determinations because the presence of hemoglobin interfered with the measurements. In 1947 Baumberger (*Abst. Int. Physiol. Cong., Oxford*) reported that the O_2 tension of whole blood could be determined polarographically if cyanide were added. Further work, however, has shown that cyanide leads to correct results only under very limited conditions. Whole blood gives approximately the same polarographically determinable O_2 tension as Ringer's solution exposed to the same gas, provided the hemoglobin is fully saturated. The O_2 tension must, however, be measured at an applied voltage of -1.5 vs. standard cal. elect. If measured at -0.5 Volt, cyanide must be added in low concentration to inhibit the catalytic decomposition of the electrolytically formed hydrogen peroxide. As the ratio of hemoglobin/oxyhemoglobin increases, the contribution of oxygen dissociated from oxyhemoglobin causes a difference to develop between blood and Ringer's (confirming Berggren). Factors such as pH and temperature that displace the oxygen dissociation curve affect the size of this difference and it would appear that a useful quantitative relationship can be worked out. When blood is sufficiently diluted (1:100) this discrepancy is not apparent as demonstrated by the correct oxygen dissociation curves for oxyhemoglobin obtained by a combination of spectrophotometric and polarographic determinations.

Intestinal motility and blood flow as influenced by arterial pressure. JOHN W. BEAN and M. SIDKY MOHAMED (by invitation) *Dept. of Physiology, Univ. of Michigan, Ann Arbor, Mich.* Isolated loops of dog intestine were perfused with heparinized dog blood. Sudden elevation of constant arterial pressure induced tonic contraction and augmented rhythmic contraction, lowered pressure also frequently increased tonic and rhythmic contractions for short periods initially, later decreased them. Occlusion of venous outflow while maintaining arterial pressure constant, immediately augmented tonus, rhythmic contractions, and venous pressure which was further increased by rhythmic contractions. Opening outflow after brief occlusion resulted in a temporary rapid flow above pre-occlusion rate but tonus and motility decreased. Arterial occlusion caused an immediate gradual drop in arterial pressure, a gradually diminished flow to zero, a decrease motility and tonus frequently after an initial increase. Arterial deocclusion immediately in-

creased the flow far in excess of either the preocclusion value or that following deocclusion of the vein, suggesting vascular dilatation during lowered arterial pressure. Simultaneous venous and arterial occlusion decreased arterial pressure, raised venous pressure temporarily, slightly increased motility initially then decreased it. Sudden elevation of arterial pressure by deoccluding the artery (vein still occluded) sharply increased motility and tonus, and venous pressure increased rhythmically. The records indicate, that changes in arterial and venous pressure *per se* influence intestinal motility and tonus appreciably, they also show that changing a constant arterial pressure to a slow pulsatile pressure alters gut motility by means other than blood flow changes. Shifting from constant to pulsatile pressure caused a reversible decrease in blood flow indicative of vasoconstriction.

Observations on congenital heart cases with the oxyhemograph. VIVIAN G. BEHRMANN, FRANK W. HARTMAN (by invitation), ROBERT F. ZIEGLER (by invitation) and CONRAD R. LAM (by invitation) *Depts. of Laby., Cardiology and Surgery, Henry Ford Hospital, Detroit, Mich.* Oxyhemography was employed during operations for tetralogy of Fallot (16 patients), patent ductus arteriosus (2 patients) and coarctation of the aorta (2 patients). Every tracing included the pre-anesthesia period, the induction and fluctuations occurring throughout the surgical procedure. A calibration for each O_2 saturation curve was obtained through Van Slyke analyses on at least two arterial blood samples. The effect of anesthetic agents, drugs and intravenous solutions administered during the operation on the arterial O_2 level are shown and discussed. The usefulness of the continuous blood O_2 curve as an index of the establishment of a more adequate pulmonary circulation is well illustrated. In 8 instances, preoperative and postoperative oxyhemograph studies, which included a determination of the O_2 saturation time and the effect of exercise, have been helpful in the evaluation of the congenital heart abnormalities as well as the benefit derived from surgery. These data indicate that continuous blood O_2 saturation recordings should prove valuable not only to the cardiologist but also to the surgeon and anesthesiologist in the treatment of congenital heart disease.

Digital skin temperature and blood flow relationship following change in environmental temperature. HARWOOD S. BELDING, JERE MEAD (by invitation) and MORTIMER E. BADER (by invitation) *The Quartermaster Corps Climatic Research Lab., Lawrence, Massachusetts.* Digital skin temperatures and blood flows in the terminal phalanx (venous occlusion plethysmography) were recorded on men wearing shorts and exposed for three hours in a room at $32^\circ C$, shifted abruptly to 13° ambient temperature and later returned to the $32^\circ C$ tem-

perature The subjects lay supine and were wheeled between the constant temperature rooms to obviate the effects of exercise

In the cold, blood flows decreased immediately and precipitously and usually reached values of 3% or less of the initial levels within three minutes Digital skin temperatures fell relatively less rapidly, and closely paralleled temperatures in digits in which arterial inflow was obstructed In the warm room, after cold exposures varying from 30 minutes to 2 hours, digital temperatures rose precipitously and initially paralleled temperature rises in arterially occluded digits Return of blood flow was strikingly less rapid, e g, in the first 90 minutes at 32°C following a 2 hour cold exposure, digital temperatures returned to within 20% of the control level, but blood flow was still 90% below the control level Not until 40 minutes later did the flow increase to control levels

Conclusions as to the state of digital blood flow derived from digital skin temperature alone, in other than constant ambient conditions, are limited by the fact that a change in ambient temperature may directly produce marked digital skin temperature changes independent of digital blood flow, and hence, of body heat economy

Body water compartments in man EUGENE Y BERGER (by invitation), MARCELLE F DUNNING (by invitation), BERNARD B BRODIE (by invitation), and J MURRAY STEELE *Depts of Medicine and Biochemistry, New York Univ College of Medicine, and Research Service, Third New York Univ Medical Division, Goldwater Memorial Hospital, New York, N Y* Recent measurements of body water by the dilution of deuterium oxide and of antipyrine have shown that the average percentage of water is formales about 53%, which figure is considerably lower than the generally accepted average of 70% Further, the proportion of water exhibits considerable variability The variability may be explained on a basis of fat content since measurements of specific gravity of the body have shown that fat and water content bear an inverse relationship When the extracellular fluid space as measured by bromide was subtracted from total body water space, the intracellular fluid space obtained by difference was obviously too small to be reasonable This suggested that neither bromide nor chloride was confined to extracellular water Hence, a substance whose volume of distribution was smaller than that of bromide was likely to be a closer measure of extracellular fluid Inulin was selected because it is known not to enter cells, yet passes the glomerular membrane and enters the pleural and peritoneal spaces The inulin and bromide spaces in nephrectomized dogs and in patients with poor kidney function were compared Inulin space was about half that of the bromide space Antipyrine space and inulin space were also meas-

ured simultaneously in normal human subjects, using an infusion technique previously described (*Proc Soc for Exptl Biol & Med*, 68 507, 1948) The results indicate that extracellular space constitutes about 15% and intracellular space about 40% of the body weight in normal subjects

Mechanisms of potassium excretion in the normal dog ROBERT W BERLINER, JAMES G HILTON, and THOMAS J KENNEDY, JR (introduced by JAMES A SHANNON) *Research Service, First (Columbia Univ) Division, Goldwater Memorial Hospital, and Dept of Medicine, College of Physicians and Surgeons, Columbia Univ, New York, N Y* Certain observed reciprocal relationships between sodium and potassium excretion suggest an exchange of K for Na ions, either directly or by secretion of K and reabsorption of Na with some common anion The existence of such an exchange can be demonstrated by obtaining in the urine larger amounts of secreted K than of anions which might have been secreted with it These conditions have been fulfilled in experiments where the chief urinary anion was ferro-cyanide the minimum secreted (excreted minus filtered) K exceeding, by considerable margins, the sum of chloride, bicarbonate, sulfate and phosphate Similar conclusions to be drawn from experiments in which thiosulfate was the major anion, require the additional assumption that sulfate is not secreted In experiments where Na_2HPO_4 , $\text{Na}_2\text{S}_2\text{O}_3$ or $\text{Na}_4\text{Fe}(\text{CN})_6$ is infused and K substituted for Na in the infusion, the initial effect is a marked increase in the excretion of K accompanied by a sharp rise in urinary bicarbonate and chloride excretion The increases occur while excreted K is well below that filtered Later pH and Cl excretion level off or drop while K excretion continues to rise, with Na excretion now dropping These phenomena suggest that the initial increase in K excretion is due to decreased tubular reabsorption rather than secretion

Alcohol and sucrose in the production of liver damage in rats C H BEST, W STANLEY HARTROFT (by invitation), C C LUCAS and JESSIE H RIDOUT (by invitation) *Banting and Best Dept of Medical Research, Univ of Toronto, Toronto, Canada* Although other investigators have studied the influence of alcohol upon the production of cirrhosis in several species of animals, no one, as far as we are aware, has examined the effect of isocaloric paired-feeding, i e, supplying the non-alcoholic control animals with only the same quantity of basal diet as was eaten by the animals consuming alcohol (15% solution replacing drinking water) and equalizing the caloric intake by adding an appropriate amount of carbohydrate (powdered sucrose) to the food of the controls About 200 white rats of the Wistar strain, initially weighing 150 grams, divided into 12 groups, were studied under these experimental conditions for 6 months, some being given lipotropic

supplements (choline, methionine or protein) Liver damage frequently attributed to alcohol appeared when sugar was substituted for it isocalorically The inclusion of 0.05% of choline chloride in the basal diet (casein 10, gelatin 5, zein 3, cystine 0.3, fat 12, cellulose 2 and carbohydrate, with adequate minerals and vitamins, including α -tocopherol) sufficed to prevent the appearance of more than slight fatty change in the livers of the majority of animals on the basal ration (total lipids under 15%) An adequate supply of choline, or precursors, in relation to the total caloric intake (including alcohol or sugar supplements) prevented liver damage Addition of sucrose to the basal diet produced as severe liver damage as did an isocaloric intake of alcohol, even to the point of fibrous tissue proliferation observed in a few rats This type of experiment is, at present, being carried out in very young rats and in older rats receiving diets in which the available lipotropic factors have been more drastically reduced

A phase-boundary mechanism for impulse formation, transmission and synaptic properties of nerve R. BEUTNER and T. C. BARNES *Dept. of Pharmacology, Hahnemann Medical College and Hospital of Philadelphia, Penna.* Previous reports (*Science* 94: 211, 1941) described the negative phase-boundary electrical potential of acetylcholine at the interface between oil and saline forming a wave like the nerve impulse (*Internat. Physiol. Congress* 390, 1947; *Federation Proc.* 6: 73, 1947) Our present experiments demonstrate the transmission of this wave A horizontal glass tube 60 \times 1.2 cm (paraffined to reduce conductivity over the glass surface) contains a continuous layer of guaiacol and resin mixture with a supernatant saline layer At each end of the horizontal tube there is a U-tube—one for introduction of the acetylcholine and one for the recording electrodes on each side of the oil layer The addition of 0.1 cc of saline containing 0.1 mg acetylcholine in the U-tube at one end produces a negative wave of 3 mv lasting 0.15 sec recorded from the other U-tube at the opposite end of the horizontal tube Multichannel electroencephalographic recording showed that the velocity is over 1 m per sec Conduction may be direct as between two nodes of Ranvier (*Stampfli, Internat. Physiological Congress* 218, 1947) The sensitivity of the synapse to acetylcholine was duplicated by adding more resin to the middle horizontal tube and filling the terminal U-tubes with oil only In this modified elongated oil-cell acetylcholine produced a transmitted wave when added to the ends or synapses but had no effect when added to the middle of the central tube or axon

Cortical response to light in the unanesthetized rabbit REGINALD G. BICKFORD (introduced by CHARLES F. CODE) *Section on Physiology, Mayo Foundation and Mayo Clinic, Rochester, Minn.* The

basic response of the cortex to a light flash of short duration in the unanesthetized rabbit consists of an initial fast diphasic deflection (*a*-wave) at 40 milliseconds, followed by a large 'slow and variable diphasic deflection (*b*-wave), swinging negative and then sharply positive, and delayed at its negative peak by 200 milliseconds from the flash The '*a*'-wave, enhanced in size and changed to a predominantly surface positive deflection by barbiturate narcosis, has been studied by previous workers Because of its variability and the fact that it is suppressed by barbiturate narcosis, the '*b*'-wave has received little attention Investigation has shown that the intermittency of the '*b*'-wave in the unanesthetized rabbit results from the disturbing effect of sensory stimuli Thus, the '*b*'-wave may be promptly suppressed (without affecting the '*a*'-wave) by adequate stimulation of the visual, auditory, olfactory or tactile systems The suppression cannot be maintained indefinitely by continued stimulation of one system Thus, stimuli appear to be effective in proportion to their 'surprise value' Weak sensory stimuli may cause suppression in a small part of the responding striate area It seems probable that the '*b*'-wave represents the synchronized response of neurons concerned in elaboration of visual material, receipt of which is signaled by the '*a*'-wave Arousal stimuli cause a temporary desynchronization of the former mechanism This phenomenon is of particular interest, since a similar suppression of the responses to light stimulation can be demonstrated in electro-encephalograms of human beings

Cardiac oxygen consumption and efficiency in man R. J. BING, and (by invitation), M. HAMMOND, J. HANDELSMAN, S. POWERS, and F. SPENCER *Dept. of Surgery, Johns Hopkins Univ. School of Medicine, and Johns Hopkins Hospital, Baltimore, Md.* The O_2 consumption and efficiency of human heart muscle were obtained *in vivo*, in patients with anemia, essential hypertension, coarctation of the aorta, and aortic stenosis and insufficiency Several patients were in cardiac failure resulting from mitral stenosis and insufficiency The coronary blood flow was determined with the nitrous oxide method and coronary vein blood was collected through a catheter inserted into the coronary sinus The O_2 consumed per 100 gm of left ventricular muscle was obtained from the coronary blood flow per 100 gm of cardiac tissue and the difference in O_2 content between coronary arterial and venous blood Left ventricular weight was obtained from the table of Smith, and the left ventricular efficiency was calculated from the work and the energy cost of the left ventricle Since figures for heart weight cannot be obtained accurately, values for the efficiency are approximations

In normal man the coronary flow averaged 65 cc/min/100 gm cardiac tissue, the arteriovenous

oxygen difference 11 vol %, and the O_2 consumption 7.8 cc/100 gm/min. In anemia the coronary flow was increased, the arteriovenous O_2 difference and the O_2 consumption were reduced. In essential hypertension the coronary flow was normal, the arteriovenous O_2 difference and the O_2 consumption were decreased. In coarctation the coronary flow, the arteriovenous O_2 difference, and the O_2 consumption were markedly increased. In cardiac failure the coronary flow was reduced but the arteriovenous O_2 difference was increased. The O_2 consumption was slightly above normal. As a result of these changes the cardiac efficiency was highest in essential hypertension and lowest in cardiac failure. The lowered cardiac efficiency in failure was primarily the result of decreased left ventricular work. Correlation between the work and the O_2 consumption of the left ventricle was good except in essential hypertension and cardiac failure.

Properties of the antidiuretic substance in the blood of normal and adrenalectomized rats J. H. BIRNIE (by invitation), W. J. EVERSOLE (by invitation), W. R. BOSS (by invitation), C. M. OSBORN (by invitation) and ROBERT GAUNT, *Dept. of Zoology, Syracuse Univ., Syracuse, N. Y.* It has been reported (Birnie, Jenkins, Eversole and Gaunt—in press) that the blood of normal rats contains an antidiuretic, chloruretic substance (ADS). It was readily detectable by a modified Heller and Urban assay procedure in 1 ml. quantities of fresh serum injected intraperitoneally. The serum lost its activity upon standing at 9°C. for 12 hours, and was present in greater quantities in adrenalectomized than in normal rats. The latter observation will presumably help explain the well-known failure of water diuresis in adrenalectomized animals. Newer experiments have shown the following: 1) ADS was not detected in 1 ml. samples of the serum of rats hypophysectomized from 4 to 11 days. 2) The ADS of normal rat serum (1 ml.) does not affect renal glomerular filtration as indicated by creatinine clearance tests and presumably therefore works by stimulating the tubular reabsorption of water. 3) The ADS is active when injected subcutaneously but less effective than by intraperitoneal administration. 4) Unlike the ADS, an isotonic gelatin solution, prepared for blood substitute use, has no effect on diuresis in 1 ml. doses. 5) Crude protein solutions, injected intraperitoneally, may inhibit diuresis by other mechanisms; they differ from ADS in that they act less promptly and are not chloruretic. All of these observations are consistent with the idea that the circulating ADS is the antidiuretic hormone of the posterior pituitary.

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assay procedure, the factors which contribute to the variability of results in assaying gastric secretory depressants were studied statistically. The day to day and hour to hour variability in the output of HCl in response to small doses of histamine administered subcutaneously every 10 minutes was determined in Heidenhain and also in total pouch dogs. The relationship between dose of histamine and magnitude of inhibition by a fixed dose of depressant as well as the relationship between magnitude of response to a fixed dose of histamine and magnitude of inhibition by a fixed dose of depressant were studied. The relationship between dose of secretory depressant and magnitude of inhibition was also studied.

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Effect of choline and inositol on hypercholesterolemia and hyperlipemia in the cholesterol-fed chick C BOLENE (by invitation), J STAMLER (by invitation), R HARRIS (by invitation) AND L N KATZ *Cardiovascular Dept., Medical Research Institute, Michael Reese Hospital, Chicago, Ill.* In experiments designed to determine the biochemical parameters of the atherosclerogenic process, we have undertaken studies on factors affecting the plasma content of cholesterol, phospholipids and fatty acids. Addition of 2% cholesterol to the normal diet of the chick results in a hyperlipemia involving all lipid elements. The degree of development of atherosclerosis is in general related to the degree of increase in these constituents in the plasma. Addition of lipotropic factors to such cholesterol-rich diets in the form of 1% choline plus 1% inositol resulted in a more marked hypercholesterolemia and hyperphospholipemia. This effect is progressive with values at 1, 3, 5 and 8 weeks increased to levels about 50% above the non-lipotrope-fed birds. This occurred despite the fact that there were no significant differences in food intake and weight gain. These results suggest that these lipotropes act to mobilize liver lipids and produce a prolonged transport hyperlipemia. Choline and inositol failed to modify or prevent atherosclerosis in birds killed after 15 weeks or diet.

Experimental studies in ascorbic acid metabolism WALTER M BOOKER, RAYMOND HAYES (by invitation) and MARIANNA SEWELL (by invitation) *Depts. of Pharmacology and Oral Medicine, Howard Univ., Washington, D. C.* Clinical studies underway in this laboratory on ascorbic acid metabolism and its relation to certain gingival disturbances have provoked many new experimental approaches to the problem of ascorbic acid metabolism and the effects of ascorbic acid on the metabolism of other substances in the animal body. In unanesthetized and anesthetized dogs ascorbic acid administered at the rate of 100 mg. hourly for 3 hours rises rapidly in the plasma and slowly in the cells. Only after the second hour is a significant increase noted in the cells. This suggests a barrier between the plasma and the cells. There is some evidence that the adreno-cortical hormone can lower the barrier, causing a more rapid rise in the cells. In both unanesthetized and anesthetized dogs, ascorbic acid administered either hourly or by constant drip causes interesting changes in the electrolytes of the blood, such as a fall in chlorides, in sodium and a rise in potassium. Urinary chlorides and sodium rise, while potassium falls. Adreno-cortical extract, administered at intervals while ascorbic acid

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5 \bar{V} cc of water by stomach tube was 820 cc/100 gm/min and the urine flow (V) was 022 cc/min. Eighteen hours after adrenalectomy the C_{Cr} was 686 and the V was 015. Five days after operation the C_{Cr} was 551 while the V was 013. When adrenalectomized rats were subjected to moderate and high water loads, the disproportionate fall in glomerular filtration rate and urine excretion rate was even more striking. When two hourly doses of water were administered (3% body surface/dose) the C_{Cr} of the dummy operated controls was 1 011 and the V 083, in rats adrenalectomized for 18 hours the C_{Cr} had fallen to 759 and the V to 041, 7 days after adrenalectomy the C_{Cr} was decreased to 701 with a V of 008. When 4 hourly doses of water were administered the C_{Cr} of the dummy operated controls was 1 010 and the V 118, 18 hours after adrenalectomy the C_{Cr} had fallen to 590 and the V to 031, 7 days after operation the C_{Cr} was down to 447 with a V of only 006. These results show that the rate of urine flow falls in all cases more than the rate of glomerular filtration. This indicates that in untreated, in contrast to salt-treated adrenalectomized rats, a decreased glomerular filtration may contribute to loss of water diuresis, but that an increase in tubular reabsorption of water is probably a more important factor.

A method for analyzing dog muscles for myoglobin WILLIAM J BOWEN *Laby of Physical Biology, Exptl Biology and Medicine Institute, National Institutes of Health, Bethesda, Md*. A method has been devised for analyzing dog muscle for myoglobin (Mb) in the presence of hemoglobin. Cleaned muscle is pulverized at the temperature of dry ice as described by Poel (*Science* 108 390, 1948). An exactly known quantity (about 1 gm) of the muscle powder is washed into a stainless steel homogenizer tube with a carefully measured volume (about 20 ml) of M/40 phosphate buffer, pH 5.9. It is thoroughly homogenized and then centrifuged at 1000 g for 10 minutes. The supernatant is heated to 56°. As soon as discrete particles appear, the extract is rapidly cooled and then centrifuged at 1000 g for 10 minutes. It is filtered through fast paper from which it emerges as a clear red solution. This is adjusted to pH 6.5 with solid K_2HPO_4 and then gassed with CO in a rotating tonometer. To insure complete conversion to MbCO, $Na_2S_2O_4$ is added to the tonometer after 10 minutes of gassing. After a total of 12 minutes of gassing the density (log I_0/I) of the solution is determined at 538 and 568 m μ . The molar concentration of Mb is calculated by substitution of the densities and the appropriate extinction coefficients for MbCO and HbCO in the Vierordt equation (*Ann Rev Biochem* 10 509, 1941). The amount of myoglobin/gm of muscle is calculated by using the molecular weight 17,300 (Bowen, *J Biol Chem* 176 747, 1948). By this method 9 samples of one heart, having an average of

3.60 mg Mb/gm of muscle, yielded a $\sigma = 0.28$ and a coefficient of variation = 8%. Also, a known quantity of dog myoglobin added to muscle homogenate was recovered 100%.

Hypoxia of 18,000 feet simulated altitude and myoglobin content of dogs WILLIAM J BOWEN *Laby of Physical Biology, Exptl Biology and Medicine Institute, National Institutes of Health, Bethesda, Md*. The left anterior trapezius muscle of 7 dogs was removed by biopsy and analyzed for myoglobin content by the method described in preceding abstract. The dogs were then exposed to 18,000 ft simulated altitude 6 hours per day, 6 days per week. Hematocrit and hemoglobin values and changes in body weight were followed as criteria of acclimatization. After 5 to 6 months of exposure the dogs were sacrificed and the trapezius muscle from the other side analyzed for myoglobin content. Final hematocrit and hemoglobin values were 119 to 170 and 122 to 170%, resp., of initial values. Body weights in each dog increased during the first month of exposure and then remained nearly constant. The myoglobin contents before and after exposure were as follows:

Dog	Mg Mb/gram of wet muscle	
	Before Exposure	After Exposure
1	3.27*	2.80
2	4.70*	4.33
3	5.20*	4.73
4	4.60	4.20
5	4.42*	4.62
6	5.07	3.75
7	3.80	4.22

These values are means obtained in repeated or duplicate analyses, except those marked (*). One value deviated 11% from the mean, another 8%. The remainder deviated 5% or less. The results indicate that exposure to simulated altitude of 18,000 ft causes no increase in the Mb content of muscle in contrast to the response of the hemoglobin of blood.

Principles of design and operation of internal standard flame photometers for sodium and potassium determination ROBERT L BOWMAN and ROBERT W BERLINER (introduced by DAVID P EARLE, JR.) *New York Univ Research Service, Goldwater Memorial Hospital, New York City*. Construction of two internal standard flame photometers for sodium and potassium has led to improvements in design and technique of operation. The basic aims have been simplicity of design and establishment of operating conditions in which minor variations of gas and air pressures, atomizing rates, etc. are maximally compensated by parallel changes in the output of cells measuring light from unknown and internal standard. Critical mechanical and electrical components were evaluated for their contribution to stability and accuracy, and extensive study made of parameters determining stability and independence of external variables.

Optimum operating conditions must be evaluated individually for each instrument by determining, separately, for sodium, potassium and lithium, a number of curves relating electrical output to atomizing rate, gas and air pressure, and ion concentration. Operating conditions are selected so that, as nearly as possible, changes in any of these will produce no effect on reading or be maximally compensated by the internal standard. Changes in gas and air pressures which may effect readings are corrected by resetting, emphasis being upon indication with sensitive gauges rather than automatic pressure regulation. Application of these principles to other instruments has been reported to be of material aid. With the present instruments over 6000 and 9000 determinations, in 14 months and 2 years respectively, have been made without interruption for repairs. Accuracy attainable with single readings of unknowns is ± 1.0 and $2.0 \mu\text{eq/l}$ for potassium and sodium respectively in the diluted sample. No interference from other substances in solution is detectable.

A new method of fibrin determination and applications to study of the thrombin-fibrinogen reaction PAUL W. BOYLES (by invitation) and JOHN H. FERGUSON *Dept of Physiology, Univ of North Carolina, Chapel Hill*. Determination of fibrin by the biuret method (J. Fine, *J Lab Clin Med* 24: 1084, 1936) was modified by use of Mehl's (*J Biol Chem* 157: 173, 1945) reagent, photoelectric colorimetry, and calibration of colorimetric readings against both gravimetric (fibrin dry weight) and macroKjeldahl (fibrin N) determinations. A commercial (Armour's) bovine plasma fraction-I, analyzing 50% fibrinogen (clottable), after reprecipitation at 2-5°C with ammonium sulfate at 25, 20, 20% saturation, successively, with subsequent dialysis, yielded fibrinogen solutions 96.7% clottable, with only minute traces of prothrombin, and no fibrinolytic protease. Clotting-times and fibrin yields were studied with variations of 1) fibrinogen concentration, 2) thrombin concentration (purified bovine thrombin), 3) temperature, 4) pH, 5) NaCl content, 6) Ca ion content, 7) added colloid (acacia), 8) heat (48°, 12 min) induced profibrin, 9) time after clotting (at 37°C). After 48 hr the fibrin yields were practically identical (aver over 95%) independent of the above variables, except for inhibitory concentrations of NaCl and CaCl_2 , which reduced yields significantly (e.g. 65% in 0.5 M CaCl_2). Clotting-times were much modified by these variables. It is particularly significant that the 48-hr fibrin yield was unaltered by varying the thrombin dilution from 1:1 to 1:250, despite a range of clotting-times, for the series, of 10 to 190 seconds. It is concluded that the thrombin-fibrinogen reaction does not follow any simple stoichiometric law but resembles the reaction kinetics of a colloidal enzymatic reaction.

Adsorption of urinary gonadotrophin on kaolin

JAMES T. BRADBURY, ELWIN BROWN and WILLIS E. BROWN *Dept of Gynecology, Univ of Louisville, Louisville, Ky, and State Univ of Iowa, Iowa City, Ia*. The following method has been devised for recovery of gonadotrophic hormones. Its main advantages are 1) reduction in toxicity of the extracts and 2) a marked saving the amount of alcohol needed.

The urine of a 12- or 24-hour specimen is adjusted to pH 4.5 with acetic acid. Add 5 volumes % of kaolin (powdered and acid washed in a 20% aqueous suspension), stir thoroughly and then allow to settle. Decant supernatant urine. Centrifuge and discard supernatant. (Kaolin may be re-suspended in water one to three times to wash it free of urine.) Add normal ammonium hydroxide in a volume equal to that of the original kaolin suspension used and stir thoroughly. Centrifuge and re-extract with a second volume of ammonium hydroxide. Combine the ammonia extracts, add acetic acid to pH 8.5 and centrifuge. Save the clear supernatant, adjust to pH 5.5 and add 4 volumes of 95% alcohol slowly while stirring. Set in refrigerator several hours to complete the precipitation. Centrifuge and dry the precipitate.

The dry precipitate is dissolved in water or phosphate buffer pH 8.0 and the solution injected into immature rats or mice for assay. The recoveries of gonadotrophins from pregnancy or castrate (post-menopausal) urines have been quantitative when compared with those obtained by the usual method of adding 4 volumes of alcohol to the original urine specimen.

Analysis of mosquito tissues for sodium and potassium and development of a physiological salt solution SARAH BRADFORD (by invitation) and ROBERT W. RAMSEY *Dept of Physiology and Pharmacology, Medical College of Virginia, Richmond, Va*. The midgut and Malpighian tubules of *Aedes aegypti* were analyzed for their content of sodium and potassium. The tissues from 1000 mosquitoes were pooled to provide sufficient Na and K for one analysis. A total of 6 such analyses were done. The Na and K content of the midguts and Malpighian tubules of 1000 mosquitoes kept on a normal diet averaged 6.91 mg Na/gm dry weight of tissue and 15.6 mg of K/gm dry weight. Both the sodium and potassium contents decrease with time on a salt free diet until after two weeks sodium is reduced to 2.5 mg/gm dry weight and potassium to 10.4 mg/gm dry weight. Malpighian tubules have a lower potassium content than midgut. No differences in sodium or potassium content due to age or sex of the mosquitoes were noted.

A physiological salt solution having the following composition was developed for mosquito tissues: NaCl-128 mM/l, KCl-4.7 mM/l, CaCl_2 -4.0 mM/l, M/150 potassium phosphate buffers to pH 6.8. Ex-

cept for the added buffer (which contributes 11.34 mM/l of K) this solution is the same as that which Bodenstein previously found best for *Drosophila*. This solution will maintain in the cold, a mosquito heart preparation alive and beating for as long as 280 hr. If small amounts of the B vitamins or p-aminobenzoic acid are added to the above the average survival is prolonged but the maximum so far found (300 hr) is about the same. Mosquito hearts can survive for as long as 30 hr in salt solutions that are very high in potassium and low in sodium provided sufficient magnesium is present.

Light tonus of the human eye G. A. BRECHER (introduced by C. J. WIGGERS) *Dept. of Physiology, Univ. of Prague, Czechoslovakia, and Dept. of Physiology, Western Reserve Univ. Medical School, Cleveland, Ohio*. The tonus of the eye muscles and thus the muscular balance of the nonfixating eye is maintained by various reflexes, among which light tonus plays an important rôle in lower animals. No such light tonus has been proven for the human eye. The influence of light upon the heterophoria of the nonfixating eye was measured in 21 normal subjects with a new method permitting the continuous recording of heterophorias under varying optical conditions. The nonfixating eye displays a greater heterophoria upon diffuse illumination than in the dark. The increase of the eye deviation is greatest immediately after the light exposure, and reaches its maximum in about 2 minutes, after which time no appreciable increase can be recorded. The heterophoria increase/time ratio follows the course of a parabola. Upon discontinuation of the illumination the heterophoria decreases to the same value which it previously had in the dark. The degree of the heterophoria is directly proportional to the light intensity. Illumination of the fixating eye causes also a heterophoria increase of the nonfixating eye, indicating the consensual nature of the mechanism involved. It is assumed that the heterophoria increase upon illumination of the retina is due to the existence of a light tonus which contributes to maintain the muscular balance of the nonfixating eye.

Chronic toxicity studies on hydroquinone N. R. BREWER (by invitation) and A. J. CARLSON *Physiology Lab., The Univ. of Chicago, Chicago, Ill.* Hydroquinone added to food fats in the amount of 0.01% decreases the rate of oxidative rancidity from 2 to 50 times, thus reducing food deterioration and food spoilage. Is this amount injurious to the health of man or animals? The following tests have been run to answer this important question. 1) Twenty weanling rats, started on a diet containing 0.25% hydroquinone, showed no evidence of toxic effects (growth, reproduction, autopsy findings) after 2 years on test when compared to 20 controls. Twenty offspring of these rats were continued on this test for a year with no signs of toxic effects.

Twenty rats were fed a diet containing 1.0% hydroquinone. After 2 years the only finding was a slight decrease in the rate of growth as measured by body weight. Fourteen rats were fed a diet containing 5.0% hydroquinone. The rats consumed very little food, lost weight rapidly, and after 9 weeks some of them developed a blood condition resembling aplastic anemia. 2) Dogs refused to eat food (apparently because of the bitter taste) containing more than 0.02% hydroquinone in the total diet. By placing the hydroquinone in sugar coated tablets in the food, one dog consumed 16 mg hydroquinone/kg/day for 34 weeks, without any evidence of injury when compared with 3 control sibs. Sixteen mg/kg/day is a 160-day supply/day, or a 100-year supply for 34 weeks, for man, if man were to ingest 1 gm fat/kg/day and all of the fat were treated with 0.01% hydroquinone. 3) Cats appear more susceptible to hydroquinone. But 10 mg hydroquinone/kg/day for 6 weeks showed no untoward symptoms to date, when given to 1 of 4 young sibs, and 15 mg/kg/day for 6 weeks showed only a small decrease in the growth acceleration curve when given to another sib. Fifteen mg/kg/day is a 150-day supply per day, a 17-year supply for 6 weeks, for man, if man were to ingest 1 gm of fat/kg/day and all of the fat were treated with 0.01% hydroquinone.

Factors influencing contractile force of the heart in open-chest mammalian experiments H. H. BRILL, M. DE V. CORRON and R. P. WALTON (introduced by T. G. Bernthal), *Dept. of Pharmacology, Medical College of South Carolina, Charleston, South Carolina*. Contractile force of a section of the right ventricle was measured directly by means of variable spring tensions inserted in the system of levers of the Cushny myocardiograph typically attached in open-chest preparations. All experiments were carried out on vagotomized dogs, with pericardium opened and with high oxygen concentrations in the mechanical respiratory system. Compensation for changes in heart size were accomplished in two ways: 1) by preliminary calibrations which determined the effect of measured changes in position of the collar which mechanically protected the muscle section from being stretched beyond the usual point of diastolic relaxation; 2) by adjustment of this collar as heart size changed. (This latter has been facilitated by construction of a micrometer screw adjustment mechanism.) Moving and re-setting this collar over wide ranges demonstrated that the muscle section could be stretched to approximately twice its diastolic resting length with an increase in contractile force several hundred percent greater than that of the control period. Within this range, progressive increments of stretch produced progressive increments of contractile force in a linear relationship. Progressive increments of venous

pressure obtained by massive saline infusions and progressive decrements of venous pressure obtained by venous hemorrhage resulted in moderate changes in contractile force which, in a few experiments, followed the linear relationships of Starling's Law but for the most part appeared to be substantially affected by other factors. Partial occlusion of the inferior vena cava lowered venous pressure, contractile force and arterial pressure, the effects apparently operating in close association. Partial occlusion of the pulmonary artery and of the aorta produced, in each case, only limited effects. Sudden release of constriction in the vena cava, the pulmonary artery or the aorta frequently produced an immediate transient increase in contractile force and stroke amplitude distinctly greater than that of the control period. Substantial decreases in contractile force (30-60%) were obtained by ligation of coronary arteries supplying the area of lever attachments.

Gravity and applied accelerations S W BRITTON and O B MURRAY, JR (by invitation) *Physiological Lab, Univ of Virginia, Charlottesville, Va*. The effects of gravitational force on the circulation have been observed to be similar to those exerted by a force of 1 *g* imposed on an animal on a centrifuge with 10-ft rotor arm. ECG, arterial blood pressure, heart rate and other determinations were made over periods up to one hour with forces directed from head to foot and *v v*, or positive and negative *g*.

Compared to the influence of 1 *g* applied by centrifuge or tilt-table continuously for 30 minutes, dogs showed similar resistance to 3-4 *g* applied discontinuously or alternately, 5 seconds on and 5 seconds off, over the same period. On the basis of *g*-minutes employed, it is noteworthy that the organism withstands about twice as much stress (in force-time) in the latter as in the former case, or 45-60 *g*-minutes compared to 30 *g*-minutes.

Discontinuous forces even as high as 6 *g*, 5 seconds on and off, were well withstood for periods of 30 minutes. Quadrupeds are nevertheless much less resistant to gravitational and high acceleratory forces than primate animals and man.

Effect of hypothermia on bromsulphalein removal RADFORD BROKAW (introduced by K E PENROD) *Dept of Physiology, Boston Univ School of Medicine, Boston, Mass*. In normothermic dogs under moderate sodium pentothal anesthesia the percentage disappearance rate of intravenously injected bromsulphalein is not constant, but is nevertheless prompt and rapid. Sixteen anesthetized dogs were injected at normal body temperature with bromsulphalein (5 mg/kg). Blood samples at 6 minutes showed an average level of 1.85 ± 0.93 mg/100 cc of plasma, and 12 minute samples averaged 0.65 ± 0.20 mg/100 cc or 35% of the 6-minute level. Following this marked initial fall

there was a more gradual decline in the concentration of bromsulphalein so that by 30 minutes 0.314 ± 0.11 mg/100 cc remained, or 17% of the 6-minute blood level. When 7 anesthetized dogs were immersed in an ice bath and the injection given intra-arterially at 35° C rectal temperature, the average plasma level after 6 minutes was 4.53 ± 1.44 mg/100 cc. In 12 minutes the average plasma level was 2.89 ± 1.62 mg/100 cc or 64% of the 6-minute level. Ten dogs received the dye injection at 30° C rectal temperature. Their 6-minute levels averaged 4.82 ± 1.25 mg/100 cc, with the 12-minute samples showing 3.24 ± 0.93 mg/100 cc, an average of 67% remaining. Both series of hypothermic dogs showed a marked retention of the dye after 30 minutes. In the 35° C group an average of 1.68 ± 1.03 mg/100 cc was obtained. These values represent 37% and 43% of the dye remaining respectively from the 6-minute plasma levels as contrasted with only 17% remaining in the normothermic controls. These data indicate that hypothermia decreases both the quantitative and relative amounts of bromsulphalein removed.

Temporal relations in the auditory pathway JOHN M BROOKHART and HARLOW W ADES *Dept of Physiology, Northwestern Univ Medical School, Chicago, Ill, and the Dept of Anatomy, Emory Univ School of Medicine, Emory University, Ga*. Unipolar recordings of responses from various stations along the auditory pathway have been recorded using anesthetized cats. Monaural and binaural click stimuli were furnished by brief condenser discharges activating hearing-aid receivers.

Introductory deflections, preceding the principal responses from the cortex, radiation and geniculate, occur at the same time as discharges from the inferior colliculus and are possibly due to electrotonic conduction from mid brain levels.

Latencies of responses to monaural stimulation from a single animal typical of the series are given below in msec.

	AUD CORT	AUD RAD	MED GENIC	BR INF COLL	INF COLL	TRAP BODY	AU TU BER
Ipsi	7.5-8.0	6.0-7.0	5.5-6.0	3.5	3.5	2.0	1.0
Contra	7.5-8.0	6.0-7.0	5.5-6.0	2.5	2.5	2.0	1.5

The lapse of 2.0 msec between the geniculate and cortical responses, and of 2.5-3.5 msec between the brachial and geniculate responses suggests either multiplicity of synapses or unusually long nuclear delays at these points. The geniculate response is similar in crest time and duration to the cortical response and seems to be unaffected by removal of the ipsilateral cortex. The differences in the latencies of ipsilateral and contralateral responses at mid brain levels suggest that one more synapse

is involved in the ipsilateral pathway. The significance of the findings with respect to the functional anatomy of the auditory system are discussed.

Analysis of the mechanism of diurnal rhythmicity in crustacea FRANK A. BROWN, JR. and H. MARGUERITE WEBB (by invitation) *Dept. of Zoology, Northwestern Univ., Evanston, Ill.* and *Marine Biological Lab., Woods Hole, Mass.* A well-defined daily rhythmicity of color change occurs in the fiddler crab, *Uca pugnax*. The rhythm persists for many weeks completely in phase with the day-night cycle in conditions of constant darkness, temperature, and humidity. A study was made of the influence of constant illumination, and changes from constant illumination to constant darkness at different times of the day-night cycle, and of alternating periods of light and darkness, different from those of the normal day-night illumination changes. An analysis of the results indicated that the phases of the diurnal rhythm could be altered abruptly by some types of light stimulation administered at certain 'sensitive' periods in the daily cycle, but could not be altered by similar stimuli at other periods. The results appeared, furthermore, to indicate clearly that two centers of rhythmicity were present, each one capable of having its rhythm altered independently of the other, but with one more basic center of inherent rhythmicity capable of influencing gradually a second center having less persistent rhythmicity.

Absorption and excretion of carbon¹⁴ as CO₂ and bicarbonate AUSTIN M. BRUES and AGNES NARANJO (by invitation) *Argonne National Lab., Chicago, Ill.* As shown previously, the mouse exposed to C¹⁴O₂ in a closed system rapidly attains a steady state in which the C¹⁴ in the animal approximates the amount originally in a volume of air ten times the volume of the mouse. This labile carbon compartment appears to be somewhat greater than the inorganic body carbon, excluding bone carbonate. Excretion rates after a 30-minute confinement period show a small portion of the body C¹⁴ to be in somewhat more stable combination.

Because of the great rapidity of absorption of C¹⁴O₂, we have measured the clearance of the tracer gas from air breathed through a tracheal cannula by rats under Nembutal anesthesia. Initially, the expired air contains less than one-half the C¹⁴O₂ concentration of the inspired air, although the CO₂ concentration is naturally much higher.

To make conditions as favorable as possible for fixation of C¹⁴, mice were injected with labelled sodium bicarbonate and allowed to rebreathe expired air for 10 hr. in a 20-liter chamber. Various tissues were analyzed at intervals up to 2 months after removal. It is estimated that less than 2% of the body carbon had exchanged with blood bicarbonate at the end of exposure. Of this retained

carbon, about 90% was lost within a week, beyond this time the rate of loss was relatively slow.

Newborn mice receiving C¹⁴ by exposure of their mothers prior to delivery lose C¹⁴ much more slowly than adult mice, but the specific activity of their body carbon decreases rapidly due to growth.

Further studies on the pepsin content of histamine stimulated gastric juice GLADYS R. BUCHER *Depts. of Biological Sciences and Clinical Science, Univ. of Illinois College of Medicine, Chicago, Ill.* All but two investigators have found pepsin in histamine stimulated gastric secretion. These workers, using acutely vagotomized dogs or cats under chloralose-urethane, report that pepsin output gradually falls to zero in 5 hours of repeated or continuous histamine stimulation. We have attempted to verify their findings, which are contrary to our own on chronic dogs, but with no success. In a dog under nembutal, receiving histamine every 10 minutes, acute vagotomy in the third hour had no influence on pepsin output which remained quite uniform through the ninth hour. The experiment was repeated on another dog, using chloralose-urethane, also with no reduction of pepsin output in 3 hours after vagotomy. There was no reduction in pepsin output in 3 dogs with vagotomized total stomach pouches observed during 7 hours of repeated histamine injections when the standard dose of chloralose-urethane was given in the third hour. In two acute experiments on dogs conducted as described by Bowie and Vineberg, (*Quart. J. Exp. Physiol.* 25: 247, 1935) and three acute experiments on cats conducted as described by Uvnas (*Acta Physiol. Scand.* 4: XI, 1943) no gradual reduction in the pepsin output was observed. In 5 hours, there was a slight tendency for the output to be increased.

The use of fraction I for separation of erythrocytes and leukocytes from whole blood EDWARD S. BUCKLEY, JR., MARVIN J. POWELL and JOHN G. GIBSON, *2nd Medical Clinic, Peter Bent Brigham Hospital and the Dept. of Medicine, Harvard Medical School, Boston, Mass.* The method of Minor and Burnett (*J. Hematol.* 3: 799, 1948) has been modified for separation of red and white cells in quantities as large as 500 ml.

Fibrinogen causes rouleaux formation of red cells, with the result that, in conformance with Stokes' Law, the sedimentation rate of the clumped red cells is accelerated. Since fibrinogen affects white cells little, if at all, their sedimentation rate is not affected. Thus, addition of fibrinogen increases the differential sedimentation rates of the two classes of cells. The fraction I used contains citrate, so that no other anticoagulant is needed.

Optimal sedimentation of red cells, and hence maximal recovery of both plasma and white cells, occurs when the fibrinogen concentration of the supernatant is about 6 mg/ml, the cell-super-

natant ratio is about 0.3, and temperature between 25° C and 12° C. About 80% of plasma, containing about 80% of the total leucocytes, are obtained after 50 minutes of sedimentation.

The method described offers the possibility of obtaining red cells relatively free from white cells, and white cells free from red cells in large quantities, and hence opens the way for critical studies of the separated formed elements of the blood.

Electrocardiographic observations following explosive decompression of dogs to 30 mm Hg BUFORD H. BURCH (introduced by F. A. HITCHCOCK) *Dept. of Physiology, The Ohio State Univ., Columbus, Ohio*. In a previous communication it was reported that dogs explosively decompressed to a terminal pressure of 30 mm Hg died as a result of ventricular fibrillation which developed about 80 seconds after the explosion. This conclusion was the result of direct observation on the heart through a plastic window sutured to the anterior chest wall. The radical surgery involved may have been a factor in producing the fibrillation, and to test this possibility a series of intact anesthetized dogs were similarly explosively decompressed and continuous electrocardiograms recorded (*lead II*). About 60 seconds after the explosion, changes in rhythm were noted. A-V block, interventricular block and idioventricular rhythms occurred but no evidence of ventricular fibrillation was seen. About 2 minutes after the explosion, a severe slowing of the heart occurred. This is considered significant since previous chest window observations have shown that cessation of the heart beat occurs within 2 minutes after such explosions. Electrocardiographic deflections were observed for as long as 50 minutes after the explosion. A second series of dogs with chest windows was run and electrocardiographic observations made. With this series there was evidence of ventricular fibrillation in about 50% of the cases. It is believed that this surgical procedure tends to precipitate ventricular fibrillation. However, electrocardiographic records indicate that a sudden slowing of the electrocardiogram is the most reliable sign of cessation of the heart beat.

Effect of moderate hypoxemia, related to intracardiac venous shunts, on the systemic blood flow HOWARD B. BURCHELL, BOWEN E. TAYLOR, JULIAN R. KNUTSON and EARL H. WOOD *Div. of Medicine and Sect. on Physiology, Mayo Clinic and Mayo Foundation, Rochester, Minn.* Determinations of resting systemic blood flow and the effect of exercise on oxygen saturation of arterial blood in 16 individuals, who had moderate hypoxemia related to congenital heart disease, were analysed. The resting values for such saturation in the group averaged 82.4% (range, 75-95). The systemic blood flow averaged 3.68 l/m²/min (range, 1.6-8). No

relationship was apparent between systemic blood flow and the resting arterial oxygen saturation. The decrease in arterial oxygen saturation with exercise averaged 22% (range 10-38). The exercise tolerance was not uniformly predictable on the basis of any of the following determinations: the resting arterial oxygen saturation, the oxygen capacity, the resting systemic blood flow, or the decrease in arterial oxygen saturation with exercise. In 2 persons who exhibited, after the initial decrease, a maintained plateau of the arterial oxygen saturation with exercise, the systemic blood flow was determined during exercise. In one, the arterial oxygen saturation decreased from 95-84%, and the systemic flow/100 cc of oxygen absorbed decreased from 1.9-0.85 l/min. In the second, the arterial oxygen saturation decreased from 86-70% and the systemic flow/100 cc of oxygen absorbed decreased from 2.3-1.3 l/min. Evident simple relationships were not found between the increased systemic blood flow that may occur with the hypoxemia of congenital heart disease and the other physiologic variables which were studied.

Blood histamine in hypoxia WILLIAM L. BURKHARDT (by invitation), DON FLICKINGER (by invitation) and HARRY F. ADLER *USAF School of Aviation Medicine, Randolph Field, Texas*. The blood histamine content of dogs, as measured by biological assay, may increase 10-fold or more in response to continuous hypoxia at a simulated altitude of 18,000 ft. The increase in histamine reached a peak on the 4th day of exposure and may be correlated with the clinical appearance of the 8 dogs which at that time appeared apathetic, lethargic and had an anorexia. However, after the peak had been reached on the 4th day the histamine level began to decline until it reached a control blood level of 0.03 to 0.06 gamma per cc about 7 or 8 days later which totaled about 12 days of continuous exposure. The decrease in histamine also seemed to be correlated with clinical symptoms in that gradually over the period of the histamine decline the animals lost their apathy and began to eat normally. When the dogs were returned to ground level for 8 days a re-exposure to 18,000 ft failed to elicit the same marked degree of histamine response noted on the first exposure. However, as compared to the longer continuous exposure, a shorter 4-day exposure to 18,000 ft followed by only a 3-day rest at ground level and then re-exposure produced an exaggerated blood histamine response. The animals' weight, food intake, blood counts and hematocrits have been followed closely during the entire experimental period.

Effect of crude renal extracts and purified renin in experimental malignant renal hypertension R. O. BURNS, JR. (by invitation), W. H. JASPER (by invitation) and G. E. WAKERLIN *Dept. of Physiology, Univ. of Illinois College of*

Medicine, Chicago, Ill Crude hog renal and liver extracts and purified hog renin were injected prophylactically into dogs subjected to simultaneous bilateral renal artery constriction previously found to produce consistent experimental malignant hypertension. Injections were made daily and intramuscularly for 3 months prior to and during survival or up to one month subsequent to renal artery constriction. Crude hog renal cortex extract containing renin was highly effective in protecting against the hypertension, at least partially effective against the arteriolonecrotic lesions and partially effective in prolonging survival time. *4 dogs* Purified hog renin was only partially effective in protecting against the hypertension and the arteriolonecrotic lesions and in prolonging survival time. *5 dogs* Crude hog whole kidney extract containing renin did not have an antihypertensive effect, and was only partially effective in protecting against the arteriolonecrotic lesions and in prolonging survival time. *4 dogs* Crude hog liver extract had no antihypertensive effect and was doubtfully effective in protecting against the arteriolonecrotic lesions of malignant hypertension and in prolonging survival time. *3 dogs* The mechanism of the antihypertensive effect is not yet determined. Antirenin may be involved. The mechanisms of the protection against arteriolonecrotic lesions and the prolongation of survival time remain to be determined. Present evidence suggests the possibility that the arteriolonecrotic lesions may be due to a renal necrotizing substance which is not renin. (A method which will produce consistent experimental malignant hypertension in the dog has been devised.)

LD-50 studies on hydroquinone HELEN BURSON (by invitation), N. R. BREWER (by invitation) and A. J. CARLSON *Physiology Lab., Univ. of Chicago, Chicago, Ill.* In rats the LD-50 of hydroquinone varied with the state of nutrition of the animal and the strain of rats. LD-50 of the Priestly strain was 1317 mg/kg, the Sprague-Dawley strain, 1133 mg/kg, and the Wistar strain, 770 mg/kg.

When the rat is in the fasting state (18 hr or more without food) the LD-50 is markedly lowered. Variations that are apparent in well nourished rats of various strains become minimized or absent when the rats are fasted. The fasted Sprague-Dawley rats had an LD-50 of 319 mg/kg. The fasted Wistar rats had an LD-50 of 313 mg/kg. Animals in the fasting state show more uniform LD-50s, but this seems not significant in this study, because when added to food fats as an antioxidant hydroquinone will never be consumed in the fasting state.

When fed adult rats are given repeated doses of 500 mg/kg of hydroquinone in propylene glycol by stomach tube the mortality, after 38 administrations, was only 7 out of 20 rats. This is supporting

evidence that 1) the toxic action of hydroquinone is not cumulative, and that 2) the LD-50 on the fasted animal is not sufficient evidence of the tolerance to this chemical as an antioxidant in food.

Two fasted dogs died after one dose of 150 mg/kg by mouth. Three dogs that were not fasted showed tremors but did not die following a single dose (by mouth) of 200 mg/kg. Four dogs that were not fasted showed similar but more severe symptoms following a single dose (by mouth) of 250 mg/kg. Two dogs were given 28 consecutive daily administrations of 100 mg/kg by mouth with the food, without noticeable effect.

The LD-50 of hydroquinone on cats in the fasting state is between 50 and 100 mg/kg. However, one young cat has to date survived for 6 weeks on 15 mg/kg by mouth, with no symptoms other than a small decrease in the rate of growth when compared to a control sib.

Effects of potassium and lanatoside-C on the failing heart in heart-lung preparations SANDOR G. BURSTEIN (by invitation), LESLIE L. BENNETT, FRANCIS E. PAYNE (by invitation), and JAMES HOPPER, JR. (by invitation) *Divisions of Physiology and Medicine, Univ. of Calif., Berkeley and San Francisco, Calif.* Dog heart-lung preparations were arranged for the continuous recording of right auricular pressure, aortic pressure, ventricular volume, and left ventricular output minus the coronary flow. Unipolar electrocardiographic tracings were taken directly from the anterior surface of the left ventricle using a wick electrode. When cardiac failure was produced by overloading the heart the administration of 16-32 mg of lanatoside-C consistently produced reduction in the right atrial pressure and in diastolic ventricular volume, and an increase in cardiac output and in aortic pressure. In four experiments the subsequent administration of potassium (as KCl) reversed the lanatoside-C effects. In five experiments the heart was not overloaded but potassium was administered to the point of production of an increased ventricular diastolic volume. In each case the subsequent administration of lanatoside-C reduced the diastolic ventricular volume. Both potassium and lanatoside-C produced prolongation of the A-V and I-V conduction times as measured from the electrocardiographic tracings. Both substances also produced displacements of the S-T segments and alterations in the T waves. The S-T segment displacements and the T wave alterations produced by a minimum effective dose of one substance could be reversed by the other if it were given in a large enough dose. It was concluded that lanatoside-C can inhibit, delay, or overcome the onset or the course of potassium-induced or overwork-induced heart failure, while high potassium levels are able to inhibit, reverse, or minimize the effects of lanatoside-C.

Alteration of neuron excitability by axon section BERRY CAMPBELL, EDGAR GASTEIGER and VERNON H MARK *Dept of Anatomy, Univ of Minnesota, Minneapolis, Minn* Associated with the chromatolysis produced by axon section is a change in the reflex pattern of the nucleus involved. Previous communications have shown the loss of the proprioceptive component of the segmental reflex, the decrease in the antidromic potential, as well as the continuance of normal conduction in the primary afferent and in the axon of the affected motor cell. This suggests a general decrease in the excitability of the neuron. The study of a pure cutaneous ('flexor') type of response, the tibial-peroneal reflex, during the chromatolytic cycle confirms this interpretation. In a series of 25 cats studied from 2 to 102 days following section of the peroneal nerve the following changes were noted: an increase in central latency, a simplification of the oscillographically recorded reflex potential curve, a decrease in amplitude and, during the height of the cycle, an absence of the reflex. The subliminal fringe, as tested by a second shock, was relatively larger in the early part of the cycle, smaller later. All effects found are consistent with a generalized decrease in excitability of the cells.

Effect of increased intracranial pressure on the circulation in relation to pulmonary lesions GILBERT S CAMPBELL (by invitation), FRANCIS J HADDY (by invitation) and M B VISSCHER *Dept of Physiology, Univ of Minnesota, Minneapolis, Minn* Studies have been made on dogs using right and left heart catheterization to measure pulmonary arterial (PAP) and venous pressures (PVP). Cardiac output, systemic arterial pressure and heart rate were also recorded. Increased intracranial pressure (IICP) was produced by inflating a balloon inserted extradurally through a small trephine opening. It was found that on the average in 18 dogs the mean PVP was 8.2 mm Hg above intrathoracic before IICP was induced. Afterward the value rose to 18.1 mm Hg. Concomitant with IICP there was bradycardia, lowered cardiac output and an elevation of PVP and PAP. Gross examination of the lungs at necropsy revealed varying degrees of pulmonary edema, congestion and hemorrhage. Atropine (1.3-1.95 mgm) was administered i.v. to 4 dogs following IICP. The control mean PVP was 5.5 mm Hg prior to IICP and the PVP following IICP was 13.9 mm Hg. The IICP was maintained following the administration of atropine, and the mean PVP fell to 6.6 mm Hg within 3 minutes. Atropine produced a tachycardia, raised the cardiac output to its control level and apparently protected the animals from the more severe pulmonary edema, congestion and hemorrhage encountered in the earlier group of 18 dogs which were not given atropine. There was no

correlation between systemic arterial pressure and these effects.

Effects of an anterior pituitary extract on the metabolism of the liver JAMES CAMPBELL and I W F DAVIDSON (by invitation) *Dept of Physiology, Univ of Toronto, Toronto, Canada* The effect of an extract of the anterior pituitary gland on the metabolism of the liver was studied by administering the extract to fasting rats and determining the rate of oxygen uptake and the production of acetoacetic acid by liver slices *in vitro*. These determinations were made at intervals throughout 6 days of fasting and treatment with the extract ($Q_{O_2}^t$ and Q_{aa}^t signify the μ l oxygen taken up and the μ g acetoacetic acid produced, respectively, per mg total solids of liver per hour). In the 24 hours following the first injection the $Q_{O_2}^t$ was less in the injected than in the control animals, but when recalculated on the basis of fat-free solids ($Q_{O_2}^f$) the values in the pituitary-treated animals were greater. After this 24-hour period both the $Q_{O_2}^t$ and Q_{aa}^t values were greater in the treated animals. The influx of lipid into the liver produced by the fat-mobilizing stimulus of the pituitary extract accounts for this inversion of the relationship during the first 24 hours. The Q_{aa}^t and Q_{aa}^f values were greater in the injected animals from the start of the experiment. The evidence indicates that a pituitary factor caused an enhanced activity of the fat-metabolising system of liver tissue. The oxygen uptake and the acetoacetic acid production by the liver per 100-gm body weight were increased by administration of the extract. The addition of octanoate to the liver slices *in vitro* produced an increased oxygen uptake and acetoacetate production, and the extra oxygen taken up in the presence of the substrate could be accounted for by the amount of extra acetoacetate formed. The liver slices from the treated animals had an enhanced ability to produce acetoacetate from the added substrate.

Parathormone and the tubular reabsorption of glucose and phosphate in man WALTER H CARGILL and A CALHOUN WITHAM (introduced by JAMES V WARREN) *Depts of Physiology and Medicine, Emory Univ School of Medicine, and the Medical Service, Lawson Veterans Administration Hospital, Atlanta, Ga* It is well known that the urinary excretion of phosphate may be increased by the administration of parathyroid hormone and decreased by the ingestion or infusion of large amounts of glucose. We have investigated the role of tubular activity in these reactions in normal human subjects. Glomerular filtration rate was determined by inulin clearance and the rates of reabsorption of glucose and phosphate obtained as the difference between the rates of filtration and excretion. Since the intravenous injection of parathormone (200-400 i.u.) did not alter filtration rate,

the increased excretion of phosphate which followed was due entirely to decreased reabsorption. The reabsorption of glucose usually decreased also. When the rate of glucose reabsorption was increased by the intravenous infusion of 10% glucose solution there was a corresponding increase in phosphate reabsorption, in addition to a decreased rate of phosphate filtration resulting from a lowered plasma level. The injection of parathormone during or immediately following the glucose infusion produced a further increase in the rate of phosphate reabsorption. These observations suggest that the mechanisms of tubular reabsorption of phosphate and glucose are closely related, and that during periods of increased glucose reabsorption the usual effect of parathormone on phosphate excretion may be reversed.

Delay of senescence infertility by dietary restriction CLAIRE J. CARR (by invitation), JOSEPH T. KING and M. B. VISSCHER *Dept of Physiology, Univ of Minnesota, Minneapolis, Minn.* A group of C₃H female mice have been studied in detail for 23 months. Ten of the original 17 are now living. Since it was known that restriction to 66% of normal calories would disturb but not prevent normal estrous cycles, these mice were restricted to 50% of a normal caloric intake. Protein, minerals and vitamins were not restricted. They remained in anestrus. Occasionally a mouse would show a subestrus response. At two different intervals several mice were given fresh pituitary implants; they responded promptly with a positive vaginal smear. After 14 months half of the group was given increasing caloric supplement with dextrose. Some mice developed positive smears with as little as 0.150 gm dextrose while others required as much as 1.0 gm. At 21 months the whole group was fed *ad libitum*. Within a week all showed positive smears. At this point they were tested with fertile males. All became pregnant. Some aborted at about the 15th day but subsequently delivered litters. To date the litters have not survived, apparently due to failure of lactation. Observations are being continued. It is apparent that prolonged, severe caloric restriction delays the onset of infertility to a very advanced age. Strain A mice restricted until 8 months old have been studied previously (Ball, Barnes and Visscher, *Am J Physiol* 150: 511, 1947).

Resistance induced by anti-thyroid compounds and by goitrogenic diets against experimental pulmonary edema in rats K. K. CARROLL (by invitation) and R. L. NOBLE *Dept of Medical Research, Collip Medical Research Lab, Univ of Western Ontario, London, Canada.* ANTU (α -naphthylthiourea) and several closely related compounds have been shown to possess a high toxicity specific for rats. Pretreatment of rats with sub-lethal doses of ANTU induces a marked re-

sistance to its toxic action (Richter 1945, 1947). The present study has demonstrated that resistance to ANTU can also be produced by pretreatment with various other compounds containing the thiourea grouping. Neither the anti-thyroid potency nor the acute toxicity of these substances is directly related to their ability to impart resistance. In addition, resistance to ANTU is found in rats fed on certain goitrogenic diets, especially those containing seeds of the *Brassica* species. This effect has been used as an assay in preparing active extracts of rape seed. Details of the extraction procedure are discussed.

Connective tissue and parenchymal changes in ovary of the rat H. R. CATCHPOLE and S. C. PAN (by invitation) *Dept of Pathology, Univ of Illinois College of Medicine, Chicago, Ill.* Two methods were applied to the study of ovarian connective tissue in immature female rats which were treated with 2-25 RU of equine gonadotrophic hormone and examined after 6-60 hours. Animals of control groups and of groups so stimulated received an injection of 0.5 ml 1:25% Evans blue (intravital) dye intravenously 10 minutes before death. Their ovaries were frozen in liquid air and frozen-dried *in vacuo*. They were then embedded in paraffin, sectioned at 30 μ , mounted in mineral oil and examined directly. Ovaries from control and gonadotrophin-treated animals were frozen-dried, sectioned at 6 μ and stained by the McManus-Hotchkiss periodic acid-leucofuchsin technique for glycoproteins. Young adult female rats at different stages of the cycle were also studied by the above techniques. In the normal rat, the extracellular distribution of Evans blue duplicates that of glycoproteins staining with the periodic acid-leucofuchsin method. There is an increased staining density of glycoprotein at pro-estrous and estrous, which is even more pronounced following gonadotrophin stimulation. Coincidentally, more Evans blue is visible in the connective tissue of stimulated ovaries compared either with controls or with normal cyclic ovaries, and the increase is proportional to the dosage or time of action of the hormone. Increased binding of Evans blue and staining density with the McManus-Hotchkiss reagent are interpreted as due to a process of depolymerization occurring in glycoprotein components of the connective tissue. These changes are thought to underlie, or to favor, structural rearrangements within the ovary occasioned by follicular growth.

Studies on bound acetylcholine DANIEL J. CAVANAUGH and JULIAN M. TOBIAS *Dept of Physiology and Toxicity Lab, Univ of Chicago, Chicago, Ill.* The coexistence of bound acetylcholine (ACh) and cholinesterase (ChE), and, in some cases, of large amounts of free ACh and active ChE (*J Cell Comp Physiol* 28: 159, 1946) in

tissue stimulates one to ponder why the enzyme does not hydrolyze the substrate *in vivo*. A variety of possibilities exists. There may be a steady state of interaction appearing to be lack of interaction, the enzyme may be in inactive form, the enzyme and substrate may be separated by a structural barrier, one or the other may be held in an inaccessible state, as by adsorption etc. Toward clarifying this situation, preliminary experiments have shown that adsorption of ACh on charcoal can protect it from demonstrably active ChE. It has also been found, using rat brain preparations, that thermal liberation of free ACh from bound precursor is rather rapid, even at relatively low temperatures, being 50% complete in 1 hour or less at 15°C. The rate of unbinding between 15–40°C is markedly accelerated over that at 0–15°C. Such adsorption protection of ACh from ChE *in vitro* and the relative ease of thermal dissociation of precursor are facts compatible with the notion that bound ACh is loosely held in precursor.

It has also been found that in a distilled water homogenate of brain (rat) the bound ACh is not thrown down at centrifugal speeds something over 20,000 r p m. It is either water-soluble or highly disperse, not associated with the denser protoplasmic constituents under these circumstances, and can be shown to be associated with a high lipid content fraction of the brain.

Effect of a psychiatric interview on renal plasma flow and finger skin temperature. JAMES H. CHALMERS (by invitation), ROBERT W. CRANSTON (by invitation), HENRY LONGSTREET TAYLOR and ANCEL KEYS *Laby of physiological hygiene, Univ of Minnesota, Minneapolis, Minn.* Thirty-eight experiments were conducted on 27 well adjusted healthy males and females ranging in age from 20–62 years, all of whom were free of cardiac and renal abnormalities. The subjects were divided into 4 groups on the basis of age and sex. Observations were made of renal plasma flow and skin temperature during a restful control period followed by a period during which emotionally charged topics were intentionally introduced by a psychiatrist familiar with the emotional backgrounds of the subjects. This resulted in moderate emotional tension in most subjects. No marked reactions were noted. A control group was studied by identical methods in which no emotional stimuli were applied. The intraindividual standard deviation of renal plasma flow of the control group (11 experiments on 6 young males) for the periods corresponding to the psychiatric interview was used to calculate T scores for evaluating the response of each of the other subjects to the psychiatric interview. Highly significant changes in renal plasma flow (T score greater than 3) occurred during the interview in 4 of 11 experiments on 7 young males, 5 of 8 experiments on 8 older males and 4 of 8 experi-

ments on 6 females. Positive as well as negative deviations from basal values were found. The degree and duration of emotional response elicited varied between individuals and in the same individual on different occasions. Significant changes in skin temperature of the finger occurred during the interview but were not correlated with changes in renal plasma flow.

Pressure changes and hemorrhage in the middle ear of monkeys resulting from decompression and recompression. HSIANG-TUNG CHANG (by invitation), RODOLFO MARGARIA (by invitation) and SAMUEL GELFAN *Laby of Physiology, Yale Univ School of Medicine, New Haven, Conn.* The pressure changes developed in the middle ear during decompression and recompression have been directly determined by means of a membrane-mirror manometer and recorded photographically. The middle ear pressure increases proportionally with gradual reduction of ambient pressure, the expanding air in the tympanic cavity escaping periodically through the Eustachian tube. The average differential pressure required for forcing the Eustachian tube open in anesthetized monkeys was found to be between 10 to 13 mm Hg. At the instant of explosive decompression the positive pressure in the middle ear increases suddenly to a value which tends to approach the pressure difference between the pre- and post-decompression altitudes. This excess middle ear pressure, however, is very quickly reduced to that of the ambient pressure.

During recompression anesthetized monkeys are unable to equalize the ambient and middle ear pressures, the negative pressure developing in the middle ear increasing with increasing ambient pressure. This negative pressure, after reaching a critical value, will cause traumatic lesions. Autopsy of explosively decompressed monkeys showed severe bleeding in the tympanic cavity and the mastoid cells of the temporal bone with the ear drum remaining intact. Puncture of the ear drum prior to decompression prevents the tympanic hemorrhage.

Artificial insemination of rabbits and transplantation of rabbit eggs (motion picture). M. C. CHANG and G. PINCUS *Worcester Foundation for Exptl Biology, Shrewsbury, Mass., and Tufts College of Medicine, Boston, Mass.* The pictures of Spallanzani (first successful artificial insemination of dogs in 1785), Heape (first successful transplantation of rabbit eggs in 1890), Hammond and Walton (who developed the technique of artificial insemination of rabbits), and Pincus (who developed the technique of transplantation of rabbit eggs) are shown. Methods of rabbit semen collection by means of a piece of rabbit skin and artificial insemination of the rabbit are photographed in color. The recovery of rabbit eggs from excised

oviducts and from the living animal by a surgical method are demonstrated. Living rabbit eggs in the four-cells stage are photographed. Surgical methods for transplanting rabbit eggs at early stages into the oviducts, and blastocysts into the uterus of recipient rabbits are shown. The storage of eggs at low temperatures and the probability of eggs (recovered at different stages, and stored at various temperatures for different lengths of time) developing into normal young rabbits are described. Albino young produced from black chin-chilla foster mothers are photographed. A historical review of sperm study and the development of artificial insemination for livestock improvement are shown in a chart. The egg study and the possibility of egg transplantation for the full utilization of female germ cells of valuable animals are illustrated for livestock improvement.

Effect of substrate concentration on the luminescent reaction of Cypridina luciferin and luciferase AURIN M CHASE *Physiological Laby, Princeton Univ, Princeton, N J, and the Marine Biological Laby, Woods Hole, Mass*. It has been stated (Moelwyn-Hughes, *Ergeb der Enzymf* 6 23, 1937) that the Michaelis constant, K_s , for the luminescent reaction of Cypridina luciferin and luciferase has a negative, and therefore theoretically meaningless, value. For this reason he analyzed and satisfactorily interpreted this reaction in terms of chain reaction theory. Since the data used by Moelwyn-Hughes represented the velocity constant rather than the initial velocity of the reaction as a function of luciferin concentration, a determination of the relationship between velocity of the luciferin-luciferase reaction and the initial luciferin concentration was undertaken in order to obtain data suitable for analysis in terms of the Michaelis-Menten theory of intermediate enzyme-substrate complex formation. It was found that the initial velocity increases asymptotically to a maximum value as luciferin concentration is increased, yielding data quite susceptible to analysis in terms of the Michaelis-Menten theory. Two enzyme concentrations were used and a 200-fold range of substrate concentration was studied. Excellent agreement with the Michaelis-Menten equation was observed and the calculated value of K_s , the dissociation constant for the luciferin-luciferase complex, was about 6×10^{-7} (the molar luciferin concentration giving $\frac{1}{2}$ maximum velocity). This order of magnitude is similar to those found for many oxidative enzyme systems which have been studied. The analysis of the data further indicated that one luciferin molecule combines with one luciferase molecule in the formation of the enzyme-substrate complex.

Effect of desoxycorticosterone acetate (DCA) on pituitary content of adrenocorticotrophic hormone (ACTH) after adrenalectomy CHI-PING

CHENG (by invitation), MARION A SAYERS (by invitation) and GEORGE SAYERS *Dept of Pharmacology, Univ of Utah College of Medicine, Salt Lake City, Utah*. The content of ACTH in the pituitary of the rat is reduced to $\frac{1}{2}$ of normal 24 hours after adrenalectomy (Cheng and Sayers, *Proc Soc Exptl Biol & Med*, in press). Six of 12 rats were implanted with six 15 mg pellets of DCA each, 48 hours later, all 12 were adrenalectomized. Twenty-four hours later, the rats were killed and the pituitaries removed. Pituitaries were also removed from untreated intact controls and from 6 intact rats 72 hours after the implantation of DCA (six 15 mg pellets each). The anterior lobes were frozen and lyophilized and the dried glands of each group pooled and ground to a homogeneous powder. The concentration of ACTH in the powders was determined by the adrenal ascorbic acid-depletion method. DCA had no effect upon the content of ACTH in the pituitaries of the intact rats. The content of ACTH in the pituitaries of the untreated adrenalectomized rats was 15% while that of the DCA-treated adrenalectomized rats was 43% of the control level. In animals with intact adrenals, non-specific stress (scald, sham-adrenalectomy) reduces the ACTH content of the pituitary by 50%. It may be concluded that DCA prevents the marked depletion of pituitary ACTH which normally follows the complete absence of adrenal cortical secretions. The results lend additional support to the concept that pituitary adrenocorticotrophic activity is regulated by the level of cortical steroids in the body fluids.

Order of recovery from excitation in dog, monkey, and human heart, nature of ST-segment and T-wave H M CHERNOFF (by invitation) and L H NAHUM *Laby of Physiology, Yale Univ School of Medicine, New Haven, Conn*. The recovery process in mammalian ventricle was studied by a) forced extrasystoles, b) heating and cooling regions of the dog ventricle, c) high altitude anoxia in monkeys and d) simultaneously recorded unipolar extremity and precordial leads in man. Practically all normal subjects show in some leads displacement of ST in the same direction as the T-wave. All extrasystoles exhibit similarly displaced ST segments. High altitude anoxia causes similarly displaced ST-segment and QT shortening. Heating localized ventricular areas produces QT shortening and similar ST-segment displacement. Cooling localized areas lengthens QT and produces ST-segment displacement in same direction as T, but displacement is opposite to that produced by heating same area. ST displacement and T-wave direction in each lead depends upon region of heart whose repolarization has been altered.

In dog, monkey and human heart repolarization is already in progress at end of S. An isoelectric

ST results from balanced repolarization in proximal and distal zones of that lead. The T-wave represents that part of the repolarization process in which the greatest imbalance of potential exists between proximal and distal zone. First stage anoxia causes accelerated repolarization, which is reflected by a specific ST-T pattern. In dog and human heart, the apical and diaphragmatic regions are the earliest to repolarize.

Vasomotor side effects of two new amines which primarily depress intestinal motility. J. R. CHITTUM, F. H. LONGINO and B. H. METCALF (introduced by K. S. GRIMSON) *Dept of Surgery, Duke Univ, Durham, N. C.* Vasomotor actions of a tertiary amino compound, dibenzylmethyl B-diethylaminopropionate hydrochloride (SC1870), which reportedly has five times the activity of papaverine in relaxing barium-induced spasm have been compared with those of a quaternary amine, ethyl dimethyl B-(9-xanthene carboxylate) ethyl ammonium chloride (SC1703), which reportedly inhibits autonomic ganglia and also has twice the anti-acetylcholine activity of atropine. Five to 7.5 mg/kg of 1870 or 2.5 to 4 mg/kg of 1703 delayed gastric emptying and reduced transit time through the ileum of unanesthetized dogs. Essentially similar doses of either drug produced reduction of blood pressure with little change or decrease of pulse rate in dogs anesthetized using chloralose. After either drug the effect of stimulation of distal ends of divided vagi upon heart rate was prevented. During the period of hypotension following either drug, increase of blood pressure normally caused by occlusion of carotid arteries did not occur. Following effective doses of either drug, anoxia failed to increase blood pressure. Stimulation of the central end of a divided vagus nerve after 1870 did produce marked increase of blood pressure. After 1703, however, the pressor response to central vagus stimulation was blocked. After either drug adrenalin increased blood pressure and heart rate. Experiments indicate that both drugs have definite vasomotor effects when given in large doses. These doses, however, apparently were well tolerated when given intravenously to unanesthetized dogs during ileal studies.

Studies of the use of intravenous quinidine orally in certain mechanism disorders. JOHN W. CHRISS (by invitation), MILTON R. HEJTMANCIK (by invitation) and GEORGE R. HERRMAN. *Univ of Texas Medical School, Galveston.* Quinidine is almost a specific drug for the interruption of serious disorders of the cardiac mechanism such as ventricular tachycardia, atrial fibrillation and atrial tachycardia. In most of our cases the sino-atrial rhythm was reestablished after 1-8 oral doses of 0.2 gm of quinidine sulfate. In rare cases that proved refractory to oral therapy the seriousness of the patient's condition was such that heroic introduction of quinidine lactate was

resorted to. We are fully cognizant of the dangers of using quinidine intravenously and certainly proceeded with great caution. Intravenous quinidine lactate and sulfate were used in several cases of ventricular tachycardia, atrial flutter, atrial tachycardia and atrial fibrillation. The few cases in which even heroic therapy failed will be discussed along with those successfully treated.

The 'motor' cortex of the dog. J. G. CHUSID (by invitation), C. G. DE GUTIERREZ-MAHONEY and FRANKLIN ROBINSON (by invitation) *Neurological Division, St. Vincent's Hospital and Lab of Physiology, Yale Univ School of Medicine, New Haven, Conn.* The cerebral cortex of 19 dogs under light Dial-urethane anesthesia was stimulated manually using bipolar Ag-AgCl electrodes and a Goodwin stimulator. 'Buried' cortex, exposed by subpial suction of tissue, was explored and found to resemble in its motor characteristics the adjacent exposed cortex. The inferior bank of the coronal sulcus was found to be the superior limit of the 'face' area, while the superior bank of the coronal sulcus was found to be the inferior limit of the 'arm' area. The posterior bank of the presylvian sulcus was found to be the anterior limit of the 'face' area, and in its inferior orbital portion was found to inhibit respiration readily. Although a relatively wide representation for 'face' and its subdivisions was apparent, repeated stimulation of this area and the adjacent posterolateral cortex failed to evoke leg or arm movements. The posterior bank of the cruciate sulcus represented the anterior limit of the 'leg' area. Extension of the 'arm' area onto the anterior bank of the cruciate sulcus occurred in its most lateral portion. Eye movements, pupillary changes and tearing were evoked from the 'buried' cortex of the presylvian sulcus in its most medial (midline) portion. Stimulation of the adjacent cortex lying medially and extending for several millimeters onto the medial aspect of the hemisphere produced the most pronounced pupillary changes; stimulation of the anterior cingulate area inhibited respiration.

Effect of intravenous injection of hypertonic saline on salivary secretion in dogs. L. J. CIZEK (by invitation) and M. I. GREGERSEN. *Dept of Physiology, College of Physicians and Surgeons, Columbia Univ, New York City.* Many investigators have shown that thirst can be induced by the intravenous injection of hypertonic salt solution. Holmes and Gregersen (*Am J Physiol* 151:252, 1947) demonstrated that the thirst so produced in man was associated with a reduction in the overall salivary secretion. This prompted us to study salivary secretion in the unanesthetized dog before and after the intravenous injection of 20% NaCl (2 cc/kg). Observations were made on 6 dogs, 3 with submaxillary fistulas and 3 with parotid fistulas. Panting in a hot box was used as a uni-

form stimulus. The submaxillary secretion was either unaffected or somewhat augmented by the injection of hypertonic saline. However, the parotid secretion, which was comparatively small but surprisingly constant during the control periods, was sharply reduced or completely absent after saline injection. The difference in response of the two types of glands is unexplained. Furthermore, the evidence so far on the dog is not conclusive in showing that the total secretion into the mouth is reduced by hypertonic saline, although that would be the inference from results on man and from the fact that intravenous saline causes both man and dog to drink.

Responses elicited by combined stimulation of pairs of fixed electrodes in the unanesthetized monkey. GEORGE CLARK and JAMES W. WARD (by invitation). *Dept. of Anatomy, Chicago Medical School, Chicago, Ill., Yerkes Labs. of Primate Biology Inc., Orange Park, Fla. and Dept. of Anatomy, Vanderbilt Medical School, Nashville, Tenn.* Electrodes were implanted aseptically upon the Betz cell area, premotor area and eye fields in each of 10 immature monkeys (*Macaca mullatta*). Selected pairs of these electrodes were stimulated with 60-cycle sine wave current either consecutively or concurrently. As compared to the effects of single stimulation the responses were either unchanged or augmented. In no case did suppression occur. It is suggested that both inhibitory and facilitatory processes may be induced by appropriate cortical stimulation and that under different physiological conditions of the animal, one or the other may be elicited.

Effect of work on renal oxygen utilization. JOHN KAPP CLARK and HAROLD G. BARKER (introduced by J. R. ELKINTON). *Dept. of Medicine and Harrison Dept. of Research Surgery, School of Medicine, Univ. of Pennsylvania, Philadelphia, Pa.* No change in renal oxygen utilization was demonstrated in 15 normal human subjects between basal states and conditions of probably changed renal work, namely saturation of PAH transport mechanism, water diuresis, osmotic (Mannitol) diuresis, 5 subjects each. Arterial and renal venous (catheter) blood was sampled simultaneously. Flow was calculated from the Fick equation using PAH. Oxygen content was determined manometrically and corrected for A-V differences in hemoglobin content. The product of blood flow and A-V difference represents oxygen utilization. 300 gm. of kidney tissue were assumed equivalent to 1.73 square meters surface area. Blood flow remained essentially constant during the tests. The mean control value for oxygen utilization in all 15 patients was 6.1 cc/100 gm/min (standard deviation ± 2.27 , standard error of the mean $\pm .607$). In 12 of the 15 cases no change over 1.5 cc/100 gm/min was observed.

TEST	OXYGEN UTILIZATION CC/100 GM /MIN	
	Mean Change	Range
PAH saturation	0.1	(-2.0 to +4.7)
Water diuresis	0.7	(-4.4 to +1.2)
Mannitol diuresis	0.4	(-1.0 to +1.4)

Small A-V oxygen differences make the experimental error large. Nevertheless the kidney seems not to vary its oxygen utilization with increased functional activity to a degree comparable to other organs or to the body as a whole where utilization may increase 16 fold during exercise.

Oxidation of CO to CO₂ by the intact animal. ROBERT T. CLARK, JR., J. NEWELL STANNARD and W. O. FENN. *Depts. of Physiology and Vital Economics, and of Radiation Biology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.* Previous experiments with isolated frog tissue in a respirometer containing 80% CO and 20% O₂ indicated that CO in this mixture invariably stimulated gas uptake in heart and skeletal muscle as compared to the rate in air. By direct analyses of the respired CO₂ using radioactive C¹⁴, this apparent stimulation was shown by us to be due to the oxidation of CO to CO₂ by the muscles. To determine whether CO would be burned in the intact animal, turtles and mice were placed in concentrations of CO in air ranging from 0.02-0.07% in air-tight containers. The amount of CO oxidized was determined by 1) measuring by a modification of the Roughton-Root technique the drop in CO concentration in the air during the experimental period and 2) determining the amount of C¹⁴O appearing as C¹⁴O₂ in the expired air. The gasometric measurements showed that considerably more CO disappeared in the presence of the animals than in the control period. In 10 days the turtles oxidized 52% of the CO present, while mice utilized CO at the rate of 0.044 mm³/g/min. Two independent measurements on mice with collection of C¹⁴O₂ indicated the rate of 0.0049 mm³/g/min. This rate of burning was greater by a factor of 10 than would be expected by extrapolation from the rate observed with isolated tissues in 80% CO and 20% O₂. Extrapolation of these rates to man would indicate oxidation of 20 cc CO per hour after breathing air containing 0.07% CO.

Occurrence of gastric secretory inhibitor activity in fresh gastric and salivary mucin. CHARLES F. CODE, HENRY V. RATEK (by invitation), GEORGE R. LIVERMORE, JR. (by invitation), and WALTER LUNDBERG (by invitation). *Section on Physiology, Mayo Foundation, Rochester, Minn., and Hormel Inst., Univ. of Minnesota, Minneapolis, Minn.* It was shown some years ago by Brunschwig and his co-workers that the intravenous in-

jection of gastric juice or alcoholic precipitates of gastric juice obtained from patients with achlorhydria produced an inhibition of the gastric secretory response of dogs to a meat meal. This observation was confirmed and extended by Blackburn and one of us (Code). They found that the inhibitor activity was associated with the mucin fraction of gastric juice, and that it was present in samples of commercial gastric mucin, whereas it was absent in preparations of pepsin.

In the present study, fresh gastric mucin was obtained by scraping the mucosa of the stomachs of hogs immediately after killing. The intravenous injection of physiologic saline extracts of this material consistently eliminated the acid gastric secretory response of dogs to rather large doses of histamine given subcutaneously every 10 minutes. The inhibitor activity was found to be precipitated by alcohol. Alcohol precipitates of saliva, like those of gastric mucin, may produce inhibition of the acid gastric secretory response to histamine, but the degree of inhibition produced by salivary preparations thus far has been less than that associated with preparations of gastric mucin. Immediate reactions, manifested often by weakness and a decrease of blood pressure and delayed reaction as indicated by emesis and an increase in temperature may occur when preparations of mucinogenous materials are injected intravenously into dogs. Although inhibition of gastric secretion has been observed in the absence of such reactions, the significance of the occurrence of these reactions in relation to the gastric secretory inhibitor activity of the preparations is not fully understood.

Renal clearances in normal dogs receiving desoxycorticosterone acetate. W. D. COLLINGS, C. F. DOWNING (by invitation) and R. E. HODGES (by invitation). *Dept. of Physiology, State Univ. of Iowa, Iowa City, Ia.* During the course of some studies of renal-adrenal relationships in dogs, it was necessary to investigate the effects of desoxycorticosterone acetate (DCA) on normal dog kidney function. Preliminary studies were made of creatinine and para-aminohippuric acid (PAH) clearances in 3 trained female dogs before, during and after treatment with DCA. Clearances were done by the usual technique of priming the animals first, then maintaining plasma levels of the cleared substances by constant intravenous infusion. Urines were obtained by catheter, and usually three consecutive 15-minute collection periods were run. Rates of urine flow obtained were 2-4 ml/min. The mean control clearances for 79 clearance periods were creatinine, 55.8 ml/min and PAH, 196.8 ml/min. The mean values for 21 clearances done while the dogs were receiving 2.5-7.5 mg DCA daily for 5-7 days were creatinine, 66.5 ml/min and PAH, 191.8 ml/min. Mean values for 26 clearances at daily DCA doses of 10-15 mg

for 6-7 days were, creatinine, 74.8 ml/min and PAH, 260.3 ml/min. Although other possibilities cannot be ruled out, increased clearances with higher doses of DCA may be related to hemodynamic effects of altered circulating blood volume. On higher DCA doses the classical changes were present, i.e. hemoglobin and hematocrit were reduced whereas both systolic and diastolic blood pressures were slightly elevated.

Cytological effect of slow neutrons. ALAN D. CONGER (introduced by ALEXANDER HOLLANDER). *Biology Division, Oak Ridge National Lab., Oak Ridge, Tenn.* The operation of chain reacting atomic piles has made a new type of radiation, slow neutrons, available in amounts large enough for considerable biologic effect and possible damage. The biologic effect of the non-ionizing slow neutrons is produced indirectly when they are captured by atoms which then emit the various ionizing radiations. Most important reactions in biologic materials are hydrogen capture of slow neutrons, forming deuterium which immediately emits a gamma ray, and nitrogen capture with the emission of a proton. The action of slow neutrons in causing a specific biologic effect, the breakage of chromosomes, has been studied in the flowering plant *Tradescantia*. Such broken chromosomes are lethal to cells possessing them when those cells subsequently divide, slow neutron exposures of about 15 minutes produced visible aberrations in 50% of the cells. Previous experiments on the same plant have shown that certain types of these chromosome breakage aberrations increase as the dosage squared when x- or gamma-radiation is used, but increase linearly with dose when a radiation of heavy particles is used. The chromosome breakage data from slow neutron treatment given in the Oak Ridge Pile show a linear increase with dose, indicating that the most important reaction of slow neutrons in this tissue is the nitrogen capture with its emission of a proton.

Cardiovascular effects of nembutal, dibenamine and yohimbine on trained dogs. GEORGE CONSTANT (by invitation), ARISTOPHANES ANDRONIS (by invitation) and ERIC OGDEN. *Dept. of Physiology, Univ. of Texas Medical Branch, Galveston, Texas.* Arterial pressure of trained dogs was measured by femoral puncture with a needle connected to a Tyco's manometer, the lowest point of the excursion being recorded. Such readings are a few millimeters above diastolic pressure. The drugs were given slowly intravenously in normal saline solutions. Nembutal (30 mg/kg) greatly increased pulse rate and affected blood pressure variably, a fall being usual. These effects lasted from 1 to 4 hours. Dibenamine (25 mg/kg) lowered the blood pressure and its effect on the pulse rate was variable, since intravenous dibenamine caused much distress and struggling these pulse rate de-

terminations are doubtful, therefore only 3 such experiments were done. Dibenzamine during Nembutal anesthesia produced a further decrease in blood pressure but slowed the pulse rate, not however to the pre-anesthetic level. Yohimbine raised the pulse rate and usually lowered the blood pressure of unanesthetized dogs. Yohimbine given to the nembutalized animal raised both blood pressure and pulse rate. These observations serve again to emphasize the difficulties of interpretation of observations of the cardiovascular system made under anesthesia. The detailed pharmacodynamic interpretation of these differences should not be attempted until a great variety of simultaneous measurements of different cardiovascular functions is available.

Proteolytic activity in cerebro-spinal fluid

GEORGE CONSTANT (by invitation), WIKTOR W. NOWINSKI, ROBERT B. WHITE (by invitation) AND JOY M. HART (by invitation) *Tissue Culture Lab and Dept of Neurology and Psychiatry, Univ of Texas Medical Branch, Galveston, Texas*. Cerebro spinal fluid from schizophrenic and neurotic patients was investigated for its proteolytic activity. This was measured by the release of tyrosine and tryptophane during incubation for 3 hours at 37°C with edestine as substrate and at a final pH 4.2. Controls were set up by incubating cerebro-spinal fluid without substrate, but under the same conditions. The estimation of released amino acids was carried out with Folin-Ciocalteu reagent using a Beckman spectrophotometer and the figures obtained were related to a standard expressed in milligrams tyrosine. It was found that in 35 of the 38 cases investigated the amount of released tryptophane and tyrosine in the spinal fluid of these individuals was 40 to 70% greater as compared with the control tubes which did not contain the substrate.

Some methods of producing traveling contraction nodes in adult frog muscle fibers (motion picture) B. A. COOKSON and FLOYD WIERCINSKI (introduced by L. V. HEILBRUNN) *Depts of Zoology, Univ of Pennsylvania, and of Physiology, Hahnemann Medical School, Philadelphia, Pa*. Small bundles of muscle fibers, teased from the adductor magnus of *Rana pipiens*, were immersed in a solution containing 1.3% NaCl and 0.75 vols % H_2O_2 . After approximately 2 minutes small contraction nodes were seen forming in various regions of the fibers. These nodes usually recurred in the same regions at regular intervals. After originating, they traveled along the fibers. Usually the node as it formed split into two nodes traveling in opposite directions. Frequently the nodes collided and canceled out. Sometimes contraction nodes were formed which involved only half the circumference of the muscle fiber. A slight increase in the H_2O_2 concentration produced, instead of traveling

nodes, large stationary areas of fiber which rhythmically contracted and relaxed. During the contraction the sarcolemma became wrinkled. A slight decrease in the H_2O_2 concentration resulted in complete absence of response. Reduction of the NaCl concentration to 65% (approximately isotonic), also resulted in an absence of response. A solution containing 1.3% NaCl, 0.75 vols % H_2O_2 , 1% glutathione and enough 0.1 N NaOH to adjust the pH to 7.4 was found to keep its potency for over 2 days, whereas the solution containing only 1.3% NaCl and 0.75 vols % H_2O_2 , due to the instability of the H_2O_2 , remained potent for only 2-3 hours. With the former solution it was possible to produce traveling contraction nodes in isolated single fibers with injured ends. Preliminary studies indicate that traveling contraction nodes can also be produced by solution of 2-methyl 1,4 naphthoquinone in Ca-free frog ringer.

Osmophilia in relation to alkaline phosphatase activity in the chloride cell (gill) of fundulus heteroclitus D. EUGENE COPELAND (introduced by C. M. WILLIAMS) *Arnold Biological Lab, Brown Univ, Providence, R. I*. Evidence has been previously presented (Copeland, *J. Morph.* 82) of a cell type responsible for the salt excretion described by Smith (*Am. J. Physiol.* 93) as occurring from the gills of marine fish. Subsequently it was suggested (Pettengill and Copeland, *J. E. Z.* 108) that the marked increase in phosphatase activity found when *F. heteroclitus* is adapted to fresh water indicated a physiological reversal of cell polarity, the cell then becoming an absorptive mechanism, accounting for the chloride absorption noted by Krogh (*Z. vergl. Physiol.* 24) in fresh-water fishes. In the present report, a similar marked increase in osmophilia is found to occur as the fish becomes fresh water adapted. Emmel (*Anat. Rec.* 91) has shown a positional relationship of phosphatase activity and Golgi zone in the absorption cells of the intestine. The chloride cell not only shows a reasonable positional similarity of the two phenomena but also a synchronous change in amounts with change in functional activity of the cell.

Ferritin effect on blood pressure and pressor response in rats A. C. CORCORAN, G. MASSON and C. SCHAFFENBURG (by invitation) *Research Division and Bunts Inst of the Cleveland Clinic Foundation, Cleveland, Ohio*. A sample of ferritin was kindly supplied us by Dr S. Granick. The effects of its intravenous injection were observed in 8 rats anesthetized with pentobarbital, 4 of the animals were bilaterally nephrectomized (to exclude participation of renal V.E.M. in the response) and 4 had experimental renal hypertension (due to silk perinephritis in 1 and to the 'endocrine-kidney' technique of Selye in 3). One carotid artery was cannulated and arterial pressure measured with a mercury manometer. Each experi-

ment consisted of successive injections of ferritin solution (0.1 or 0.2 cc containing 200 to 400 μ g of ferritin) preceded and followed at intervals of 5 to 10 minutes by injections of adrenaline (0.12 μ g, intravenously). Injection of ferritin had no consistent immediate or delayed effects on arterial pressure or adrenaline response. Ferritin or its protein moiety has been identified as V.D.M. and found to inhibit the effect of local adrenaline on the terminal arterioles of the rat mesoappendix for several minutes in a dosage of about 0.005 μ g intravenously (Mazur and Shorr, 1948). From our experiments it seems that these segments of the vascular tree do not participate significantly in the pressor response to intravenous adrenaline. Further, these observations do not support the view that the presence or absence of responsiveness to adrenaline in this segment is closely related to the level of arterial pressure.

Conditions influencing explosive decompression injury E. L. COREY *Univ. of Virginia Medical School, Charlottesville, Va.* In an attempt to evaluate the multiple factors operating in conventional experiments on explosive decompression (expansion of intrapulmonary gases, anoxia, aeroembolism), over 300 tests were performed in which rats were placed in a circulating atmosphere of compressed air for varying time periods (2-30 atmospheres, 10 sec-30 min), and subsequently decompressed to ambient atmospheric pressure in c. 0.5 sec. Anoxia was thus prevented, and the effects of rapid gas expansion alone could be observed following exposures to pressure. The effects of anoxia *per se* were studied by placing animals in an atmosphere of nitrogen for suitable time intervals. In rats maintained under positive pressure for 30 min prior to decompression, 100% mortality was not attained until decompression from 11 atmospheres. In these cases pulmonary lesions were observed in only 15% of the animals, while all exhibited marked aeroembolism. Of 99 animals which succumbed in experiments of this type, 26% gave evidence of pulmonary damage, while 87% showed gross aeroembolism. Obviously time under pressure constituted a potent factor in determining survival after decompression. Thus at 30 atmospheres a time-under-pressure difference of 40 seconds was found to alter the mortality rate from 100% to zero. Aeroembolism thus appeared to be the major cause of injury in these experiments. Anoxia resulted in pulmonary hemorrhage in 50% of animals tested. Results of these tests indicated that anoxic anoxia and aeroembolism far outweigh the physical expansion of intrapulmonary gases as lethal factors in explosive decompression.

Comparative diuretic effects of neutral and alkaline sodium salts in hypoproteinemic edema SAMUEL A. CORSON and ELIZABETH O'LEARY (by invitation) *Dept. of Physiology, School of Medi-*

cine, Howard Univ., Washington, D. C. Edema was produced in trained unanesthetized dogs by means of a low protein diet and periodic massive plasmaphereses. Edema (as measured by an increase in thiocyanate space) could be produced by this method within a period of several days and maintained for several weeks. Hypertonic solutions of Na salts were administered intravenously (in the postabsorptive state) by means of a constant infusion pump at the rate of 2.5 ml/min, injecting a total dose of 10 ml/kg of body weight. The diuretic response was measured until the rate of urine flow began to approach that of the control period. A 1.4M solution of disodium salts of succinate and fumarate increased the rate of urine flow 10-fold (from a control value of 0.5 ml/min to an average value of 5 ml/min), this diuresis persisting for about 300 minutes. Subtracting the quantity of fluid injected and the fluid that would have been removed as a result of the normal urine flow, one obtains a figure of 76 ml/kg as the average net quantity of urine excreted following the administration of the alkaline salts. The injection of either 2M (isosmotic with a 1.4M disodium salt) or 2.8M NaCl (equivalent in Na content to a 1.4M disodium salt) tripled or quadrupled the rate of urine flow for a period lasting for about 300 minutes. The average net fluid output under these conditions was 24 ml/kg. Thus, intravenous administration of either neutral or alkaline sodium salts leads to the removal of large quantities of fluid in experimental hypoproteinemic edema, the alkaline salts invariably producing a diuresis approximately 3 times as great as that induced by isosmotic or equivalent solutions of NaCl.

Development of Turbulence in Flowing Blood N. A. COULTER (by invitation) and JOHN R. PAPPENHEIMER *Dept. of Physiology, Harvard Medical School, Boston, Mass.* The relations between pressure, flow and electrical conductivity of bovine blood were determined over a wide range of flows, corpuscular volumes, and temperatures in glass tubes 2.52 mm and 6.80 mm in diameter. The viscosity (calculated from Poiseuille's Law) decreases with increasing flow—a phenomenon known previously to occur in capillary tubes. Presumably change of viscosity with flow is associated with orientation of red cells in the flow stream. This orientation is beautifully demonstrated by changes of electrical conductivity along the axis of the flow stream. At zero flow the (non-conducting) cells are randomly oriented and the electrical resistance is high; as flow progresses, the cells orient to present least hydrodynamic resistance and both the (apparent) viscosity and the electrical resistance diminish in regular fashion toward constant values. Turbulence begins at an apparent Reynolds number of about 1000 as with homogeneous fluids. However, the electrical resistance remains un-

changed even at Reynolds numbers up to 2500. We, therefore, infer that turbulence occurs only in the plasma layer, while viscous flow continues in the central core of oriented cells. It appears possible to consider the apparent viscosity of blood as the resultant of two real viscosities: a) the viscosity of plasma alone in the peripheral sleeve, b) the viscosity of cells plus plasma in the central core. Evidence suggests that the rate of shear in the peripheral sleeve is so high compared with that in the central core that most of the energy required to maintain flow is dissipated in the plasma layer.

Pulmonary blood flow and alveolar ventilation-perfusion relationship following resection of one lung in man. A. COURNAND, R. L. RILEY and A. HIMMELSTEIN (by invitation). *Dept. of Medicine and Surgery, Columbia Univ. College of Physicians and Surgeons, New York City.* Pulmonary blood flow and pulmonary arterial pressure were measured at rest in 9 subjects, 14 to 72 years of age, from 1 month to 11 years after resection of one lung. In 6 the same measurements were repeated during moderate exertion. Although the pulmonary arterial pressure at rest was but slightly altered, it rose invariably during exercise. This finding is in contrast with observations in normal individuals during much more severe exercise (*Am J Physiol* 152: 372, 1948). The pulmonary artery pressure rise was small in cases where the remaining lung appeared normal on the basis of physiological measurements, while the rate of blood flow exceeded the blood flow through each lung of normal individuals during severe exercise. The maximum load which can be taken up by a normal vascular bed was therefore tested. The pulmonary artery pressure rise was larger in individuals where moderate emphysema developed in the remaining lung, although the increase in blood flow was smaller. The relationship between alveolar ventilation and perfusion was studied in these same individuals according to the method presented in these proceedings by two of the authors. They were normal in 6, in the remaining 3 there was evidence of perfusion of poorly ventilated alveoli and/or of ventilation of poorly perfused areas.

Effect of a psychiatric interview on the blood pressure response to cold stimuli. ROBERT W. CRANSTON (by invitation), JAMES H. CHALMERS (by invitation), HENRY LONGSTREET TAYLOR, AUSTIN HENSCHEL and ANCEL KEYS. *Laby of Physiological Hygiene, Univ. of Minn., Minneapolis, Minn.* The blood pressure responses to a 'cold pressor' test of 9 well-adjusted healthy young men 18 to 24 years old were studied on six occasions during a control period of 7 months. The cold test was standardized as to time of events, relation to meals and activity and psychological atmosphere. Blood pressures were measured by means of a sphygmomanometer and stethoscope

with the subject supine during a suitable control period and during the immersion of the hand in ice water for one minute. Each subject was interviewed by the psychiatrist for 5 to 8 hours during the 7-month control period to learn his psychodynamics. When preliminary observations were completed, an interview was arranged in which emotionally charged topics were intentionally introduced by the psychiatrist. There was a mild emotional response of hostility and resentment in the majority of cases but no marked emotional reactions. The interview produced variable but small changes in the resting blood pressure. At the end of the interview, the blood pressure response to cold was determined. The grand average of the mean individual increases in the systolic blood pressure during the control period was 16.03 mm Hg. The mean response to cold after the psychiatric interview was 24.2. This difference was significant at the 1% level.

Sodium and potassium depletion in rats by means of cation exchange resins mixed with food. J. M. CRISMON. *Dept. of Physiology, Stanford Univ. School of Medicine, Stanford, Calif.* Maintenance of negative sodium balance with the aid of ingested cation exchange resins as suggested by Dock (*Trans. Assn. Am. Physicians* 59: 282, 1946) has received little study and few clinical trials have been reported. Intakes of sodium and potassium and fecal loss of these cations from 10 rats were measured every other day for 23 days. After a 9-day control period, 10% by weight of acid-treated -200 mesh Dowex 50, a sulfonated polystyrene resin was added to the diet. After addition of resin 100 gm of diet contained, by analysis, 30.3 mEq of sodium and 16.0 mEq of potassium. On the 18th day sodium in the diet was reduced to 18.9 mEq/100 gm and potassium to 13.7 mEq/100 gm. During the control period 96% of ingested sodium and 94% of ingested potassium were absorbed from the gut. During high sodium intake, plus resin, absorbed sodium decreased to 70% and potassium to 82% of the amount ingested. On reduced sodium intake, absorbed sodium decreased to 34%, potassium absorption was still 82%. The low sodium diet containing resin was fed for an additional 10 days, and the animals were killed. Plasma sodium varied from 142 to 167 mEq/l with a mean value of 152. Potassium concentrations were slightly low with a mean of 3.2 mEq/l, the range was 2.3 to 3.9. Muscle sodium, potassium and chloride were low with the following mean values in mEq/100 gm of fat-free dry muscle: Na = 6.68 (4.46 - 9.26), K = 36.8 (32.1 - 40.6), Cl = 5.37 (4.65 - 5.98).

Action potential of heart muscle. HOWARD J. CURTIS. *Dept. of Physiology, Vanderbilt Univ. Medical School, Nashville.* By microdissection methods it has been found possible to isolate small,

homogeneous bundles of muscle fibers from the turtle heart in which the fibers are all parallel and there are very few anastomoses between fibers. These bundles are transferred to mineral oil and stimulation and electrical recording is effected by means of microelectrodes. At about 10°C spontaneous activity is almost absent. When both recording electrodes are on active muscle, the conducted action potential is monophasic, as contrasted with the diphasic action potential of skeletal muscle under the same circumstances. The monophasic wave is followed by a T-wave which may be either positive, negative or diphasic. When one end of the bundle is crushed or treated with isotonic KCl a resting potential of 10 millivolts or more is recorded. The action potential now falls from the level of the resting potential to zero and remains there for as long as 2 seconds and then relatively rapidly returns to the level of the resting potential. Preliminary estimates indicate that the period of depolarization corresponds closely with the period of contraction and with the S-T interval. Thus the initial part of the action potential represents conduction in the individual muscle fiber presumably by the same mechanism as for skeletal muscle, but unlike skeletal muscle repolarization is delayed for a relatively long time. The T-wave is not a conducted response and represents true cellular re-polarization.

Attempted blockade of the adrenotrophic mechanism of the pituitary in starvation. SAVINO A. D'ANGELO *Dept of Biology, Washington Square College of Arts and Science, New York Univ, New York City*. It has been demonstrated (D'Angelo, Gordon, and Charipper *Endocrinology* 42: 399, 1948) that starvation in the guinea pig fires the adrenotrophic mechanism of the anterior hypophysis and causes marked enlargement of the adrenals. Although reciprocal regulatory control between anterior pituitary and adrenal cortex is shown by adrenal depression with cortical hormone overdosage in the fed animal, it appears that in the guinea pig the cortical hypertrophy of starvation cannot be blocked. Female guinea pigs (300-350 gm) were subjected to acute starvation during which cortical hormone was maintained at presumably high levels in the body fluids by administering subcutaneously aqueous cortical extract (Upjohn) in 3 cc doses every 3 hours, 4 times daily, for 9-11 days, and by giving cortical extract in the drinking water as well. Each animal received approximately 150 cc of the cortical extract over the starvation period. Adrenal enlargement occurred with mitosis frequently encountered in the outer fasciculate region of the cortex. Cytological study of the pituitary indicated high rather than suppressed secretory activity. Two marked peripheral effects were observed in the cortical-treated animals, 1) oliguria and 2) marked leuco-

cytosis characterized by a pronounced increase in the numbers of neutrophils showing advanced stages of nuclear lobulation. Further studies on the peripheral blood and on the marrow following high cortical dosage are being made.

Effects of steroids on the endocrine system of the starved guinea pig. SAVINO A. D'ANGELO, ALBERT S. GORDON and HARRY A. CHARIPPER *Dept of Biology, Washington Square College of Arts and Science, New York Univ, New York City*. In female guinea pigs acutely starved to 40% body weight loss by complete food deprivation (water allowed), the thyroid lost at a lesser rate (18%), the ovary at about the same rate (44%), and the cervical lymph nodes at a greater rate (68%) than did the body. In contrast, the adrenal underwent absolute hypertrophy (60%) with commensurate increase in solid contents. The results obtained following the subcutaneous administration (6-11 days) of various steroid preparations in high daily doses (Progynon B, 600 RU, progesterone, 15 mg, desoxycorticosterone acetate, 18 mg, lipoadrenal extract, 1 cc, aqueous cortical extract, 10 cc) indicate that estrogen, desoxycorticosterone and lipo-adrenal, but not progesterone, augmented the adrenal hypertrophy of starvation. The solid content of these enlarged adrenals was correspondingly increased and microscopic examination further indicated a true increase in cortical mass. In the fed animal only the aqueous cortical extract affected adrenal weight (by depression) but desoxycorticosterone and aqueous cortical extract both caused involution of the zona glomerulosa in the adrenals of fed animals. A marked difference on the adrenals was found between the steroid vehicles used. Peanut oil given in 1-cc daily injections for 9-11 days caused marked adrenal enlargement in fed or starved animals, whereas, sesame oil in equivalent or greater amounts was without effect. The various steroids employed did not appreciably influence thyroid and ovarian weights in either the starved or fed animal, and the regression of the lymph nodes in starvation bore no apparent relationship to the adrenal hypertrophy. The involvement of the anterior pituitary in the end organ response to starvation is considered.

Rise in serum precipitable or protein-bound iodine following massive doses of inorganic iodide. T. S. DĄWOWSKI and J. H. GREENMAN (by invitation) *Dept of Research Medicine, Univ of Pittsburgh School of Medicine, Pittsburgh, Penn*. Riggs, Laviates and Man (*J Biol Chem* 143: 363, 1942) demonstrated that both inorganic and protein-bound iodine is present in plasma. The work of Taurog and Chaikoff (*J Biol Chem* 165: 217, 1946) with rats indicated that, within limits, increases in the intake of inorganic iodide were accompanied by a rise in the organic iodine of the thyroid gland and plasma. The maximum level of

circulating total iodine recorded was 33.6 gamma %, and the changes in the circulating protein-bound iodine, though definite, were quite small.

This report deals with the relationship between the intake of inorganic iodide and the level of circulating serum precipitable or protein-bound iodine found in 4 patients to whom massive doses of potassium iodide (up to 3 gm daily), or of a saturated solution of potassium iodide (up to 7 cc daily), had been given during 4 to 18 weeks. This resulted in total serum iodine levels of 2,100 to 18,100 gamma %. Serum precipitable iodine measured by the method of Barker (*J Biol Chem* 173:715, 1948) slightly modified, rose progressively as to high as 31.7 gamma %. Removal of any traces of inorganic iodide which would yield false high values was assured by obtaining checks on two separate sets of duplicate or triplicate aliquots of precipitated serum, one set of which was washed with 14 and the other with 18 successive changes, 25 cc each, of double distilled water. Iodism did not develop nor were these high levels of protein-bound iodine associated with hypermetabolism.

Age and osmotic work in urea excretion D F DAVIES (introduced by H A SCHROEDER) *Section on Cardiovascular Disease and Gerontology, National Inst of Health, Bethesda, Md and Baltimore City Hospitals, Baltimore, Md* Previously reported results on degenerative changes in renal function with age have indicated a primary vascular defect. The possibility was considered that this reduction in renal function was caused by lowered metabolic rate and decreased work requirement of the kidney in old age. If this were true, the kidney could be said to undergo an atrophy of disuse having no deleterious effect on other body functions. To examine this hypothesis data on basal urea production, tubular reabsorption of urea, urea clearances and blood and urine urea concentrations were examined for their relationship to their contribution to osmotic work in adult men between the ages of 20 and 90. Urea clearance decreased with age at a rate comparable to that of glomerular filtration rate and tubular excretory capacity after the 5th decade. Tubular reabsorption of urea followed a progressive decline during the seven decades studied. Most of this fall is explained by decreased filtration rate. Urea production (excretion) showed no significant correlation with age. Blood urea concentration showed a moderate increase with age. Calculation of renal osmotic work of urea excretion showed that there is an increase in the amount of osmotic work per unit of functioning kidney tissue with increasing age. Unless a significant and consistent decrease in salt intake occurs during the aging process, involutional changes due to decreased work requirement cannot be the cause of the decrease in effective renal blood flow and excretory functions associated with aging.

Theophylline ethylene diamine and renal function in control subjects and decompensated cardiac patients JAMES O DAVIS (by invitation) and NATHAN W SHOCK *Section on Cardiovascular Diseases and Gerontology, National Heart Inst, National Inst of Health, Bethesda, Md, and the Baltimore City Hospitals, Baltimore, Md* Inulin, diodrast and sodium clearances were determined during 3 control periods and during 5 periods after the intravenous administration of 0.48 gm of theophylline ethylene diamine (TED). In control subjects, an elevation in GFR and Cl_{Na} was sustained for 50-60 minutes. The rise in effective RPF was usually transitory. Statistically, the ratio E_{Na}/Cl_{Na} was more variable and of significantly greater magnitude after than before the injection of TED. This finding indicates that increased sodium excretion cannot be regarded as solely the result of augmented GFR and suggests that TED acts upon the renal tubules to decrease sodium reabsorption.

Patients in congestive heart failure were divided into two groups according to the level of Cl_{Na} before TED administration. In the low Cl_{Na} group, GFR was usually within normal limits but effective RPF was consistently low. In the elevated Cl_{Na} group both GFR and effective RPF were reduced. This evidence indicates that a low GFR is not the primary mechanism for salt retention in patients with cardiac failure. The response in GFR and effective RPF in heart failure patients ranged from a negligible elevation to an increase greater than 100%. Unlike the response in controls, the elevation in GFR lasted for 10-20 minutes only, the increase in Cl_{Na} was usually sustained for 50-60 minutes. The lack of correlation between the duration of the elevation in GFR and Cl_{Na} indicates that the sustained increase in Cl_{Na} is the result of decreased tubular reabsorption of sodium.

Role of the adrenal cortex in the abnormal insulin and adrenaline response of hypophysectomized dogs R C DE BODO, S P KIANG (by invitation) and I H SLATER (by invitation) *Dept of Pharmacology, New York Univ College of Medicine, New York City* It was previously suggested (*Federation Proc* 7:116, 1948) that the gradual development of insulin hypersensitivity and resistance to adrenaline hyperglycemia in the hypophysectomized dog might be related to the secondary atrophy of the adrenal cortex and thyroid. Hypophysectomized dogs manifesting maximal insulin hypersensitivity (more than 60 fold) were given potent adrenal cortical extract (ACE). The total dosage, route and time of administration were varied. Administration of ACE both on the previous day and again 4-5 hours before insulin gave the optimal effect. While 1500 DU had little effect, 3500-4000 DU (for 15- to 20-kg dog)—an amount many times the maintenance dose for

adrenalectomized dogs—produced the following effects 1) it elevated somewhat the post-absorptive blood sugar, 2) after insulin (0.025 U/kg i.v.) it prevented the fall of blood sugar to the usual low level—hypoglycemic signs did not develop—and markedly accelerated the recovery to post-absorptive levels. It did not, however, alter the rate of the blood sugar drop. Though ACE in massive doses exerted some 'anti-insulin' action, reversion to a normal response was never achieved. In each case the insulin-induced hypoglycemic response in the ACE-treated animal closely resembled the response obtained in the animal 2-3 days after hypophysectomy.

Similar studies with ACE were made on the adrenaline response of hypophysectomized animals showing resistance to the hyperglycemic action of adrenaline. In every instance, the effect was small and in no case was a return to a normal response noted.

Protein utilization in hypophysectomized dogs
R. C. DE BODO, S. P. KIANG (by invitation) and I. H. SLATER (by invitation) *Dept. of Pharmacology, New York Univ. College of Medicine, New York City*. Houssay showed that hypophysectomized dogs when compared with normals have a markedly decreased nitrogen excretion on fasting, but a normal nitrogen output on meat diet. Soskin found that meat-fed hypophysectomized dogs were able to sustain a normal blood sugar and that after fasting recovery from hypoglycemia occurred with meat feeding. Considering the importance of these observations which might suggest a distinction between 'endogenous' and 'exogenous' protein metabolism in hypophysectomized dogs, it was felt that re-examination of the problem was warranted. We have found that hypophysectomized dogs were able to maintain and even increase their post-absorptive blood sugar level and increase their body weight when fed a trypsin hydrolysate of casein (with vitamin supplement) for a period of three weeks. In a second series of experiments when hypophysectomized animals were fasted, prompt recovery of the blood sugar from hypoglycemic levels was noted within one hour after the feeding of the hydrolysate.

During fasting the hypophysectomized dogs showed a decrease in nitrogen excretion to about $\frac{1}{4}$ of that noted in normal animals after comparable or even longer periods of starvation. These hypophysectomized animals showed also a diminished output of potassium and phosphate with unchanged serum levels, a further indication of impaired breakdown of intracellular protein. Thus, we have been able 1) to confirm and extend the previous observation, that while the metabolism of body protein is defective in the hypophysectomized animal, the utilization of fed protein is adequate and

2) to demonstrate that the conversion of amino-acids to carbohydrate is very rapid.

A constant delivery pipette particularly designed for analysis of small volumes
R. C. DEERING (introduced by H. C. BAZETT) *Dept. of Physiology, Univ. of Pennsylvania Medical School, Philadelphia, Penna.* Direct determinations of cardiac output require special methods for analyzing small blood samples for which a modification of the Guest pipette possesses advantages. The pipette consists of a calibrated glass tube joined at each end by two-way stopcocks. The capillaries of the upper stopcock connect the calibrated tube to a) a cup similar to that of the Van Slyke apparatus or b) a smaller overflow cup. The capillaries of the lower stopcock connect it to a) a sample inlet tube or b) a stem having a rubber tip. The pipette serves to introduce a sample into a Van Slyke apparatus after which standard procedure is followed. The stem, supported by a rubber stopper, is placed in the analyzer cup. For analyses of 0.2-ml. samples, gas-free lactic acid is run into the upper cup, the cocks are reversed and the calibrated section washed and dried. The pipette is filled directly from collecting syringes, the blood sample, preceded by a mercury 'cap,' being forced up the pipette section to the overflow cup. The cocks are reversed cutting off a contained volume. The blood, followed by a mercury drop and the lactic acid, is run into the chamber, thus introducing the whole sample. Present experience indicates a greater accuracy of sample-introduction, so that consistency with 0.2-ml. samples is equivalent to that commonly obtained with 1 ml. With 0.5 ml., conditions are improved and the procedure simplified. Accuracy attained will be illustrated with data obtained on samples from the right heart.

Influence of work on the amount of K-strophanthoside stored by muscle
E. C. DEL POZO, G. ANGUIANO (by invitation) and E. G. PARDO (by invitation) *Inst. de Salubridad y Enfermedades Tropicales Mexico, D. F., Mexico*. It is generally thought that striated muscle is able to store only very small amounts of digitalis glycosides. The heart, on the other hand, can take up much greater amounts per gram of tissue. The cause of this selective storage of digitalis bodies by the heart is not yet settled. While the values known for skeletal muscle have been obtained by perfusion experiments on inactive muscle, the values for the heart have been measured in preparations in which the heart is working. There is the possibility that the absence of muscular work may be a factor in determining the difference observed between the storage by the heart and the storage by striated muscle. In view of this, the influence of activity on the amount of K-strophanthoside taken up by skeletal muscle was analyzed. This was done by determining the *dosís certa letális* (d.c.l.) of said

glycoside for cats under dial anesthesia. In one half of the series of 80 cats the muscles innervated by both sciatic nerves were continuously stimulated at a frequency of 5/sec. The average d.c.l. for the cats whose muscles were thus stimulated was significantly higher than that for the control animals. This seems to indicate that the muscles in activity took up greater amounts of the glycoside. These results could have a bearing on the selective storage of digitalis bodies by the heart, which is in constant activity.

Differences in the effects of cold environment and of muscular work on adrenal function. A. DESMARAIS (introduced by L. P. DUGAL) *Dept. d'Acclimatation, Inst. de Biologie Humaine, Univ. Laval, Quebec, Canada*. It has been shown by Selye (*Arch. internat. de pharmacodyn. et de therap.*, 55: 432, 1937) that adrenalectomized rats adapted to muscular work (revolving cages) are able to perform more work than non-trained, non-adrenalectomized rats. Since cold temperature is being regarded as an alarming stimulus having the same effects on adrenal function as muscular work, it was of interest to observe if adrenalectomized rats adapted to cold would resist better than non-adapted, non-adrenalectomized animals. The results show that the latter group, contrary to what one could expect, is significantly more resistant to cold than the adrenalectomized, adapted group. The difference between both types of stress is striking for the above experiment, but less so if one compared adrenalectomized animals adapted and non-adapted.

Further observations on the hypotensive effects of intravenous injections of hypertonic solutions. INGRITH J. DEYRUP (by invitation) and WILLIAM W. WALCOTT *Dept. of Zoology, Barnard College, and of Physiology, College of Physicians and Surgeons, Columbia Univ., New York City*. It was reported previously (Walcott and Deyrup *Am. J. Physiol.*, 155: 1948) that, in the anesthetized dog, the intravenous injection of hypertonic solutions is followed by a fall in blood pressure which is consistently different from a marked and characteristic hypotension resulting from injection of mixtures of hypertonic solutions with homologous blood. Thus, whereas the injection of 12 to 30 ml. of 5 to 7% NaCl results in a diphasic fall in blood pressure, there is a profound and rapid single phase fall following injection of an equivalent volume of an equally concentrated mixture of blood and hypertonic NaCl. A related phenomenon has been observed in rabbits (nembutal, ether) but not in cats (nembutal). The striking difference between the cardiovascular response to injection of mixture of blood with hypertonic solutions, as compared with the effects of hypertonic solutions alone, suggests that the hypotension following the injection of such solutions may result, at least in part, from

the interaction of some component of the blood with the hypertonic solution. In line with this hypothesis, we have obtained evidence that, under specifically defined conditions, a depressor substance may be demonstrated in approximately isotonic solutions derived from mixtures of hypertonic solutions with whole blood or erythrocytes suspended in 0.9% NaCl. An attempt has been made to characterize the depressor substance thus obtained, and to compare it with other depressor substances (various ions, histamine, compounds related to choline and adenosine).

Carbohydrate utilization and lactate formation in the chicken retina. H. F. DIERMEIER and V. F. LINDEMAN (introduced by Robert Gaunt) *Dept. of Zoology, Syracuse Univ., Syracuse, N. Y.* The retina has one of the highest rates of glycolysis of any tissue of the body. In order to find the source of the lactate produced by this tissue the following determinations were conducted on chicken retina kept at 37.5°C: glycogen content before and after incubation for one hour; glucose utilization with varying concentrations of glucose in the incubation media; lactate production. Chemical assays were carried out by photometric analysis. All experiments were conducted under aerobic conditions. Ten experiments on matched retina are reported. Weights are given as wet weight. The average glycogen content (Morris, *Science* 107: 1948) immediately after removal from the eye was 0.0032 mg glycogen (glucose equivalents) per mg retina. After one hour of incubation in Krebs-Ringer solution with 0.1% glucose as substrate the value was decreased to 0.00227 mg/mg of retina. The incubated retina consumed an average of 0.0205 mg of glucose/mg of tissue from the substrate and produced 0.00919 mg of lactate/mg of tissue.

In an attempt to account for the utilized carbohydrate not in the form of lactate, oxygen consumption measurements were made using the direct method of Warburg. The average oxygen consumption in the Krebs-Ringer solution with 0.1% glucose added was 0.507 cmm/mg of tissue (wet wt.) per hour. Oxygen consumption with no glucose added as substrate was 0.279 cmm/mg tissue (wet wt.) per hour. Oxygen consumption does not account for the remainder of the carbohydrate utilized and further experiments are in progress.

Identification of chloramphenicol (chloromycetin) degradation products by paper chromatography. WESLEY A. DILL (by invitation) and ANTHONY J. GLAZKO *The Research Lab. of Parke, Davis and Company, Detroit, Mich.* The paper strip chromatographic methods of Consden, Gordon and Martin (*Biochem. J.* 38: 224, 1944) were applied to the separation of chloramphenicol (Parke, Davis) and its degradation products in urine. Using *N*-butanol with 2.5% phenol and 2% pyridine added it was possible to separate chloramphenicol (I), a

hydrolysis product (II) and a conjugate (III) on a single strip of paper. After developing the chromatogram, colored derivatives were formed on the strip by reduction with titanous chloride, oxidation of excess reagent with bromine, diazotization with butyl nitrite and coupling with the Bratton-Marshall reagent. Small amounts of I and II were found in the urine of man by chromatography after administration of chloramphenicol, with large amounts of III being present. Dog urine showed larger amounts of II than man. III was identified in the urine of man, dog, and rat, with the compound having approximately the same R_f value in all cases. The conjugate (III) was isolated by use of the counter-current extraction technic, and provisionally identified as the monoglucuronide. Following hydrolysis of III with β -glucuronidase, chloramphenicol (I) and free glucuronic acid were identified separately by paper chromatography. Two-dimensional paper chromatograms of urine were also prepared using *n*-butanol with 3% acetic acid and again with 3% ammonium hydroxide added. I, II and III were identified by comparison with known compounds, and, in addition, at least one other derivative of chloramphenicol was found to be present.

Potential gradient vs current density in electrical control of axial polarity in regenerating *Dugesia tigrina* JEAN DIMMITT (introduced by GORDON MARSH) *Zoological Laby, State Univ of Iowa, Iowa City, Ia*. Sections of *Dugesia tigrina* were exposed to direct current for 120 hours (original posterior end facing the cathode), in solutions of specific resistance from 478.4 to 52,157 ohm-cm, at 21°C. One hundred % mortality was found below 1000 and above 15,000 ohm-cm. For media of 1031 (previously reported), 2400, 7237, 10,605 and 12,600 ohm-cm, the potential gradients in mv/mm across the regenerating pieces were constant for the following morphological effects, although the current densities varied 12-fold (values in parentheses are for 1031 ohm-cm, others are averages for all data): 178 (177) for regressive bipolars, in which head structures appear on both ends but disappear from the original posterior end within 3 days after removal from the current, 185 (193) for permanent bipolars, 198 (200) for progressive bipolars in which the head on the original anterior end is reorganized into a tail after removal from the current, and 217 (212) for complete reversal of axial polarity. Since constant current density is not the determinant of a given morphogenetic effect it may be concluded that control of polarity and differentiation is not determined by a stable relationship between the regenerating piece and its environment, nor by a critical rate of internal ion transport, i.e., by features of capacity. Constancy of potential gradient may be interpreted as the attainment of a critical discharge voltage (valence

change) for some essential ion, orientation of molecules having a characteristic dipole moment, establishment of a liminal counter-E M F or the overcoming of an inherent potential difference.

Antifibrillatory properties of N-methyl-N-(3,4-dimethoxybenzyl) β -(4-methoxyphenyl)-ethylamine hydrochloride JOSEPH R. DiPALMA, JOSEPH J. LAMBERT (by invitation), RICHARD A. REISS (by invitation) and JOHN E. SCHULTS (by invitation) *Dept of Physiology and Medicine, Long Island College of Medicine, Brooklyn, N. Y.* By use of a thyatron stimulator set at 600 impulses/min and electrodes attached to the right auricle of intact open-chest cats the fibrillation threshold for the new compound was determined. Quinidine and α -Fagarine were also assayed for comparative purposes. The new compound proved to be 2.3 times more active than quinidine and 1.3 times more active than α -Fagarine. The average acute lethal i.v. toxicity for cats was less for the new compound as compared to quinidine and α -Fagarine. On i.v. injection the new compound caused a transitory slowing of the pulse and a fall in blood pressure. In this respect and in the EKG changes produced with higher doses it resembled quinidine. It was also demonstrated that the refractory period of frog skeletal muscle was markedly prolonged by the new compound. In humans with normal hearts the new drug in equivalent doses 1 m. caused a greater lengthening of the Q-T interval than did quinidine. Also in human chronic fibrillation the f-f interval was increased more by the new compound than by quinidine. The interesting features of the new compound may be listed as follows, it does not have a quinoline structure yet it is remarkably potent, its activity apparently stems in part from the proper substitution of methoxy groups, its structure is simple enough to offer important leads as to the basic chemical moiety necessary for antifibrillatory action and finally it may prove to be superior to quinidine and α -Fagarine for the therapy of fibrillatory states in human beings.

Separate inhibition of resting and active oxygen consumption of functioning nerves R. W. DORF (by invitation) and R. W. GERARD *Dept of Physiology, Univ of Chicago, Chicago, Ill.* To study resting and active respiration, a modified capillary microrespirometer was developed to permit measurement of action potential and oxygen uptake of 10 separate nerves. The Q_{O_2} of nerves from one frog agree within 10%. The action of Na azide and methyl fluoroacetate (MFA) has been studied, yohimbin explored. Drugs were applied by soaking for an hour before mounting a nerve, stimulation was kept maximal at 120/sec, temperature range was 20-25°C. Azide, 0.1 mM, and probably yohimbin, 0.01 mM, can depress the increased Q_{O_2} of activity while leaving the action potential height

normal Resting Q_{O_2} may be somewhat depressed with azide, in which case conduction has gradually failed, but when normal, and with no increase on stimulation, conduction can continue unimpaired for at least 4 hours (confirming Bronk *et al*) MFA, by contrast, is able to depress resting Q_{O_2} without diminishing the increase on stimulation At 1 mM, resting Q_{O_2} is decreased 25% while the active increase is normal At 5 mM, resting Q_{O_2} is halved and the active increase may be intact, depressed or even abolished In any case, action potential may remain at normal height, one nerve, with the active increase present, being tetanized for 7 hours In interpreting these results, the presence of respiring non-conducting elements in a nerve trunk must be considered

'Saturation tension' and alveolar-arterial pO_2 gradient as expressions of 'venous admixture' in the lungs J C DOUGLAS (by invitation), R L RILEY and A CURNAND *Dept of Physiology, Univ of Western Ontario Medical School, London, Ontario, and of Medicine, Columbia Univ College of Physicians and Surgeons, New York City* The 'saturation tension' as measured with the Millikan oximeter during high oxygen breathing and the alveolar-arterial pO_2 gradient as measured by analysis of arterial blood and expired air during ambient air breathing have been studied in normal subjects and in patients with chronic pulmonary disease Both methods provide information from which 'venous admixture' in the lungs can be calculated The 'saturation tension' may be defined as the partial pressure of oxygen in the inspired air required to produce complete saturation of the mixed arterial blood The required increase in pO_2 above atmospheric, in any given patient, is due chiefly to the lowering of the mixed arterial pO_2 by addition of blood which has been inadequately exposed to oxygen before entering the left heart This blood has been called 'venous admixture' The mixed arterial blood may be completely saturated in spite of a considerable proportion of 'venous admixture' Increasing the inspired pO_2 raises the oxygen tension in poorly ventilated alveoli until at a certain inspired pO_2 all ventilated alveoli saturate the blood in contact with them In addition blood perfusing non-ventilated portions of lung (or separated from alveolar gas by an impermeable barrier) is saturated by the excess dissolved oxygen in the blood from well ventilated areas 'Venous admixture' as calculated from the alveolar-arterial pO_2 gradient during ambient air breathing involves the same factors but probably weights more heavily the effect of poorly ventilated alveoli

Spectrophotometric determinations of Brilliant Vital Red and T-1824 in serum PHILIP DOW and RAYMOND W PICKERING (by invitation) *Dept of Physiology, Univ of Georgia School of Medicine, Augusta, Ga* Hamilton and collaborators (1932,

1940) have demonstrated that Brilliant Vital Red can be accurately and conveniently determined in the supernatant of an ethanol precipitation of serum by colorimetric comparison with similarly treated standards, T-1824 has universally been found to go down with the precipitate Anomalies encountered in adapting the method for photoelectric colorimetry led to an investigation by Beckman spectrophotometry As progressively more dilute ethanol (always 10 volumes) was used to clarify the serum (dog), a larger fraction of both dyes remained in solution, roughly paralleling the albumin solubility The two dyes differed quantitatively, BVR being only 60% removed by absolute, not at all by less than 88% ethanol, T-1824, 93% removed by absolute, negligibly only at 80% ethanol Below 82% ethanol, inconstant cloudiness vitiated determinations

As previously emphasized, the ethanol precipitation provides a much clearer and more constant blank in the face of considerable variations in color and cloudiness of the original serum Hemoglobin as such is removed completely Some ethanol supernatants show no color whatever, even from severe hemolysis In others an as yet unidentified derivative of hemoglobin (probably a ferriporphyrin) is extracted in small amounts It absorbs comparatively little light at 508 or 625 $m\mu$ (the dye peaks) and has a high sharp peak at 404 $m\mu$ where the dyes absorb only slightly Correction can thus be easily made by reading at two wave-lengths and applying the customary formula Other methods of minimizing this contamination are being studied

Cardiac changes in monkeys following explosive decompression to extreme altitudes ROY DOWLING (by invitation) and SAMUEL GELFAN *Laby of Physiology, Yale Univ School of Medicine, New Haven, Conn* Cardiac changes following explosive decompression to simulated altitudes of as high as 75,000 feet have been studied in anesthetized monkeys by means of unipolar lead electrocardiography, recording simultaneously from three extremity leads Recompression of the animals at free fall rate began immediately after the sudden decompression The first observable change in the *ecg* complex is the displacement of the S-T segment, which occurs between 5 to 30 seconds after the sudden decompression This change is quickly followed by an increase in the P-R interval and simultaneously an absolute shortening of the Q-T interval, even during the marked bradycardia that develops The Q-T interval shortening indicates an acceleration of repolarization and the elevation of S-T segment in the VL and VF leads and depression in VR leads indicates that it is the left anterior ventricle that repolarizes faster than the rest of the heart The increase in irritability of this part of the heart is also indi-

cated by the extra systoles that arise from the left anterior ventricle. Heart block ranging from first degree to complete block were also observed. The e c g complex returns to normal after recompression to sea level.

These cardiac changes are due to the anoxia rather than the pressure changes. The same alterations have been observed in 100% N₂ anoxia without pressure changes. Similarly, sudden decompression from sea level to 40,000 feet in O₂, at which altitude the anoxia is insignificant, does not alter the e c g complex. Roentgenograms show a dilation of the heart during the anoxia following decompression and also following N₂ anoxia.

Studies in pancreatic function II Statistical studies of secretin responses in individuals without pancreatic disease DAVID A. DREILING (by invitation) and FRANKLIN HOLLANDER *Gastroenterology Research Lab., The Mount Sinai Hospital, New York City*. Amylase, bicarbonate and volume responses to secretin were studied in 172 patients without pancreatic disease, to set up criteria of normalcy. One series of data, with Secretin-Wyeth alone, and another series with several different preparations were compared with data published by other investigators. Duodenal contents were collected by continuous suction through a double lumen gastroduodenal tube for 80 minutes following intravenous administration of 1 l u of secretin/kg body weight. This collection time was used, rather than the shorter intervals advocated by others, because it represents the entire period of secretory activity in response to hormone stimulation. Best agreement among the various series was shown by the means for maximum bicarbonate concentration in mEq/l, also, the corresponding coefficients of variation were least. Poorest agreement and greatest scatter were shown by the amylase data, in activity u/cc or total nu/80 min/kg. Volume data in cc/80 min or cc/80 min/kg were intermediate. The following norms were established for future performance of this test with Secretin-Wyeth: maximum bicarbonate concentration, 92-124 mEq/l, total volume, 2.0-4.4 cc/80 min/kg, total amylase secretion, 8.0-20.4 u/80 min/kg. Bicarbonate and volume responses are more valuable than amylase determination, due partly to unknown pancreozymin content of different secretin preparations, and partly to alterations of vagal tone during the test. Nevertheless, amylase activity manifests sufficient statistical homogeneity to merit its inclusion in the study of pancreatic function.

Site of action of mercurial diuretics JOHN J. DIGGAN (by invitation) and ROBERT F. PRITS *Dept of Physiology, Syracuse Univ College of Medicine, Syracuse, N Y*. Two lines of evidence indicate that organic mercurial diuretics block

distal tubular absorption of sodium. 1) When saline is infused into normal dogs at such a rate that 800 to 1200 μ Eq of sodium are excreted per min, the intravenous administration of 60 mg Hg as mercurhydrin, increases sodium excretion to 2000 μ Eq/min. Doubling the dose to 120 mg Hg increases sodium excretion to 2400 to 2500 μ Eq/min. Quadrupling the dose to 240 mg does not increase it further. The administration of BAL returns excretion to the control range. It is apparent that mercurhydrin can block the absorption of a maximum of 1500 μ Eq of sodium per min, a quantity roughly equal to 15% of that absorbed during the control periods and after BAL. This finding is consonant with the view that 80 to 85% of the sodium which is absorbed is absorbed in the proximal tubule (not mercurial-sensitive), and 15 to 20% in the distal tubule (mercurial-sensitive). 2) The administration of 45 mg Hg as salyrgan to a normal dog increases sodium excretion some 800 μ Eq/min. The infusion of 500 mU of pitressin per hour increases excretion to a variable degree, i.e. from 200 to 500 μ Eq/min. The addition of salyrgan during pitressin naturesis increases sodium excretion only to the level attained with salyrgan alone. Apparently, pitressin and salyrgan block sodium absorption at the same site, presumably in the distal segment.

Effect of l-arterenol on the peripheral circulation in man D. DUNCANSON (by invitation), T. STEWART (by invitation) and O. G. EDHOLM *Univ of Western Ontario, Ontario, Canada*. Forearm and hand blood flow were measured together with blood pressure and a continuous recording of heart rate with a cardi tachometer. L-Arterenol HCl (nor-adrenaline) was injected intravenously with a constant infusion apparatus, at a rate of 2, 5 and 10 μ g/min for 5 minutes in each instance. The forearm blood flow showed no change with 2 μ g/min but with 5 or 10 μ g/min the blood flow showed a slight reduction in flow at a time when both systolic and diastolic blood pressure were raised. Hand blood flow showed a marked and consistent decrease with 5 or 10 μ g/min. In most cases 2 μ g/min had no definite effect. The heart rate slowed consistently with infusions at the rate of 5 or 10 μ g/min. In a few instances 2 μ g L-Arterenol also produced a definite slowing.

Relation of the adrenal and spleen in regulation of the leucocyte picture of the rat ABRAHAM DURY and HABEEB BACCHUS (by invitation) *Dept of Physiology, George Washington Univ School of Medicine, Washington, D C*. The relation of the adrenal and the spleen as a factor in the regulation of the total white cell count, and the absolute number of circulating lymphocytes, neutrophils, and eosinophiles was investigated by determining whether statistical differences existed in groups of rats before and after adrenalectomy, splenec-

tomy, adrenalectomy-splenectomy, and sham operations as regards these leucocytes. A similar procedure was followed in these categories of operated groups of rats to determine the effect of adrenaline on the components of the leucocyte picture. It was found that the total white cell count was significantly increased only following splenectomy or following adrenaline treatment of the adrenalectomized rat. None of the operative procedures altered the absolute number of lymphocytes, but these cells could be significantly depressed following adrenaline treatment only when both the adrenal and the spleen were in situ. The neutrophils were significantly increased following either adrenalectomy or splenectomy but were unaffected by adrenalectomy-splenectomy. Following adrenaline treatment, the absolute number of neutrophils was not significantly increased only in the splenectomized group of rats. The number of eosinophiles was found to exhibit great variability from group to group of normal rats, but the evidence was clear that both the adrenal and the spleen were required if a significant depression in the circulating eosinophiles were to follow adrenal treatment.

Arterial and venous potassium levels in man D P EARLE, S J FARBER (by invitation) and J D ALEXANDER (by invitation) *Dept of Medicine, New York Univ College of Medicine, New York City*. When proper precautions were observed the normal resting venous plasma potassium level in man was found to be approximately 0.5 mEq/l less than generally reported. Blood was drawn into oiled syringes, transferred to tubes containing dried heparin and centrifuged immediately. Results were not affected by the use of a tourniquet to assist in obtaining blood, but muscular activity in the extremity raised the plasma K level. Hemolysis and allowing the cells to remain in contact with the plasma more than 10 minutes caused an increase in the plasma K level. At rest, arterial and venous plasma usually contain the same amount of K although at times there was more in the venous sample. Muscular activity raised the plasma K acutely and venous plasma from the extremity contained more K than the arterial. During intravenous infusions of glucose, venous plasma also contained more K than arterial, a difference which persisted following insulin in spite of a fall in both venous and arterial plasma K levels.

Measurement of cold and warmth thresholds of subjects exposed to environments from 18°C to 38°C FRANKLIN G EBAUGH, JR and RUDOLF THAUER (introduced by EUGENE F Du Bois) *New York Hospital and the Depts of Medicine and Physiology, Cornell Univ Medical College, New York, N Y*. Using Hardy's radiation method, cold and warmth thresholds were measured on the

forehead and trunk of subjects exposed to environmental temperatures from 15°C to 38°C. Subjects were nude and to allow for adaptation remained for 2 to 3 hours at the same temperature and experimental conditions while measurements were made. The wall temperatures were not significantly different from the air temperatures. The cold thresholds remained at a mean value of $27 \cdot 10^{-5}$ cal/sec/cm² in air temperatures of 15°C to 25°C, but increased with the air temperature from 25°C until at 38°C the cold threshold ($67 \cdot 10^{-5}$ cal/sec/cm²) was more than twice that of the cooler environments. By contrast, the warmth thresholds remained at the mean value of $30 \cdot 10^{-5}$ cal/sec/cm² throughout the range of air temperatures studied. This rise in the cold thresholds could not be explained by a change in the skin temperatures because at temperatures below 25°C the cold threshold remained constant while the skin temperature gradually increased with the air temperature. However, the plot of room temperature against cold thresholds closely followed the curves of increasing peripheral blood flow with temperature published by Hardy and Soderstrom. Thus, it is possible that hyperemia of the skin increases the threshold of cold sensation but has no effect on the threshold of warmth sensation.

Effects of over and underperfusion upon coronary arterial blood flow R ECKEL (by invitation), R W ECKSTEIN, M STROUD (by invitation), and W H PRITCHARD (by invitation) *Western Reserve Univ, Dept of Medicine, Cleveland, Ohio*. If the size of the coronary vascular bed and its resistance to blood flow depends upon the oxygen supply and demand, it should be possible to show resistance changes when the vessels are perfused at various pressures with cardiac work constant. With a special perfusion apparatus the left descendens, or left circumflex or total left coronary arteries of anesthetized, heparinized, open-chest dogs were perfused with blood at various pressures which were suddenly changed. Aortic and perfusion pressures were measured with Gregg manometers and coronary inflow with a Shipley recording rotometer or a Gregg and Green differential flow manometer. In some cases, when the total left coronary artery was perfused, sinus outflow was also measured with a second rotometer. The resulting flows show a rapid marked increase in vascular resistance within a few seconds following perfusion at pressures above aortic. Likewise, when perfused at pressures below aortic, there is a marked sudden reduction in vascular resistance. Since these changes, although slightly delayed, occur also in coronary sinus outflow, it is believed that they represent automatic changes in vascular resistance which serve to meet the metabolic demands of the myocardium.

Response of coronary blood flow following stimulation of cardiac accelerator nerves R W ECKSTEIN, M STROUD (by invitation), CHARLES V

DOWLING (by invitation), ROBERT ECKEL (by invitation), and W H PRITCHARD (by invitation) *Western Reserve Univ, Dept of Medicine, Cleveland, Ohio* In the anesthetized, open chest and heparinized dog cardiac output, coronary artery inflow or coronary sinus outflow, and aortic blood pressure were measured before and after stimulation of the left cardiac accelerator nerves The blood pressure was recorded with a Gregg optical manometer The coronary flow was measured with a Shipley recording rotometer Cardiac output was measured with a large Shipley recording rotometer placed in the thoracic aorta prior to ligation of the major proximal aortic branches Cardiac output was varied and controlled by inflating a balloon in the left auricle Control and experimental A-V differences between arterial and coronary sinus blood were determined

Stimulation of the accelerator nerves produces an increase in vigor of contraction, cardiac output, cardiac work, coronary blood flow, and oxygen consumption Simultaneous nerve stimulation and inflation of the left auricular balloon to reduce external cardiac work to below the control value is likewise followed by increased vigor of contraction, increased coronary flow, and increased oxygen consumption Therefore it is suggested that the adrenaline-like substance released by nerve stimulation markedly increases myocardial O_2 consumption and reduces cardiac efficiency Although direct effect of nerve stimulation upon coronary vessels must be considered, we have no evidence of increases in coronary flow in the absence of rising O_2 consumption

Effects of x-radiation on water and electrolyte metabolism in the rat ABRAHAM EDELMANN *Biology Dept, Brookhaven National Lab, Upton, L I, N Y* The intakes of distilled water, 1% NaCl solution, and food and the excretion of urinary Na, K, Cl and water by male albino rats were determined daily previous to and following exposures to 100, 250, 500, 800 and 1000 R of x-rays Polydipsia and polyuria occurred during the first and fifth 24 hour period following radiation The urinary excretions of Na, K, and Cl were increased at these times Following doses of 500 R or more there is a relative and absolute decrease in intake of the salt solution This is a monophasic response reaching its minimum at about 3 days The salt solution intake returns to previous levels at 5 days After doses of 100 or 250 R, the 1% NaCl solution intake follows the biphasic curve of water intake Food intake decreased for 2 days following radiation and then returned to normal in about 2 days Body weight changes paralleled the food consumption

Responses to vagal stimulation during hyperthermia H E EDERSTROM (introduced by A B Hertzman) *Dept of Physiology, St Louis Univ School of Medicine, St Louis, Mo* Dogs under anes-

thesia were heated to the lethal point by means of lamps During the rise in body temperature the peripheral ends of the cut vagi were stimulated and electrocardiograms and optical recordings of blood pressure taken The period of cardiac arrest due to vagal stimulation was reduced from several seconds at normal temperatures to a fraction of a second when rectal temperatures approached 45°C The fall in systolic blood pressure induced by vagus stimulation was relatively smaller at high body temperatures 'Vagus escape' was observed at all temperatures, but at $44-45^\circ\text{C}$ the rate of escape beats was about 3 times greater than those occurring during the same stimulation at normal body temperatures Stronger stimuli gave similar results In EKG tracings escape beats were characterized by absence of P waves when the right vagus was stimulated Beats recorded during left vagus stimulation were more like the normal pattern Bizarre and irregular QRST complexes were common in beats occurring during stimulation of the vagi at any temperature

Effect of haemorrhage on forearm blood flow and heart rate in man O G EDHOLM *Dept of Physiology, Univ of Western Ontario, London, Ontario, Canada* Sixteen normal subjects were bled from 250-1300 cc The withdrawn blood was retransfused approximately 4 hours later Forearm blood flow was measured with a plethysmograph, and graphic records were obtained throughout the experiments with a cardi tachometer During the period of haemorrhage, which lasted up to 30 minutes, there was no evidence of a diminished forearm blood flow except in one case whose control flows were high In the 7 subjects who fainted, the usual vaso-dilatation occurred during the faint Following the haemorrhage, in 9 non-fainters the forearm blood flow remained close to the control level for periods up to 4 hours In 3 subjects, the blood flow diminished significantly below control values, but only during a period when the mean blood pressure was reduced In the 6 subjects who fainted, the forearm blood flow remained at substantially control levels in 4 subjects In 2 subjects the mean blood pressure was reduced throughout the period of the experiment and the forearm flow was likewise diminished It was considered that these experiments indicated that the muscle blood vessels do not constrict to a significant extent after haemorrhage, and that there is no marked difference in this respect between fainters and non-fainters

Effects of certain physical factors on the in vitro gastric secretion of the dog CAROLAN TROWBRIDGE EDWARDS and LESLIE E EDWARDS (introduced by ROBERT W RAMSEY) *Dept of Physiology and Pharmacology, Medical College of Virginia, Richmond, Va* The method, previously reported, for in vitro gastric secretion has been improved and standardized, using the mucosa of the dog Dissec-

tion techniques and a method for pressure control are described by which it is possible to obtain more consistent data. It has been shown that secretion can take place in one direction only as reversal of the membrane results in complete failure of secretion. This method has been used to show the effects of temperature on the secretion rate.

Acid secretion by gastric mucosa of the bull frog in vitro LESLIE E. EDWARDS and CAROLYN TROWBRIDGE EDWARDS (introduced by ROBERT W. RAMSEY) *Dept. of Physiology, Medical College of Virginia, Richmond, Va.* Using the method for securing pure gastric secretion in vitro previously reported by the authors (*Federation Proc.*, 1948), acid secretion by the mucosa of the bull frog (*Rana catesbeiana*) has been obtained. pH values (as low as 1.5), free acid and total acid concentrations and titration curves are comparable to those for mammalian gastric juice. After an initial delay of about 1 hour, secretion with a pH of less than 2 can be obtained continuously for approximately 3 hours. Subsequently the pH gradually rises while the volume of secretion increases. Total chloride determinations indicate the presence of neutral chlorides as well as HCl.

Comparison of intracardiac and intravascular temperatures with rectal temperature LUDWIG W. EICHNA, ADOLPH R. BERGER and BERTHA RADER (introduced by DAVID P. EARLE, JR.) *Dept. of Medicine, New York Univ. College of Medicine, New York, N. Y.* In a study of the thermal gradients and deep tissue temperatures of 12 afebrile patients, thermocouples were introduced into the rectum, into the femoral artery and vein, and, by catheterization, into the right heart, pulmonary artery, superior and inferior venae cavae and the hepatic, subclavian and internal jugular veins. The data are presented as differences between the temperature of each intravascular site and the rectal temperature. Within the pulmonary artery, right ventricle, right atrium and femoral artery the temperatures usually were identical and averaged slightly less (0.2°C) than the rectal temperature. This difference, though small, was consistent and in 10 of 12 comparisons the intravascular values fell below the rectal values by 0.13°C to 0.34°C . Within the superior vena cava the temperature was lower than in the right heart, and in the subclavian vein its average value was 0.48°C below rectal temperature. Within the inferior vena cava the temperatures were variable and their average approximated closely that of the right heart. The temperatures deep in the hepatic vein and high in the internal jugular vein were considerably above the values in the veins into which these vessels drain and these hepatic and internal jugular vein temperatures equalled the rectal temperature. The data demonstrate the thermal gradients within parts of the vascular tree, indicate sites (liver, brain) of in-

creased heat formation at rest, and suggest that femoral arterial temperature is perhaps a more desirable index of 'mixed' deep tissue temperature than the usually accepted rectal temperature.

Photo-electric measurements of the arterial O_2 saturation dynamics in man for study of cardiopulmonary function J. O. ELAM (by invitation), J. L. EHRENHAFT (by invitation), W. N. ELAM, JR. (by invitation), and H. L. WHITE *Laby of Applied Thoracic Physiology (Surgery) and Dept. of Physiology, Washington Univ. School of Medicine, St. Louis, Mo.* Evidence favoring the use of histamine iontophoresis of the ear lobe for oximetry technique suggested a reevaluation of the saturation responses currently employed in the assay of cardiopulmonary function. Observations were made with the ear spectrophotometer and several experimental oximeters on the histamine flushed ear lobe and the heated pinna of normal subjects. The lung-to-ear circulation time measured from the inspiration of helium to the initial saturation decrease averaged 4.1 seconds when the histaminized lobe was used and 5.2 seconds when the heated pinna was used. Ear-to-ear circulation time measured from the initial saturation decrease with a single inspiration of helium to the secondary decrease after recirculation averaged 2.1 seconds with the histaminized lobe and 2.4 seconds with the heated pinna or lobe. The rate of saturation increase when subjects changed from air to oxygen was found to be significantly greater when the histaminized lobe was employed. In these measurements the period required for maximal saturation increase (saturation time) ranged from 15 to 30 seconds when determined from the histaminized lobe depending upon the minute respiratory volume. Using the heated pinna the saturation times ranged from 73 to 148 seconds in agreement with the Millikan data reported by others. The histaminized lobe technique provided significant saturation deflections within 4 to 7 seconds when hyperventilation, breath holding, and single inspirations of oxygen or nitrogen (interspersed during quiet air breathing) were performed. The technique using the histaminized ear lobe affords information more representative of arterial dynamics than does the conventional Millikan procedure.

Blood coagulation studies in relation to menstruation and menorrhagia RICHARD M. ELGHAMMER (by invitation), BURTON J. GROSSMAN (by invitation), PETER V. MOULDER (by invitation), ARTHUR K. KOFF (by invitation) and J. GARROTT ALLEN *Dept. of Surgery, Univ. of Chicago, Chicago, Ill.* A series of 183 coagulation studies were made on 52 women during their menstrual cycles. Sixteen women were considered normal in reference to menstruation, and 36 suffered from menorrhagia without apparent cause. The studies included the whole blood clotting time, prothrombin time, protamine

titration, platelet count, and observation for gross evidence of lysis. In the menorrhagic group, during menstruation, 82% showed a mild to moderate thrombocytopenia (29% with less than 100,000/mm³), 35 of 36 showed a prolonged whole blood clotting time, and 35 of 36 had an increased protamine titration. The intermenstrual studies on these women showed thrombocytopenia in 46% (8% below 100,000), 15 of the 36 had a prolonged whole blood clotting time, and 14 of 36 had an increased protamine titration. All patients had prothrombin values of 80% or better except two who had 72% and 76%. During menstruation 46% of the normal controls showed a mild thrombocytopenia. Nine showed slightly prolonged clotting times, and 5 of 16 had increased protamine titrations. Between menstrual periods 21% of the controls had mild thrombocytopenia. Six of the 16 had increased whole blood clotting times and 5 had abnormal protamine titrations. Evaluation of the extent of bleeding was made from careful clinical histories. Menstrual pads were not collected and analyzed for hemoglobin content.

Blood changes associated with cold diuresis
JOHAN W. ELIOT, RICHARD A. BADER and DAVID E. BASS (introduced by H. S. BELDING) *Quartermaster Corps, Climatic Research Lab., Lawrence, Mass.* Blood changes associated with cold diuresis were studied in conjunction with experiments on the renal and hormonal mechanisms of cold diuresis. The order of successive 2½-hour exposures to 27°C and to 15°C was alternated between men and in each man from day to day. In 23 experiments plasma proteins rose an average of 0.5 gm/100 cc, and the hematocrit rose 2.9% in the cold, in 12 experiments the corrected erythrocyte sedimentation rate rose 76% in the cold. In the experiments in which a warm exposure followed the cold these values fell an average of 0.5 gm/100 cc, 1.8% and 41% respectively. In 18 experiments on 5 men, the average increase in urine output in the cold over output in the warm was 287 cc, which corresponded to an accompanying average plasma loss of 277 cc as calculated from increases in plasma proteins and hematocrit and an estimated plasma volume of 50 cc/kg. However, a series of 15 experiments in which cold diuresis was largely or completely abolished by small doses of pitressin, but plasma proteins rose 0.4 gm/100 cc and hematocrit rose 2.6%, indicates that there is no direct connection between changes in urine output and plasma volume in the cold. Further indication is supplied by considerable individual variations, well outside the error of the methods, between increased urine output and calculated plasma volume loss. Plasma chloride levels showed no significant change during cold diuresis, despite the fact that in 11 experiments average total chloride output in the cold was 2330 mg, compared to 1279 mg in the warm.

Non-utilization of mannitol in uremic patients
J. RUSSELL ELKINTON *Dept. of Internal Medicine, Yale Univ. School of Medicine, New Haven, Conn.* Although 100% recoveries of mannitol in urine have been reported (Elkinton, *J. Clin. Invest.* 26: 1088, 1947; Clark and Barker, *Proc. Soc. Exp. Biol. & Med.* 69: 152, 1948), evidence has been presented for utilization or clearance from plasma by non-renal excretory routes (Berger *et al.*, *Proc. Soc. Exp. Biol. & Med.* 66: 62, 1947; Dominguez *et al.*, *J. Lab. Clin. Med.* 32: 1192, 1947). In 4 uremic patients given 25-45 gm of mannitol intravenously, little evidence of mannitol utilization was found for periods of 6 hours to 3 days. Mannitol utilized was calculated as the difference between the mannitol retained and the product of the conc. in serum water \times the original mannitol space corrected for changes in chloride conc. and balance. In patients 2 and 4 (anuric) utilization was less than the analytical error, and in patients 1 and 3 was less than 12% of the non-renal excretory clearance rate of 21 cc/min. calculated by Dominguez *et al.* Masking of utilization by an identical rise in serum blank, or failure of diseased kidneys to metabolize mannitol are alternative explanations.

PATIENT	MANNITOL				
	Time	Conc. Serum H ₂ O	Re-tained	Calc. in ECF	Utilized
	hr	mg %	gm	gm	gm
1	3	128.0			
	6.5	118.4	25.16	24.30	0.86
1	4	78.1			
	6.2	77.2	24.90	24.86	0.04
3	4	83.2			
	23	77.7	24.96	22.38	2.58
4	4	55.0			
	60	52.2	25.36	25.84	-0.48

Alterations in reflex muscular contractions induced by positive intrapulmonic pressure
LEONARD H. ELWELL (by invitation) and JOHAN W. BEAN *Dept. of Physiology, Univ. of Michigan, Ann Arbor, Mich.* The knee-jerks of young adult men, elicited by constant stimulation at 4-second intervals by a Lombard hammer, were recorded kymographically, together with spirometer tracing, thoracic and abdominal movements, and changes in blood oxygenation as determined by oximeter applied to the ear. Positive intrapulmonic pressure (20 cm H₂O) was applied through a rebreathing tank filled with room air and provided with soda lime canister and a pressure spirometer. Positive pressure was maintained for periods of about 3 to 7 minutes duration. Gentle application of pressure caused an immediate augmentation of the knee-jerk which persisted throughout the period of increased pressure, in several experiments the knee-jerk became

pendular in character. Respiratory minute volume was increased by a deepening and slowing of breathing, in some subjects frequency as well as depth was increased. The thorax was expanded in both its inspiratory and expiratory positions, in some cases the maximum inspiratory position was attained after several respiratory cycles rather than immediately. Thoracic respiratory movements were augmented. Changes in the abdominal region were in general similar to, but much less pronounced than, those of the thorax, except for a few cases where the abdominal circumference decreased rather than increased with positive pressure application. Changes in oximeter readings were so small as to be considered of little or no significance as causative factors in the reflex changes. All these effects were rapidly reversed on return to normal intrapulmonic pressure.

Separation of natural estrogens by counter-current distribution. LEWIS L. ENGEL (by invitation), WILSON R. SLAUNWHITE, JR. (by invitation), PRISCILLA CARTER (by invitation) and IRA T. NATHANSON. *Medical Labs. of the Collis P. Huntington Memorial Hospital of Harvard Univ. at the Massachusetts General Hospital, and Dept. of Biological Chemistry, Harvard Medical School, Boston, Mass.* Separation of the naturally occurring estrogens and in particular the separation of estrone from estradiol are difficult procedures. The development of the countercurrent distribution technique for the separation of closely related substances promised to aid in the simplification of this problem. Solvent systems consisting of ternary and quarternary mixtures were found in which a satisfactory spread of the partition coefficients of estrone, estradiol and estriol may be obtained. This makes possible a separation of the three urinary estrogens using a 24-plate distribution in the Craig countercurrent distribution apparatus. Other solvent systems were devised in which the partition coefficient of any one of the three compounds was nearly unity, thus placing the material in the optimum region for the detection of impurities. The partition coefficients of the three estrogens have been measured in the solvent systems employed in the isolation of the phenolic fraction from acid hydrolyzed urine. For these studies a fluorometric method based upon the procedures of Jailer and Bates has been used. With a Coleman Model 12-A Electronic Photofluorometer and a filter system consisting of a 436 m μ incident light filter and a 525 m μ interference filter for the emitted light, a full scale deflection of the instrument is obtained with 0.26 μ g of estrone, 0.36 μ g of estriol and 0.50 μ g of estradiol. The reaction is carried out by heating the estrogen in 1 ml of 90% sulfuric acid and diluting with 65% sulfuric acid. An accuracy of $\pm 10\%$ is obtained.

The hepatic venous circulation with special ref-

erence to the sphincter mechanism. H. E. ESSEX and W. D. THOMAS (by invitation). *The Mayo Foundation, Rochester, Minn.* The existence of a sphincteric or throttle mechanism in the hepatic circulation of the dog has long been recognized. Mautner and Pick believed the mechanism resided in the smaller hepatic veins. Bauer, Dale et al. held that it consisted of muscle rings in the hepatic veins near their confluence with the vena cava. The latter view has gained wide acceptance. A series of experiments made in this laboratory by Grana, Essex and Mann has shown that intact dogs are killed by intravenous doses of extracts of *Ascaris suum* that have little if any effect on a hepatectomized dog. It was also shown that the flow of blood through an isolated liver was arrested or markedly reduced by injections of such extracts into the portal vein. Casts made by injections of vinyl acetate into the hepatic blood vessels have shown that intravenous injections of extracts of *Ascaris*, histamine, digitoxin, horse serum into sensitized animals (anaphylactic shock) and occlusion of the hepatic artery for two hours cause intense spasm of the hepatic veins giving a cork-screw or spiral appearance, particularly to the casts of the smaller vessels.

In a series of exploratory experiments similar spasm of the hepatic veins has been produced in some but not all the cats injected and also in white rats. The data are insufficient to warrant drawing a final conclusion concerning this question except in the dog. In contrast with what occurred in the hepatic venous system the portal venous system shows marked engorgement.

Cholinergic nature of the vestibular receptor mechanism forced circling movements. C. F. ESSIG (by invitation), J. L. HAMPSON (by invitation), P. D. BALES (by invitation) and H. E. HIMWICH. *Medical Division, Army Chemical Center, Maryland.* Freedman and Himwich observed that the intracarotid injection of di-isopropyl fluorophosphate (DFP) produced righting movements in rabbits; the head turned away from the injected side and forced circling movements also in an adverse direction ensued while the homolateral pupil became miotic. Subsequent work revealed that the intracarotid injection of appropriate doses of freshly prepared DFP in normal saline under local procaine anesthesia, produced similar results in other species: guinea pigs, dogs, cats, and monkeys. Preliminary experiments indicate that the internal ear must be intact for the altered head posture and forced circling to occur; for labyrinthectomized rabbits failed to show the characteristic response following the injection of DFP on the labyrinthectomized side. A rabbit with unilateral section of the eighth nerve and a similarly prepared cat failed to exhibit the characteristic responses to DFP. Extirpation of the cerebral cortex on the in-

jected side did not prevent the syndrome if the vestibular apparatus was intact. The mechanism of vestibular stimulation is indicated by the drug used to produce the turning seizures for DFP is a powerful anticholinesterase agent which facilitates the accumulation of acetylcholine. Atropine and scopolamine which shield the organism from an excess of acetylcholine corrected the abnormal posture and stopped the forced circling movements.

Experimental production of femoral fractures

F. GAYNOR EVANS, HERBERT E. PEDERSEN, JOHN F. HAYES, and HERBERT R. LISSNER (introduced by M. MASON GUEST) *Dept. of Anatomy and Engineering Mechanics, Wayne University, Detroit, Mich.* Sixteen experiments were performed on 13 femora of white males and 3 of Negro females ranging in age from 45 to 85 years. 'Stresscoat' deformation patterns, with a lacquer sensitivity of 0.00125 inches per inch, and fractures resulted from static torsion loading, loading of the greater trochanter and cross-bending loading of the center of the shaft. Torsion loading produced spiral fractures of the shaft, cross-bending loading gave transverse comminuted fractures of the shaft, loading of the greater trochanter resulted in intertrochanteric, transverse neck, subcapital and abduction fractures of the neck and trochanteric region. In trochanteric loading bones from females fractured at 548.3 lb (average), males at 885.6 lb (average). Elevating the trochanter increased the load supported without fracture because the effective bending force on the shaft is decreased and the direct compressive force increased. Under similar test conditions in our small series, there was an inverse relation between load producing failure and age of the individual from whom the bones were obtained. Loads producing failure with torsion and cross-bending loading were approximately equal in magnitude and considerably less than those producing fracture when the greater trochanter was loaded. All fractures closely resemble similar types seen clinically. 'Stresscoat' deformation patterns indicated that all types, with the possible exception of the rare abduction fracture, resulted from failure of the bone under tensile stress.

In vitro metabolism of bone marrow in Ringer-bicarbonate medium containing no glucose. JOHN D. EVANS and ROBERT M. BIRD (introduced by EUGENE F. DuBOIS) *Dept. of Physiology, Cornell Univ. Medical College, New York, N. Y.* The aerobic metabolism of rabbit bone marrow has been studied *in vitro* at pH 7.3 in Ringer-bicarbonate media containing no glucose. The rate of oxygen consumption under these conditions is 3.6 microliters/mg of cell protein per hour. This is essentially the same rate as observed when glucose has been added to the system. Total aerobic acid production is small averaging 0.4 microliters/mg/hour. In the absence of glucose, small amounts of lactic

acid appear to be consumed, averaging 1.0 micrograms/mg/hour. The R.Q. averages 0.84 as compared with one of 0.95 obtained in the same system to which glucose has been added. The amount of total reducing substances in marrow under our experimental conditions is small. Its utilization averages 1.1 micrograms/mg/hour. Analyses for total amino acids and non-protein nitrogen give no evidence for protein breakdown. From these data it appears clear that carbohydrate and protein breakdown are not sufficient to account for the oxygen consumption of marrow in a medium containing no glucose. It is inferred that the breakdown of fatty substances is primarily responsible for this oxidation.

Lymph flow during edema formation. GEORGE FAHR and HERMAN KOSCHNITZKE *Minneapolis Gen. Hospital, Minneapolis, Minn.* The rate of lymph flow is a very important factor in reducing the rate of accumulation of edema fluid when ultrafiltration of plasma fluid into the interstitial spaces is accelerated. One of the three large lymph vessels near the ankle of a dog was cannulated and connected to the barrel of a tuberculin syringe before plasmapheresis. After plasmapheresis had been carried on for a period of 12 hours the colloid osmotic pressure was 5.8 mm Hg. The colloid osmotic pressure of the lymph collected from the cannula was 3.7 mm Hg. Before plasmapheresis it was only possible to get a lymph flow out of our cannula at the rate of 1 cc. in 9 minutes and 25 seconds when passive motion of the limb was carried out. Lymph flow after plasmapheresis was spontaneous and the rate was 1.5 cc. in 15 minutes. As there were 3 large lymph vessels the total flow of lymph from the foot of the dog was 0.3 cc. per minute. The weight of the foot from the point of cannulation down was 246 gm. or 1.2% of body weight. If we assume that the mean filtration pressure in the capillaries of the dog during these experiments was 22 mm Hg. and at the same time assume that the tissue pressure under these conditions of edema formation was 5 mm Hg. and then make use of Pappenheimer's filtration constant (Pappenheimer and Soto-Rivera, *Am. J. Physiol.* 152: 480, 1948) we calculate that 5 cc. of fluid would have been filtered per minute in the hind legs of a cat whereas we find 3 cc. lymph flow.

Resuscitation from fresh water drowning. DAVID C. FAIRER (by invitation), CLEMENT G. MARTIN (by invitation), HENRY SCHWARTZ, and A. C. ILLI *Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago, Ill.* Studies on fresh water drowning have been made on dogs. The first stage lasting 68 seconds (mean), is characterized by struggling. The second stage, lasting 56 seconds (mean) is characterized by apnea or irregular respiratory movements. The third stage starting 124 seconds (mean) after submersion is ushered in by a precipitous fall in blood pressure which levels off

and reaches zero 259 seconds (mean) after submersion. No dogs survived after the precipitous drop in blood pressure occurred. Using an endpoint of 4 to 10 seconds after the cessation of struggling, no significant difference in survival was noted in dogs with a cannulated femoral artery if they received no resuscitation (survived 10, died 11), manual artificial respiration (survived 12, died 12) or artificial respiration with a mechanical alternating positive and negative pressure resuscitator (survived 12, died 8). In dogs without femoral artery cannulation, no significant difference in survival was noted if they received no resuscitation without postural drainage (survived 9, died 3), no resuscitation with postural drainage (survived 10, died 2) or artificial respiration with the alternating positive and negative pressure resuscitator (survived 4, died 2). Regardless of the measures employed, dogs having little or no staining of the lungs with methylene blue (added to the water used in drowning) survived, while those having dense staining died. About one-half of those dogs with moderate staining died. We interpret these results as indicating that mechanisms other than simple asphyxia play a role in drowning.

Oximeter measurements in pulmonary and cardiac disease J K W FERGUSON, D M FINLAYSON and I M HILLIARD *Dept of Pharmacology, Univ of Toronto, and the Toronto Western Hospital, Toronto, Canada*. Three measurements were investigated: 1) the difference in oximeter readings breathing air and breathing oxygen (A-O diff), 2) the time for the oximeter reading to change $\frac{1}{2}$ way to its final reading on changing from oxygen to air ($\frac{1}{2}$ R time), 3) the effect of exercise on the oximeter reading. The following groups of subjects were examined: a) 41 normals, b) 7 congenital heart cases, and then a group of miscellaneous pulmonary affections excluding tuberculosis and detectable cardiac disease, subdivided as follows: c) 20 lobectomies and pneumonectomies, d) 28 others showing no fall in O_2 saturation (less than 1%) on exercise, e) 27 others showing more than 1% fall in O_2 saturation on exercise. Group c included the most dyspneic. Out of 20 in group c, 12 had a fall in O_2 saturation on exercise. Average values for A-O diff \pm S.E. were as follows for each group: a) 4.0 ± 0.17 , b) 4.9 ± 0.24 , c) 3.17 ± 0.29 , d) 3.86 ± 0.24 , e) 4.94 ± 0.38 . For $\frac{1}{2}$ R time (in minutes) values were: a) 1.7 ± 0.07 , b) 0.9 ± 0.04 , c) 1.88 ± 0.22 , d) 2.82 ± 0.28 , e) 3.31 ± 0.23 . Group b had the greatest fall in O_2 saturation on exercise (8-22%, av 13.3%). Group c illustrates well the difference between pulmonary reserve and pulmonary 'efficiency'. Reserve is low, particularly in those who show a fall in O_2 saturation on exercise, while 'efficiency' is high in the same group, as shown by the low A-O diff and short $\frac{1}{2}$ R time.

Significance of activation of prothrombin in

serum JOHN H FERGUSON *Dept of Physiology, Univ of North Carolina, Chapel Hill, N C*. In spontaneous clotting of blood a variable and often considerable amount of prothrombin escapes conversion and persists, unlike the activated thrombin which is lost by the antithrombic mechanisms. In the presence of the suboptimal calcium content of serum, successive additions of optimal thromboplastin (brain extract) each activate some residual prothrombin to thrombin, which gradually disappears. The incomplete activations are proved by the repeated enhancement of coagulant activity, without exhausting the prothrombin even after 6 or more thromboplastin additions. However, if optimal Ca is added, complete exhaustion of prothrombin occurs after only 2 or 3 thromboplastin treatments. That more than one thromboplastin addition was found necessary suggests some 'antithromboplastic' activity of serum. The substrate for measuring thrombin activity in the above experiments was tested prothrombin-free ($BaSO_4$ -adsorbed fibrinogen). If a prothrombin-containing fibrinogen or plasma was used as substrate, an additional thrombin formation occurred which could be fully accounted for (as shown by control tests, minus serum) by transfer of Ca and thromboplastin in the serum activation mixture. Tests with suboptimal (1.2 mM) $CaCl_2$ were particularly significant since the extra thrombin from the substrate, in this case, was minimal. $BaSO_4$ -adsorbed serum (prothrombin-free) yielded no thrombin on treatment with Ca and thromboplastin (tests on prothrombin-free fibrinogen). With its natural suboptimal Ca and optimal added thromboplastin, this serum had no influence on the weak thrombin formation in prothrombin-containing substrates.

Tissue respiration in the polar cod (*Boreogadus saida*) as a function of temperature JOHN FIELD and C N PEISS (by invitation) *O N R Arctic Research Lab, Point Barrow, Alaska and Dept of Physiology, Stanford Univ, Calif*. The polar cod found off the north coast of Alaska is active at environmental temperatures as low as $-1.5^\circ C$. At such temperatures many temperate zone poikilotherms are in cold narcosis. The present study was designed to throw some light on the metabolic factors which must underlie the capacity for functional activity at these very low temperatures. Brain and liver were removed from over 100 polar cod of similar body size by the moist cold box technique. Measurements of the Q_{O_2} of whole brain mince and of liver slices were made at 0° , 5° , 10° , 15° , 20° and $25^\circ C$ by the Warburg method. For each organ the log Q_{O_2} -Centigrade temperature curve was approximately rectilinear over the range tested. Moreover the slopes of the curves were nearly the same. Comparison of the log Q_{O_2} -Centigrade temperature curve for polar cod brain with a similar curve for black bass brain mince (*Huro*

salmoides, habitat temperature about 18°C) established the following differences between these arctic and temperate zone forms 1) At each temperature the Q_{O_2} of polar cod brain was several times greater than that of black bass brain 2) The log Q_{O_2} -Centigrade temperature curve for bass brain showed a change in slope at 10°C, below this the slope was steeper (increase in Q_{10}), whereas the corresponding curve for polar cod had a constant slope from 0° to 25°C Both these differences as exhibited in the polar cod brain curve are advantageous in arctic adaptation

Blood production and iron reserves in man
STUART FINCH, DONALD HASKINS and CLEMENT A FINCH (introduced by M O LEE) *Dept of Medicine, Harvard Medical School, the Medical Clinic, Peter Bent Brigham Hospital, Boston, Mass* Five normal subjects were phlebotomized at weekly intervals During 7 weeks of bleeding, between 1 and 1½ gm of iron were removed from each subject About 800 mg of iron were mobilized for hemoglobin production during this time Measurements were made of the rate of blood production and of the various hematological changes during the period of bleeding and the period of recovery Available iron would appear to be the limiting factor in hematopoiesis Patients with large iron stores do not develop the degree of anemia shown by normal subjects However, previous prolonged iron feeding in 2 of the subjects had no effect on their ability to tolerate phlebotomy In those subjects who were allowed to spontaneously recover, serum iron values had not returned to normal in a period of 6 months These studies provide information regarding the rate of hematopoiesis in normal subjects under stress of phlebotomy, of the amount of available iron and of the time necessary to replenish the depleted iron stores

An anti-coagulant present in testicular extracts
S FISCH and E J TOWBIN (introduced by E F ADOLPH) *Dept of Physiology, Univ of Rochester, Rochester, N Y* Some crude hyaluronidase commercial preparations from bull testes were found to inhibit blood coagulation However, the anti-coagulant is not hyaluronidase but a non-dialyzable material precipitated by various concentrations of ammonium sulfate It is inactivated by heating at 65°C for 10 minutes The most potent preparation yet obtained, which has a very low hyaluronidase content, has an activity such that 5 mg of the dialyzed and dried precipitate will produce a 13-fold increase in clotting time of 1 ml of recalcified citrated human plasma or freshly drawn human blood The anti-coagulant is not heparin, for titration with salmine over a wide range of concentrations has no effect Moreover, the effects of these substances, in themselves anti-coagulants, are additive to those of the testicular substance It does not act by binding calcium It does not appear to

be fibrinogenolytic nor fibrinolytic It is neither anti-thrombic nor anti-prothrombic, these conclusions lending further support to the contention that it is not heparin It appears to delay clotting by acting as an antithromboplastic agent Only one other such agent has been described (Tocantins)

Electrophoretic study of the water soluble proteins of normal and atrophied muscles
E FISCHER, N J COPENHAVER (by invitation), B GALESKI (by invitation), and K W RYLAND (by invitation) *Baruch Center of Physical Medicine, Medical College of Virginia, Richmond, Va* Electrophoresis of the water-soluble proteins of normal gastrocnemii (rabbit) reveals 5 main fractions, of which 4 could be tentatively identified as myogen (43.6%), myosin β (24.3%), myosin α (15.1%), and myoalbumin (9.6%) The migration velocity at pH 7.5 increases in the above order, and the small unidentified fraction has a velocity between that of myosin α and myoalbumin During denervation atrophy, myoalbumin increases from the beginning, while myogen and the myosin fractions diminish considerably only after a delay of 10 to 12 days After 3 weeks of denervation, the myogen fraction and the myosin fractions are nearly halved, while myoalbumin is increased three to four times After tenotomy, myogen decreases and myoalbumin increases within 5 to 6 days as much as after 2 weeks of denervation Simultaneous denervation and tenotomy changes the electrophoretic pattern considerably less than simple denervation or tenotomy Enzymatic studies of the extracts confirmed that the combined operations damage the muscle less than the single operations

Sensibility of man to light anoxia
P E FISET (by invitation) and L P DUGAL *Dept d'Acclimation, Institut de Biologie Humaine, Univ Laval, Québec, Canada* Standard and objective psychological tests (concerning mechanical ability, space relations ability and survey of object visualization) were used in order to establish a difference in susceptibility to light anoxia (10,000 ft, 4 hours a week, 14 weeks) between 2 groups of human subjects receiving respectively, by demand valve system, oxygen and compressed air The results show that the progress in performance from the 1st to the 2nd trial is significantly better for the group receiving oxygen with all the tests used Confirmation using the same tests, and others, in the same conditions, has been obtained with 20 other human subjects

Production of constrictive pericarditis in dogs
A P FISHMAN, L H RUBENSTEIN, L W SENNETT, K KURAMOTO (introduced by L N KATZ) *Cardiovascular Dept, Medical Research Institute, Michael Reese Hospital, Chicago, Ill* Incident to the investigation of the cardiodynamics in congestive heart failure in dogs, it became necessary to establish a consistently effective method for the production of

constrictive pericarditis It was found that an envelope of polythene placed around the surface of the heart resulted in a rapidly progressive constrictive pericarditis with effusion Within 2 weeks neither coronary vessels nor auriculoventricular grooves could be identified The surface markings of the heart were completely obliterated Within 4 to 6 weeks, distended neck veins, hydrothorax, pulmonary congestion, hepatomegaly, ascites and peripheral edema uniformly ensued The predominance of any individual back-pressure manifestation varied with the site of intra-cardiac obstruction The polythene did not enter into the inflammatory reaction and could be readily removed from between the thickened, scarred pericardial surfaces If the polythene was not removed, the dogs died of constrictive pericarditis in 4 to 6 weeks An attempt was also made to induce pericarditis in the intact animal Thorotrast, injected under fluoroscopic vision, was used to outline the pericardial sac Sodium morrhuate was then injected intra-pericardially A sero-fibrinous pericarditis, without constriction, resulted The use of polythene provides a simple effective method for the production of a rapidly progressive constrictive pericarditis in dogs

Enzymes in the grasshopper egg LAURENCE ROCKWELL FITZGERALD (introduced by J H BODINE) *Zoological Labys, State Univ of Iowa, Iowa City, Iowa* The alkaline phosphatase of the developing egg of the grasshopper, *Melanoplus differentialis*, is shown to be apparently formed by the serosa and localised in the extra-embryonic fluid In comparing this with other enzymes studied during the development of this same material, it is found that the enzymes of the grasshopper egg fall into 3 groups a) Enzymes apparently of maternal origin, including methyl butyrase and tributyrinase These enzymes decrease in amount (or total activity) during development b) Enzymes which develop principally in the embryo This group includes cytochrome oxidase and cholinesterase c) Enzymes which appear during development, and which are found in extra-embryonic materials This group includes alkaline phosphatase, tyrosinase, and catalase The members of this last group are either undetectable, or barely detectable, in the embryo during development, but are swallowed, along with the extra-embryonic fluid just as the hatching process begins The failure to detect these enzymes in the embryo does not necessarily mean that they play no part in development, as Haldane has shown, using data on saccharase, that a relatively small number of enzyme molecules may be sufficient for the normal functioning of a cell

A small plastic tubing technique for right and left heart catheterization H F FITZPATRICK, T G SCHNABEL, JR, and L H PETERSON (introduced

by H C BAZETT) *Dept of Physiology, Univ of Pennsylvania Medical School, Philadelphia, Penna* By the use of small plastic tubing, cardiac catheterization can be accomplished by a simplified procedure without skin incision and without apparent risk of venous thrombosis In dogs, a routine venipuncture of the right external jugular is done with a special 18-gauge needle the lumen of which permits the passage of a small plastic catheter The needle then is withdrawn As the catheter is advanced slowly toward the heart, continuous ink recordings of venous pressure gradients can be obtained with a capacitance manometer Without fluoroscopic guidance satisfactory measurements of right auricular and ventricular pressures may be made From a manifold of 3-way stopcocks, blood samples for analyses can be withdrawn at a rate of 1 ml or more per minute Left ventricular pressures in dogs can be obtained by an open method, using a long, 18-gauge, olive tipped needle which is introduced, via the common carotid artery, to a level 1 inch above the aortic valve Through this needle, a small plastic catheter is advanced beyond valve and into left ventricle The needle is then withdrawn The characteristics of this relatively short and very small plastic tubing permits reliable recordings of pressure waves which probably are not inferior to those obtainable by the much larger and longer catheters commonly used This method of right heart catheterization is being evaluated on man with the purpose of determining cardiac output Simultaneous electrocardiograms are made

Thiocyanate and mannitol space and native ionic balance in acute adrenal insufficiency JAMES B FLANAGAN (by invitation) and R R OVERMAN *Division of Physiology, Univ of Tennessee College of Medicine, Memphis, Tenn* It has been suggested that the urinary loss of Na and Cl in untreated adrenalectomized dogs is insufficient to account for the total reduction of these ions in extracellular fluid Similarly, it has been proposed that urinary water loss in this condition is insufficient to explain the apparent extracellular dehydration Simultaneous measurement of the volume of fluid available for the dilution of NaSCN and mannitol combined with flame photometric analyses of Na and K and chemical analysis of Cl in plasma and erythrocytes makes possible the calculation of the total amount of 'extracellular' Na and Cl in 1) normal dogs, 2) dogs in acute adrenal insufficiency, and 3) adrenalectomized dogs maintained on DCA Together with Na, Cl and water intake and output studies, it was calculated that on the average, the total 'extracellular' Na of adrenalectomized dogs maintained on DCA was 568 mm The average total 'extracellular' Na of dogs in acute adrenal insufficiency was 347 mm Thus the average total reduction in 'extracellular' Na was 221 mm Since the dogs showed an

average negative Na balance (intake minus output) of but 50 mm, an average of 171 mm of Na remains unaccounted for. Similarly the total 'extracellular' Cl averaged 433 mm in dogs maintained on DCA and but 273 mm in dogs in acute insufficiency. The difference between these figures is 160 mm. However, the dogs showed an average negative Cl balance of but 79 mm, thus leaving 81 mm of Cl unaccounted for.

Persistence of hypertension after removal of the causative ischemic kidney. JACK FLASHER (by invitation) and DOUGLAS R. DRURY, *Dept of Physiology, School of Medicine, Univ of Southern California, Los Angeles, Calif.* Persistent hypertension develops in the rabbit as a result of unilateral renal ischemia. If this ischemic kidney is removed after the hypertension has lasted some time, the blood pressure does not necessarily revert to normal, but may continue above normal for several weeks. A control group of normal rabbits subjected to unilateral nephrectomy did not show any significant change in blood pressure after the operation. The findings support the view that a hypertension that has lasted some time tends to be self-perpetuating, persisting for an appreciable time after the original cause has been removed.

Comparison of heptazone (4,4-diphenyl-6-morpholinoheptanone-3-hydrochloride) with other analgesic drugs. LARS FLATAKER (by invitation) and CHARLES A. WINTER, *Merck Institute for Therapeutic Research, Rahway, N. J.* Comparisons have been made between heptazone, dl-methadone, dl-isomethadone, and morphine, for analgesic activity, toxicity, analgesic tolerance, and side effects. Heptazone, orally or parenterally, proved to be about twice as potent as methadone in rats, though of relatively short duration, but only half as potent as methadone in dogs. Isomethadone gave less consistent results than the other drugs. The outstanding advantage of heptazone was its low acute toxicity. LD₅₀ in mice (subcutaneously) averaged 210 mg/kg, compared with 33 mg/kg for methadone. When dogs were dosed twice daily (heptazone 4 mg/kg, other drugs 2 mg/kg) analgesic tolerance appeared in about a month. Side effects, such as narcosis, sedation and general depression, were most marked with methadone and morphine, much less with heptazone, and least with isomethadone. Nausea and vomiting occurred frequently in non-tolerant dogs given morphine, occasionally in methadone-treated dogs, but was never observed after heptazone or isomethadone. After analgesic tolerance was established, dose for all drugs was doubled. The increased dose was well tolerated by all dogs except those receiving methadone. Dosing was continued for 4 months, the drugs withdrawn for 1 month, then a second course of injections was given. After analgesic tolerance was

established the second time, cross-tolerance studies were made. With the doses used, dogs tolerant to morphine were also tolerant to heptazone and isomethadone, but not to methadone. Those tolerant to methadone or isomethadone were tolerant to all the other drugs. Dogs tolerant to heptazone were tolerant to morphine and isomethadone, but only partially tolerant to methadone.

Fractional conditioning of behavior based on electrically induced convulsions. STEPHEN FLECK (by invitation) and W. HORSLEY GANTT, *Pavlovian Lab., Phipps Psychiatric Clinic, Johns Hopkins Univ., Baltimore, Md.* Three dogs were subjected to electric convulsions through 2 cephalic jelly plate electrodes. Two auditory stimuli were given: one 15 seconds before shock (inhibitory), the other 5 seconds before shock (excitatory). Approximately 30 experiments/dog were made during 2 months. The dogs became increasingly resistant and negativistic to the experimental situation. Specifically there was fighting with other dogs, hostility towards the experimenters and increased motor activity outside the camera. Inside in connection with environmental stimuli the dogs manifested extreme motor activity with struggling in the hammock and running movements, at times indistinguishable from motor phenomena observed during and after convulsions. The animals disregarded injury and the movements seemed purposeless. Stimuli that brought on this behavior were 1) the experimenter leaving the camera and 2) the 'inhibitory' stimulus. The 'excitatory' stimulus caused no specific reaction except occasional orienting responses. The respiratory tracings often paralleled the motor excitement following stimuli 1) and 2) cited above, i.e. the tracings approached the pattern seen during convulsions. Occasionally jerking of the hind legs occurred after these stimuli. The negative reaction to the 'excitatory' stimulus with preservation of the reaction to the 'inhibitory' stimuli is evidence of amnesia for the 5-second pre-convulsive period—comparable to the retrograde amnesia in patients after shock and after head injuries. The conditioning of certain motor phenomena with failure to condition the total convulsive behavior demonstrates fractional conditioning, rather than complete duplication of the UR by the cr.

Studies on colchicine derivatives: toxicity and antimitotic effect. W. FLEISCHMAN, A. GOLDIN (by invitation), B. GOLDBERG (by invitation), L. G. ORTEGA (by invitation), A. D. BERGNER (by invitation) and G. E. ULLYOT (by invitation), *Medical Division, Army Chemical Center, Maryland, Dept of Preventive Medicine, Johns Hopkins Univ. School of Medicine, and the Smith, Kline and French Labs., Philadelphia, Penna.* Colchicine arrests mitosis in metaphase. In view of the great

toxicity of this compound it seemed of interest to investigate the antimutagenic effect of some of its derivatives. Toxicity was determined by intraperitoneal administration. Mice were implanted subcutaneously with sarcoma 180. Tumors were 7 days old at the time the animals were sacrificed, 6 hours after injection of various compounds. Corneal mitoses were studied on flat preparations. Spermatogonia were studied on pre-fixed smears stained with acetic orcein. The following compounds were studied: Colchicine (1), trimethylcolchicinic acid methyl ether d-tartrate (2), colchicine (3), trimethylcolchicinic acid (4), N-benzoyltrimethylcolchicinic acid methyl ether (5), N-benzoyltrimethylcolchicinic acid (6), and N-acetylcolchicinol (7).

COMPOUND	LD ₅₀ mg/kg	LOWEST DOSE (MG/KG) AFFECTING MITOSIS		
		Cornea	Spermatogonia	Sarcoma 180
1	3.5	0.35	0.35	0.88
2	64.0	3.2	3.2	3.2
3	84.0	84.0	21.0	21.0
4	200.0	inactive	200.0	200.0
5	20.0	35.0	1.0	5.0
6	>700.0	inactive	inactive	inactive
7	56.0	28.0	14.0	—

The relationship between chemical structure, toxicity and antimutagenic activity will be discussed.

Intravenous aminophylline and coronary blood oxygen. ELWOOD L. FOLTZ, ALAN J. RUBIN and WILLIAM A. STEIGER (introduced by CARL F. SCHMIDT) *Dept. of Pharmacology, Univ. of Pennsylvania, Philadelphia, Penna.* In 10 normal intact dogs under morphine-nembutal anesthesia, coronary sinus catheterization was accomplished under fluoroscopic guidance (*Am J Physiol* 152: 341, 1948). Simultaneous arterial and coronary venous blood samples were collected during control periods and after injection of either saline or aminophylline. Following intravenous aminophylline, coronary venous O₂ saturation fell from 30.2% to 17.7% (mean values) and the unsaturation (A-V difference) rose from 62.1% to 74.1%. Compared with results where saline was injected, these data showed significant differences. No marked change was noted in mean arterial blood pressure, but the mean pulse rate increased from 145 to 165 beats per minute. Electrocardiographic records revealed no significant change in pattern as a result of catheterization or experimental procedures. Since previous studies demonstrated that the myocardial stimulating effect of aminophylline increases cardiac output and cardiac work, it is suggested that aminophylline increases cardiac oxygen consumption more

than the augmented coronary flow increases the delivery of oxygen.

Narcosis and refractory phase in certain cerebral mechanisms. A. FORBES, A. F. BATTISTA (by invitation), P. O. CHATFIELD (by invitation) and J. P. GARCIA (by invitation) *Harvard Medical School, Boston, Mass.* Stimulation of the sciatic nerve in cats under deep barbiturate narcosis has previously been shown to evoke a primary electric response in the sensory leg area of the cortex and a secondary discharge, more widespread in the cortex, with latencies from 30 to 80 milliseconds. When a series of stimuli is applied at more than 4/second, each stimulus evokes a primary response, but only the first evokes the secondary discharge. A rest of nearly a second is needed before another stimulus can evoke the discharge. This behavior resembles the Wedensky inhibition in peripheral nerve, in which a partial block stops all but the first of a series of impulses so frequent that each impulse after the first falls in the relative refractory phase following its predecessor and is therefore subnormal.

Our experiments dealt with the question whether the failure of all stimuli after the first of a rapid series to evoke a secondary discharge is likewise due to subnormal impulses encountering a partial block in the cerebrum. Increasing the depth of narcosis greatly prolonged refractory phase in the mechanism of secondary discharge and decreased the stimulus frequency required to hold the discharge in abeyance. We found nothing inconsistent with the view that the effect is essentially a Wedensky inhibition. Depression of blood pressure, respiration and cortical waves was related to blood analysis of narcotic, and from the combination of these observations the rate of elimination of narcotic was estimated, this provided a measure of the depth of narcosis.

Comparison of pharmacological activities of several antihistaminic agents. ROBERT T. FORD, SHIRLEY L. MCHUGH, VIRGINIA M. O'ROURKE, DOROTHY J. NIXON and GERALD Q. O'NEILL (introduced by KARL H. BEYER) *Medical Research Division, Sharp and Dohme, Inc., Glenolden, Penna.* Tripeleminamine hydrochloride, Pyranisamine Maleate, Antergan, Thenylpyramine hydrochloride, Prophepyramine hydrochloride, R.P. 3277, and several other compounds have been evaluated on the basis of their local anesthetic, antihistaminic, antiacetylcholine, and antihyaluronidase activities. The antihistaminic tests include the antagonism of the effect of histamine against the isolated guinea pig ileum, the isolated guinea pig tracheal chain, and the histamine blood pressure depression in intact dogs. The local anesthetic measures include the rabbit cornea and the electrical stimulation of the isolated rat nerve-

diaphragm preparation. The hyaluronidase test was the inhibition in rabbits of the effect of hyaluronidase on the intradermal spread of India ink. The antiacetylcholine test was the antagonism of acetylcholine stimulation of the isolated rabbit ilium. The data suggest that of these measures, other than the strictly antihistamine tests, the inhibitory effect of these agents on the spreading induced by hyaluronidase may be a relatively important factor in anticipating clinical efficacy.

Indirect estimation of cardiac output in man from carbon dioxide exchange. CYRIL A. FORSSANDER (by invitation), HERBERT KRAMER (by invitation), C. J. MARTIN (by invitation), COLIN WHITE (by invitation) and H. C. BAZETT. *Dept. of Physiology, Univ. of Pennsylvania Medical School, Philadelphia, Penna.* Recent reports have demonstrated a wide range in arteriovenous differences in blood from various organs, with a consequent contrast between the compositions of superior and inferior caval blood. Any vaso-motor reflex, or change in mechanical conditions, is liable to alter the composition of mixed venous blood by changing the relative proportions of different streams, consequently in estimating venous alveolar air tensions it is probably important not to ask the subject either to make any effort or to change his respiratory pattern. Simple rebreathing of a high oxygen mixture, adopting these precautions, sometimes gives indications of a short plateau in CO_2 tension after some 20 seconds, but this is particularly difficult to demonstrate in shallow (and relatively rapid), also in deep (tho relatively slow) breathers. Attempts are being made to demonstrate plateaus in all types of subjects by reducing the volume of gas used for rebreathing to a minimum (in which Scholander's micro method for gas analysis aids) by utilizing a double bag system to accelerate mixing in the lungs (according to Gladstone's Principles), and thirdly by use of various experimental mixtures of oxygen and carbon dioxide. Cardiac outputs have been calculated from such estimates of virtual venous alveolar air, arterial alveolar air and carbon dioxide output. The values so obtained have usually been higher than those reported by users of the old acetylene method.

Electrolyte and protein changes during acute pneumococcal infection in rats. CHARLES L. FOX, JR. and GILBERT ASHWELL (introduced by ALFRED GILMAN). *Dept. of Bacteriology, College of Physicians and Surgeons, Columbia Univ., New York City.* Metabolic balance studies of Na, Cl, K, N and H_2O were made on individual rats infected intraperitoneally with a minimal dose of virulent pneumococci to ascertain some of the chemical changes that occur in a typical bacterial infection,

terminating in death in 4 to 5 days. Within the first 24 to 48 hours after infection, the excretion of Na, Cl and K increased 250 to 1000% above the pre-infection levels. On the 3rd and subsequent few days of survival, the Na and Cl excretion dropped to much below the pre-infection values, and occasionally to nil, in marked contrast, the greatly increased K excretion continued until death. There was a steady loss of body weight of from 12 to 19% and nitrogen excretion decreased progressively. The rats reduced their intake of food and water after the second day and urine output gradually diminished. All balances after infection became negative. The net loss of K was especially large and increased steadily until death occurred. It is of interest that in one instance of spontaneous recovery, these changes occurred initially but on the 4th day the weight loss ceased, K excretion approached zero and, as the K balance became positive, body weight returned to normal. Tissue analyses were made of samples taken from moribund rats at a time when other rats had already died. Tissues from uninfected rats carried on the same diet to control the metabolism studies were also analyzed. Muscle tissues from infected animals showed a marked elevation in K content, the intracellular compartment appeared to have increased and the extracellular components decreased.

Electrolyte, nitrogen and protein changes that follow massive removal of red cells and plasma protein. CHARLES L. FOX, JR. and BETTY B. FREEMAN (introduced by ALFRED GILMAN). *Dept. of Bacteriology, College of Physicians and Surgeons, Columbia Univ., New York City.* In a study of the chemical sequelae of acute blood loss and hypoproteinemia in dogs, from $\frac{3}{4}$ to $\frac{1}{2}$ of the blood was removed in 3 hours and replaced with mixtures of salts and water. The concentrations and balances of H_2O , Na, Cl, K, nitrogen and protein were determined before and after the massive hemorrhage. A most noticeable result was a 50% decrease in plasma K concentration followed by excretion of considerable K in the urine during the first 24 hours after bleeding. One to two days later, K excretion became extremely low while nitrogen excretion, which had been subnormal at first, rose sharply. Thus the K/N ratio initially became very high (10 to 15/1), then became very low (0.8/1) and after a few days returned to normal (3-5/1). The Na and Cl values were high or normal depending upon the volume and composition of solution infused but equilibrium was achieved rapidly. The massive blood removal resulted in hematocrit values ranging around 10% and plasma proteins ranging around 1.3 gm/100 cc, nevertheless the dogs survived, no edema resulted and both red cells and proteins regenerated. When K was in-

cluded in the replacement solution (5 mEq/l) the fall in plasma K was only partly counteracted and the K excretion was unaltered, the added K, however, seemed to reduce both the tachycardia and the volume of infusion needed to prevent circulatory collapse and renal failure

Growth and O₂ consumption, as affected by X-irradiation, using the bacterium, *Serratia marcescens* ANN C FOWLE (by invitation) and KENNETH C FISHER *Univ of Toronto, Toronto, Canada* The growth of the bacterium, *Serratia marcescens*, is accompanied by an increased rate of oxygen consumption, from which presumably it derives energy for growth. When the bacteria are subjected to X-irradiation various disturbances of growth and cell division are produced. It has been found that although the bacteria which grow and divide after irradiation do so at a rate considerably slower than normal, they do so without any appreciable interference with the amount of oxygen associated with growth. Thus it appears that the primary effect of X-irradiation does not involve the energy reactions specifically concerned with growth.

Factors affecting constancy of vasomotor response to repeated intra-arterial injections of mecholyl and of epinephrine LEWIS FRANKLIN (L I M R Fellow) (by invitation), HOWARD WAYNE (by invitation), J MAXWELL LITTLE and HAROLD D GREEN *Dept of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N C* In evaluating vasomotor responses induced reflexedly or directly by chemicals, in dogs anesthetized with sodium pentobarbital, we have compared these responses with responses to standardized quantities of mecholyl or epinephrine. Venous outflow was measured at 10 sec intervals from the physiologically isolated hind leg, perfused from the dog's own arterial system. To determine the constancy of response 116 intra-arterial injections of 0.08 μ g of mecholyl were made in 9 experiments and 35 injections of 1.0 μ g of epinephrine in 6 experiments. In intact preparations the coefficient of variation (coef var) for the maximum change of flow per 10 sec interval was 12-41% (av 22.6%) for mecholyl and 7-53% (av 28.5%) for epinephrine. Severance of both vagi and both carotid arteries or the sciatic and femoral nerves did not improve the coef var. Expressing the response as percentage change increased the coef var to 29.4%, when expressed as total change in flow (cc of blood flow gained or lost) the coef var was 27.0%. At constant control flows, the response to both drugs, expressed as maximum change of flow, decreased with decreasing perfusion pressure. At constant perfusion pressures, the response to mecholyl decreased but to epinephrine increased with

increase in control flow. Enough data are not yet available in which results were obtained at constant control pressures and flows to draw definite conclusions but the coef var appears to be about 10%. Another cause of variability may be the development of refractoriness near the end of prolonged experiments.

Further lowering of glucose tolerance following an induced hypothyroid state in Yale rats THOMAS F FRAWLEY and CHARLES W BISHOP (introduced by GEORGE F KOEPF) *Dept of Medicine, Univ of Buffalo School of Medicine, and Medical Research Labs, Buffalo General Hospital, Buffalo, N Y* A significant percentage of Yale-strain rats have been shown to have an abnormally low glucose tolerance. Sayers and Sayers (*Am J Physiol* 141:466, 1944) postulated that this diminished tolerance might be due to an inherited hyperactivity of the adrenalmedullary system. Studies were undertaken to determine the influence of thyroidectomy or propylthiouracil on this impaired glucose tolerance. The goitrogens provide a more specific method for studying the influence of hypothyroidism on carbohydrate metabolism heretofore equivocal with thyroidectomy due to the high percentage of aberrant thyroid tissue. A 35% solution of glucose (350 mg/100 gm body weight) was administered intraperitoneally and blood glucose determined at intervals over a 5-hour period. One group of 5 Yale rats weighing 200-400 gm was thyroidectomized and to another similar group propylthiouracil (0.10 gm/100 gm body weight) was administered daily by stomach tube. Glucose tolerance was determined at weekly intervals over a 5-week period. The average values (mg %) for each group are shown in the following table.

HOURS	0	$\frac{1}{2}$	1	2	4	5
(T) Before	90	326	228	134	104	97
(T) After 5 weeks	89	395	420	295	200	164
(P) Before	80	280	243	154	102	119
(P) After 5 weeks	80	354	310	228	136	114

(T) = Thyroidectomy group

(P) = Propylthiouracil group

Quantitative measurement, by external counting, of the I¹³¹ content of the thyroid gland in man A. STONE FREEDBERG, ALVIN URELES and MARVIN VAN DILLA (introduced by HERRMAN L. BLUMGART) *Med Res Lab, Beth Israel Hospital, Dept of Medicine, Harvard Medical School, and Dept of Physics, Mass Inst of Technology, Boston, Mass* We have recently described a method for quantitative measurement of I¹³¹ content of the thyroid gland in man by external counting. Independence of size and location of the thyroid gland (depth geometry and absorption) was achieved by con-

necting in parallel 4-6 platinum cathode Geiger-Mueller counters, arranged in a horizontal plane in circles of varying radii. With these counters in a circle 36-120 cm in diameter, a central source of radiation was moved about within a sphere 8-32 cm in diameter concentric with the circle of counters, the maximum variation in counting rate was 5%. When the source was surrounded by muscle, bone, water and saline in quantities approximating the human neck there was no significant change in counting rate. With a constant amount of radioactivity in volumes varying from 5-350 cc in different shaped containers no significant change in counting rate was observed. Identical amounts of I^{131} in 5 cc and in 2,000 cc of water placed in gallon jugs showed a decrease of 5% in the counting rate in the latter instance.

Preliminary studies on thyroid uptake in patients with varying gland size are consistent with mock up experiments. With a circle 52 cm in radius $10 \mu c$ in the thyroid gland gives a counting rate 2.5 background. Following a standard oral dose of $150 \mu c$, I^{131} carrier-free, the thyroid uptake and urinary I^{131} excretion was studied in 22 subjects. Thirteen thyrotoxic patients demonstrated, at varying times up to 48 hours, a maximum uptake in the thyroid gland from 46-84% and averaged 66. In 7 euthyroid subjects the uptake ranged from 13-39% and averaged 30. In 2 myxedema patients the thyroid uptake was 1 and 4%. Preliminary studies on the rate of uptake in the thyroid gland have also been carried out and are described.

Effect of Eck fistula formation and meat intoxication on serum phosphatase and dye clearance of adult dogs. SMITH FREEMAN, *Dept of Exptl Medicine, Northwestern Univ Medical School, Chicago, Ill.* The average preoperative Rose-Bengal dye-clearance of 19 adult dogs was 107 units and their serum phosphatase averaged 2.4 Bodansky Units. The minimum dye clearance after Eck fistula formation averaged 53 units and the corresponding phosphatase values averaged 9.9 units. Meat intoxication was produced in 10 of 11 dogs of the preceding series. The average serum values prior to meat intoxication were dye clearance of 51 units and phosphatase of 6.6 units. A diet of raw horse meat (50 gm/kg) produced meat intoxication in an average of 9 days. The average dye clearance decreased to 34 units and the serum phosphatase increased to 18.8 units. These changes are construed as evidence of further liver injury in the Eck fistula dog.

Summation of stimuli studied with the aid of anticholinesterases. JEANE SISKEL FREY (by invitation) and ROBERT GESELL, *Dept of Physiology, Univ of Michigan, Ann Arbor, Mich.* The carotid nerve was stimulated repetitively at frequencies ranging from 10 to 50/sec and the effects of this

stimulation on breathing recorded with the aid of a Hutchinson spirometer. Progressive increase of frequency of stimulation was attended by an increase of temporal summation of stimuli as shown by the increased respiratory response. Similar gradation of frequency of stimulation following administration of anticholinesterases (physostigmine, DFP, X substance (Army Chemical Center) and CO_2) was attended by an appreciably greater summation of stimuli at all frequencies. Potentiation of temporal summation by anticholinesterases agrees with an acetylcholine sparing action at synapses and a consequent intensification of neurocellular current which are believed to generate nerve impulses (current report of Hunter, Lillie and Gesell). Since anticholinesterases impair conduction of impulses in nerve fibers (Gilman, Nachmansohn, and Lowe and Gesell) it is unlikely that they potentiate reflexes by improved conduction of nerve impulses in the dendrites and cell body. For the reason that these reagents potentiate rather than depress central nervous activity, our experiments support earlier conclusions of Lowe and Gesell that impairment of impulse conduction in nerve and muscle by anticholinesterases is incompatible with the theory of synaptic conduction of nerve impulses. It is suggested that our experiments agree with the concept of specialization of function in the neuron.

Can transfer rate be calculated from urine isotope ratio? HERBERT D. FRIEDLANDER (by invitation) and WALTER S. WILDE, *Physiology Dept, Tulane Univ School of Medicine, New Orleans, La.* The expression for calculating transfer rate is $P^*/P = \Delta n_1^*/\Delta n_1 = \Delta n_2^*/\Delta n_2 = \dots = \Delta n_n^*/\Delta n_n$. P^*/P is the instantaneous ratio for tagged and for total but exchangeable chemical in the plasma. $\Delta n^*/\Delta n$ is the ratio of the respective chemicals, as infinitesimals, accumulated in the organs 1, 2, etc., or in an excreted fluid e, urine, saliva, etc. The amount of exchange back and forth of intrinsic substance, Δn , cannot be measured directly in most systems. It must be calculated from the equation. Certain excreted fluids such as urine, however, represent a continuous one-way sampling of Δn . Thus we measure Δn^* and Δn directly in urine collected over the transfer period and substitute them in the equation to calculate Δn_1 in an organ. This obviates estimation of P^* as \bar{P}^* from the area under a plasma time-concentration curve for tracer.

\bar{P}^*/P and $\Delta n^*/\Delta n$ were compared in 16 rats after subcutaneous tracer phosphate. Before 35 minutes \bar{P}^*/P exceeds $\Delta n^*/\Delta n$. Non-tracer phosphate lying in the urinary channels at injection time probably contaminates Δn . Later $\Delta n^*/\Delta n$ for the full period exceeds \bar{P}^*/P often threefold even at 150 minutes. Govaerts (*Arch Int Pharmacodyn*

75 261) presents evidence or non-exchangeable dog plasma phosphate. Calculations from his data agree with ours \bar{P}^*/P in plasma acid filtrates of the rat equals that in magnesium precipitates prepared in the cold. Faster urination of phosphate during the plasma peak hardly explains the result. Radiocalcium data sent by Govaerts yield similar \bar{P}^*/P and $\Delta n^*/\Delta n$ values.

Inactivation or removal of insulin by the liver
A. FRIEDMAN (by invitation), H. F. WEISBERG (by invitation) and R. LEVINE, *Dept. of Metabolic and Endocrine Research, Medical Research Inst., Michael Reese Hospital, Chicago, Ill.* Under normal circumstances insulin, after leaving the pancreas, reaches the liver before it goes into the general circulation. We wished to determine whether any inactivation or removal of the hormone occurred during its passage through the liver. For this purpose we have injected insulin into dogs at a constant rate I.V. ($0.1 \mu\text{g}/\text{kg}/\text{hr}$) over 2-hour periods, by two injection routes—the splenic vein and the femoral vein. Blood sugar values were estimated at 15–30 minute intervals. Each dog served as its own control. The results show that insulin infused via the splenic vein had, in all cases, a significantly smaller effect on the arterial blood sugar levels than did the insulin infused via the femoral vein. It is estimated from the areas of the curves that the liver led to removal or inactivation of about 40% of the injected insulin. These experiments were made with Lilly insulin which contains variable amounts of a hyperglycemic factor which might have been the cause of the lessened effect of intrasplenic insulin. However, identical experiments with a brand of insulin (NOVO) which we found not to have the hyperglycemic factor, also gave the lessened intrasplenic response.

Gastrointestinal motor effects of intestinal extracts
M. H. F. FRIEDMAN, *Dept. of Physiology, Jefferson Medical College, Philadelphia, Penna.* The experiments were performed on dogs under sodium pentobarbital, ether, or urethane anesthesia. Motor activities were recorded by means of tandom balloons and water manometers from the antrum, duodenum, terminal ileum, and colon. All tissue extracts were administered by vein. Certain preparations of intestinal mucosa, in doses of 2 to 10 mg/kg, always increased the tonus and force of contractions. This effect apparently occurred throughout the whole gastrointestinal tract but not in other smooth muscles. The response was obtained within a latent period of 2 minutes and was sustained for 10 to 45 minutes. Some evidence of tachyphylaxis was obtained. The motor effects were not abolished by bilateral vagotomy, splanchnectomy, separation of the intestine into segments, or large doses of atropine. No synergism between effective extracts and pilocarpine or mecholyl was

noted. The motor effects were not due to histamine and probably not to choline. The effective principle was not found in extracts of gastric mucosa, or in other types of intestinal mucosa extracts. It could be concentrated from intestinal extracts made by the method of Greengard et al. for the preparation of enterogastrone, and was found present in a commercial preparation of "purified enterogastrone" which is available for the treatment of peptic ulcer. The results suggest, but do not prove, the presence of a gastrointestinal motor 'hormone' in the intestinal mucosa.

Role of the adrenal cortex in the excretion of purines
MEYER FRIEDMAN, DONALD BERNSTEIN (by invitation), and SANFORD O. BYERS (by invitation), *Harold Brunn Inst. for Cardiovascular Research, Mount Zion Hospital, San Francisco, Calif.* Twenty-eight rats (maintained on water containing sucrose 5%) were given 100 μg of an extract of adrenal cortex (Lipo-Adrenal Cortex-Upjohn) by intramuscular injection. It was found that the total renal excretion of purine increased approximately 25% during the first 24 hours following the above injection. Six rats, injected with DOCA (5 mg/kg), showed no appreciable increase in purine excretion during the next 24 hours. Twelve rats were nephrectomized and given the same amount of Lipo-Adrenal Cortex. Four nephrectomized rats were given DOCA. The total purine content of the blood of these 16 animals 24 hours after injection was no greater than that of 14 control nephrectomized rats. These observations suggest that Lipo-Adrenal Cortex effects in rats an increase in the excretion of purines which is of renal origin.

Emotional state on acquired and inborn reactions: satiation on cardiac conditional reflexes and unconditional reflexes to food
W. HORSLEY GANTT, *Pavlovian Lab., Phipps Psychiatric Clinic, Johns Hopkins Univ., Baltimore, Md.* In the laboratory the conditional reflexes maintain a mechanical constancy comparable to the physiological reflexes so that they can be expressed even in a formula. Outside the laboratory the reactions are characterized by extreme variability and apparent lawlessness. The explanation is to be found chiefly in the strict control of the state of the animal used in the laboratory. Our previous investigations showed that the salivary conditional reflexes (*crs*) were dependent upon the state of hunger of the animal, falling from a maximum to zero immediately after satiation. The cardiac *cr* has been measured in this laboratory for many years to study the inner emotional state of the animal contrasted with the more specific external *crs*. Several dogs with well established food *crs* were allowed to eat to satiation after taking control salivary and cardiac measures to the condi-

tional signals for food. An immediate decrease of the cardiac *cr* resulted from the satiation. The cardiac component of the unconditional reflex (*UR*) was only slightly affected (the animal would not eat after satiation unless the food were forced into the mouth). Satiation caused an elevation of control cardiac rate. For example before satiation 'Sechs' control heart rate = 85, *cr* = 110, *UR* = 110, after satiation control heart rate = 100, *cr* = 108, *UR* = 105. In 'Peik' before satiation control heart rate = 65, *cr* = 98, *UR* = 95, after satiation control heart rate = 85, *cr* = 95, *UR* = 110. The cardiac component of the inhibitory *crs* was not affected. It is thus evident that not only the specific salivary *crs* but the more generalized emotional *cr* components, represented by the heart rate, may be immediately abolished by a change of emotional state of the animal while the *URs* are much less affected.

Anoxia on conditional reflexes in dogs W. HORSLEY GANTT, GEORGE W. THORN and C. DORRANCE. *Pavlovian Lab., Phipps Psychiatric Clinic and Dept. Medicine, Johns Hopkins Univ., Baltimore, Md.* Changes in higher cerebral function caused by single exposures to moderate altitudes (18,000 ft) are difficult to measure because of inadequate testing methods. Two dogs aged 11 and 4 years were exposed 4 hrs. to both 18,000 and 25,000 ft, either by reducing pressure or reducing oxygen content. One dog was exposed in a pressure chamber 6 times, the other 5 times. Responses measured were based on food ('Sechs') or pain ('Connie'). Sechs at 18,000 ft showed a reduction of the conditional reflex (*cr*) from 238 σ control in the pressure chamber to 170 experimental, inhibitory *cr* remained 0. At 25,000 ft the disturbance was more severe, excitatory *cr* = 150 σ , inhibitory = 135. After 24-hr recovery impairment persisted, excitatory *cr* = 205, inhibitory = 48 σ . Connie having *crs* to painful stimulus, gave as control 83% correct motor responses to excitatory *cr*, 3% to inhibitory *cr*. At 18,000 ft no impairment, at 25,000 ft excitatory *cr* = 90% correct but inhibitory *cr* = 60%—disturbed differentiation. After 24 hr there was complete recovery. Although differences according to age, temperament and kind of reflex measured, in both dogs there was marked impairment at 25,000 ft, but in the younger dog not at 18,000 ft. *Cr* measurements can be useful in evaluating efficacy of therapy for altitude tolerance. The *cr* impairment appears when there is no other observable neurological change.

Spinal cord pathways and connections for sensory fibers from the knee joint of the cat ERNEST GARDNER (introduced by M. MASON GUEST). *Dept. of Anatomy, Wayne Univ. College of Medicine, Detroit, Mich.* Recordings were made from the dorsal surface and ventral roots of the

spinal cord following electrical stimulation of the posterior nerve from the knee joint of the cat. With single shocks, potential changes characteristic of internuncial activity were recorded from approximately the 3rd lumbar to the 1st or 2nd sacral segments. These potentials were maximal in the 6th and 7th lumbar levels. Reflex discharges were recorded from ventral roots of those segments in which considerable internuncial activity was observed. Recordings were also made of action potentials in the posterior nerve conducted antidromically after single shocks to dorsal funiculi at various levels. Results indicate that articular fibers, after entering the spinal cord, synapse with internuncial neurons in several cord segments. These neurons in turn relay to motor neurons and also to lateral spinothalamic tracts. In addition, collaterals from the larger of the entering fibers ascend in dorsal funiculi as far as the medulla oblongata, beyond which they were not traced. In the lumbar cord, the fastest conduction rates were 90–100 m/sec. At the cervical cord, however, conduction was from 35–50 m/sec. The endings from which these ascending fibers are derived are Ruffini-type endings in the capsule of the posterior part of the joint.

Physical and chemical properties of emulsifying agents in urine JOSEPHINE B. GARST (by invitation) and HARRY B. FRIEDGOOD. *Dept. of Medicine, Univ. of Calif. at Los Angeles, Calif. Inst. for Cancer Research, Los Angeles, Calif.* The urinary material which stabilizes oil-in-water emulsions encountered in extraction of urine with organic solvents has been separated by full saturation with ammonium sulfate at pH 5. The flocculent brown precipitate which forms can be separated more rapidly by addition of ether and shaking. The precipitate is separated from the urine and reprecipitated twice from distilled water by addition of acetone to 80% concentration. It is then washed at the centrifuge with small amounts of water. When centrifugation no longer sediments the material, it is precipitated finally with acetone. The precipitate is then dried in vacuo over sulfuric acid. The product is hydrophilic, has a total nitrogen content of 10%, gives positive Molisch, xanthoproteic and biuret tests. A solution of the separated precipitate in distilled water stabilizes an ether-in-water emulsion. On the other hand, the residual urine at pH 5, freed of the above material, does not form emulsions with ether. Electrophoresis of the product has been conducted at pH 8.6 in 0.1 molar barbiturate buffer. The soluble fraction consisted of two components in the ratio of approximately 3 to 1. The characteristics of the material suggest that it consists predominately of glyco-protein.

Electrocorticograms of the cytoarchitectural

areas of macaca mulatta JOHN S GARVIN and L V AMADOR (introduced by PERCIVAL BAILEY) *Dept of Neurology and Neurological Surgery, Univ of Illinois College of Medicine, Illinois Neuropsychiatric Institute, Chicago, Ill* Investigators have noticed differences in electrical activity from various cytoarchitectural areas during other electroencephalographic experiments. Specific studies have been made on rabbits and cats. The cortex of *Macaca mulatta* was exposed and direct recordings made in anesthetized, unanesthetized and curarized animals. Electrical activity from various areas revealed marked differences. There were minor variations between animals, but in general the following holds. The tracings from areas *FA* (Area 4) and *PC* (Area 1) are quite distinctive, but indistinguishable from each other. The entire occipital region is lower in amplitude than any other region. *OC*, *OB* and *OA* (Areas 17, 18, 19) are practically alike except that the amplitude of *OA* is slightly higher. Activity is similar throughout all of *TA* (Area 22), and is not characteristic. The activity of *FB* (Area 6) is of high amplitude and resembles the activity from the inferior parietal region, while the activity from *FD* (Areas 10, 9) and *FD delta* (Area 46) is of rather low amplitude with some faster superimposed activity. Tracings from some areas, notably *PEm* (Area 5), *PE* (Area 7), *PF* (Area 40) and *PG* (Area 39) were indistinguishable, but were characteristic for those regions. Multiple simultaneous recordings within *FA* revealed 'Dial bursts' at one point and not at another and at times almost synchronous bursts throughout the area. No particular difference between the unanesthetized, curarized and anesthetized monkeys was observed except that the frequency in *FA* and *PC* appeared faster under curarization and without anesthesia.

Action of D O C A and adrenal cortical extract on body water and kidney function MARIO GAUDINO and MARVIN F LEVITT (introduced by HOMER W SMITH) *Dept of Physiology, New York Univ College of Medicine, New York City* Three normal dogs were injected with 30 mg of D O C A for 3 weeks and the changes in extracellular space (inulin vol of distribution), total body water (D_2O), intracellular space (difference between D_2O and inulin vol), plasma vol (T-1824) and renal function (inulin and PAH clearances, Tm_{PAH}) were studied frequently. There was a marked increase in the extracellular space with no change or slight decrease in the total body water, resulting in a significant decrease in the intracellular space. Plasma volume slightly increased or showed no change. Inulin clearance increased, PAH clearance increased very slightly and Tm_{PAH} was significantly decreased. The first change to appear was the increase in extracellular space. A fairly

good correlation exists between the inulin clearance and the inulin space. With the prolongation of treatment the above changes tended to disappear, despite an increase in the dose of D O C A. After the injections were discontinued the return to normal was complete. Two dogs treated with 15 cc of total adrenal cortical extract (Upjohn) revealed a completely different effect. The extracellular space did not change significantly, but the total body water increased markedly, reflecting an absolute increase in the intracellular space. Plasma volume, inulin and PAH clearances did not change. Tm_{PAH} increased in one case and decreased in another. All changes were reversed after the treatment was discontinued.

Arterial oxygen saturation and intracardiac pressures during acceleration in relation to cardiac damage O GAUER (by invitation), J P HENRY, E E MARTIN (by invitation), P J MAHER (by invitation) *Aero Medical Lab, Air Materiel Command, Wright-Patterson Air Force Base, Dayton, Ohio* A fall in arterial oxygen saturation has been predicted during acceleration in view of the marked disturbances in lung circulation observed in X-Ray motion pictures. Therefore, in both human and animal experiments oxygen saturation in arterial blood has been continuously recorded during acceleration. A Kramer glass cuvette oximeter with an automatic syringe has been employed together with Van Slyke determinations of blood oxygen content. A fall from 97% to 90% in oxygen saturation was measured in a human exposed to 4.5 g positive acceleration for 15 seconds. Experiments in anesthetized dogs submitted to both positive and negative acceleration showed changes of the same order during brief acceleration and even greater changes during prolonged runs (2 min). Intracardiac pressures were simultaneously recorded in both ventricles of nembutalized dogs during negative acceleration, using modified pressure sensitive tip Wetterer catheters. Carotid and jugular pressures were also recorded in these experiments. These measurements were an attempt to correlate the above mentioned fall in arterial saturation with the frequent development of subendocardial ecchymoses in the left and right ventricles during negative acceleration. During the acceleration there were changes in the contour of the right intraventricular pressure wave suggesting that at certain periods during the cardiac cycle the changes in right intraventricular pressure may fall out of phase with those occurring in the left ventricle. Such pressure differentials between the heart chambers during acceleration indicate a possible mode of origin of the above mentioned ecchymoses.

Utilization of fructose by the perfused brain of the living cat A GEIGER, J MAGNES, R M

TAYLOR and H. WAELSCH (introduced by H. GRUNDFEST) *Depts of Biochemistry and Psychiatry of New York State Psychiatric Inst., New York City* Fructose penetrates into the narcotized cat's brain up to concentrations of 60-100 mg/100 gm, if the fructose concentration of the blood is kept very high (15-2%) for an hour or more. When the normal blood circulation of the cat's brain was switched over to perfusion with a glucose-free suspension of erythrocytes in a Ringer solution containing 100 mg of fructose and 7 gm bovine serum albumin/100 cc the following was observed. The oxygen consumption of the brain was about half as great as with the same perfusion fluid containing glucose. The glucose from the brain disappeared at a rate corresponding roughly to the rate of oxygen consumption. As long as glucose was present in the brain the fructose concentration in it remained unchanged. Only after the complete disappearance of glucose did the fructose concentration slowly diminish. The amount of oxygen consumed during this period was larger than that required for the oxidation of fructose. The larger part of the oxygen consumed during the glucose-free period could be as preliminary experiments indicate, accounted for by the disappearance of glutamine and glutamic acid from the brain cortex.

Differences in cortically and subcortically induced convulsions. ERNST GELLHORN *Lab of Neurophysiology, Dept of Physiology Univ of Minnesota, Minneapolis, Minn* **Cortically induced convulsions.** Convulsive activity induced by topical application of strychnine does not interfere with the independence and asynchrony of different cortical areas either under control conditions or in asphyxia. **Subcortically induced convulsions.** First, strychnine is injected into either hypothalamus (mammillary body) or thalamus (dorsomedian or ventrolateral thalamic nucleus) in 'Dial' cats and local spikes are recorded. Hereafter asphyxia is initiated which causes the spikes to disappear first and the normal potentials later. During this time the asynchrony existing between various cortical and subcortical areas persists unchanged. After cortical potentials have disappeared a synchronous outburst is recorded in cortex, thalamus, basal nuclei and hypothalamus and lasts for 10 to 30 seconds. This discharge consists of convulsive spikes and is accompanied by overt convulsions in the non-curarized cat. The experiments suggest that the subcortical focus does not control cortical activity as long as the cortical excitability remains normal. If, however, cortical potentials disappear the subcortical focus seems to be released (removal of cortical inhibition) and leads to a generalized synchronous discharge. **Systemic convulsions.** If generalized convulsive

activity is induced by intravenous injection of picrotoxin asphyxia leads to synchronous cortical and subcortical discharges as observed in experimental group 2.

A skin resistance recorder or sudomotor. WILLIAM A. GEOHEGAN (introduced by Joseph C. Hinsey) *Dept of Anatomy, Cornell Univ Medical College, New York City* Measurement of skin resistance provides a convenient index of sudomotor activity, and recording of skin resistance changes is of great value in determining nervous pathways. While in some cases an approximate measure of skin resistance is desirable, in others, it is important to record small changes due to an applied stimulus. An instrument has been developed which permits measurements of both kinds, is simple to operate and is safe for application to human subjects in the operating room. A set of interlocking push buttons permits connection of any one of 5 electrodes to a bridge and vacuum tube voltmeter which is calibrated directly in ohms. The voltmeter output is connected through a stable d.c. amplifier to an Esterline Angus recorder. Sensitivity and stability are adequate for recording changes as small as 1%, and permit detecting presence of relatively few fibers in a stimulated pathway. A convenient balancing arrangement is provided to center the recorder for any resistance value from 1000 ohms to 100 megohms. The instrument is operated from the 115 volt a.c. line and employs no batteries. Electrical and mechanical isolation of the input circuit insure against accidental application of high voltage to the patient.

Cholinesterase activity of intact nerve, DFP inhibition. R. W. GERARD, B. LIBET and D. CAVANAUGH *Dept of Physiology, Univ of Chicago and Marine Biological Lab., Woods Hole, Mass* The inertness of ACh applied to nerve has been attributed to lack of penetration. Also, an objection raised to the measured ChE inhibition, on grinding a nerve soaked in DFP, has been that the inhibitor and enzyme react after structural breakdown. The following experiments invalidate these points. A frog sciatic or a single squid giant axone is threaded through a capillary, with cut ends outside. The capillary is filled (about 20 cu mm) with a dilute ACh solution, or (squid axone) a measured drop of the solution is moved to and fro along the nerve. After desired intervals at room temperature the ACh solution is removed and ACh assayed. With unpoisoned nerve or axone, 65 to over 80% of the ACh present (0.1 to 1.28) had disappeared in 20 minutes. Controls show that no ChE leaves the tissue, so ACh must have reached the enzyme in intact fibers. Despite adverse conditions (low ACh concentration, long diffusion distances, part time contact) this corresponds to a QChE of over one-fourth the full value of a squid axone. When

10 mM DFP was present in the ACh solution, no ACh loss was found in 60 minutes with frog nerve. Conduction remained normal.

Species reactivity of staphylocoagulase and staphylokinase EARL B GERHEIM (by invitation) and JOHN H FERGUSON *Dept of Physiology, Univ of North Carolina, Chapel Hill, N C* A study was made of sheep, cattle, rat, dog, horse, guinea pig, chicken and rabbit plasmas in connection with the staphylococcal factors concerned in coagulation (prostaphylocoagulase) and lytic activity (staphylokinase). The lengthened staphylocoagulation times, as well as the complete incoagulability of some plasmas was due to 1) an inhibitor to the active clotting agent, staphylocoagulase, 2) destruction of fibrinogen by proteolytic activity due to activation of a proenzyme by the factor staphylokinase, and 3) a possible low co-factor content. Fractionation of the plasmas with ammonium sulfate indicated that the inhibitor was quite ubiquitous, with the globulin fractions containing the most. No fraction of any species except rabbit contained as much cofactor as could be obtained from human plasma or serum. The fractionation procedure seemed to inactivate a great deal of the inhibitor. Parallel tests for fibrinolysis with staphylokinase and streptokinase were made. Staphylokinase, which has previously been shown to activate a proenzyme in human serum, was reactive with a proenzyme found in rabbit, guinea pig and dog serum. Streptokinase did not activate any proenzyme of the species tested, except human. Staphylokinase differs from streptokinase in requiring a long incubation period for maximal lytic activity. The greatest proteolytic activity was developed in dog serum.

Biophysics of the cochlea B E GERNDT (by invitation), J S RIESCO-McCLURE (by invitation) and H DAVIS *Central Inst for the Deaf, St Louis, Mo* Aural microphonics were recorded simultaneously from the round window of guinea pigs and from another electrode placed in a very small hole drilled into the scala vestibuli or media of the apex or one of the intermediate turns. Pure tones of various frequencies were adjusted in intensity to give a standard response of 20 microvolts at the round window. The necessary sound intensity was noted, and the corresponding voltages of the microphonic at the other electrode were measured. In several experiments the cochlea was then injured through another small hole at some intermediate position and measurements repeated. The logarithms of responses at apex (with round window response constant) plotted as a function of log frequency yield a smooth curve that is nearly horizontal below 1000 cps and falls off more or less rapidly as frequency is increased. Apical and round-window responses are usually equal at about

3000 to 4000 cps. Intermediate positions of the electrode yield similar curves with less low-tone emphasis. Injury abruptly increases the intensity required to produce a standard response to the frequencies that correspond in position to the position of injury. The relations of position of electrodes and of injuries to frequency correlate well with those previously established by Stevens, Davis and Lurie. Even a severe injury, however, rarely raises the necessary intensity more than 20 db. The significance of these and related observations to the biophysics of the cochlea are discussed.

Fractionation of enterogastrone concentrates with N-butyl alcohol LEON L GERSHBEIN (by invitation), HUGH M MILLEN (by invitation) and A C IVY *Dept of Clinical Science, Univ of Illinois College of Medicine, Chicago, Ill* A number of enterogastrone concentrates purified by way of the tannate (ET) as well as by subjecting the latter to picric acid treatment (EP), were submitted to alcohol fractionation. The extraction of these products with successive portions of aqueous alcohol and absolute methyl alcohol led to high recoveries of the starting materials with little enrichment of the hormone, as based on the continuous histamine assay method. However, at least a two-fold enrichment occurred with n-butyl alcohol. In fact, 50 mg of one concentrate which gave a gastric stimulatory response in excess of 80% yielded a butanol extract eliciting 43% and 52% inhibition with 25 mg doses. The yields of these fractions aggregated 13-14%. The procedure employed is exemplified by the following run. A mixture of 2.00 gm of ET-16 and 200 ml of butyl alcohol was mechanically stirred for 20 minutes at 32°-38°C. After filtration by centrifugation, the supernatant liquid was concentrated to 16 ml and treated with 110 ml of acetone. The precipitate which was washed by centrifugation with portions of acetone, then 1:1 acetone-ether and finally ether, weighed 268 mg (13.4% recovery). Further fractionation of the butyl alcohol-soluble product was afforded by treatment with methyl alcohol. The 22% soluble portion was highly stimulatory to the dog, whereas the methylalcohol-insoluble fraction in a 25 mg dose inhibited gastric secretion to the extent of 70% in the same dog.

Heating of tissues by near and far infrared radiation JEROME W GERSTEN (by invitation), KHALIL G WAKIM, RICHARD W STOW (by invitation) and ADRIEN N PORTER (by invitation) *Sections on Physical Medicine and Physiology, Mayo Foundation and Mayo Clinic, Rochester, Minn* It has been widely accepted that near-infrared radiation is more efficient in heating human tissues than is far-infrared. Because the evidence does not seem convincing a comparative

study of the heating effects of irradiation with energy in the far-infrared and near-infrared ranges was made. Tissue temperatures were recorded by means of copper-constantan thermocouples, and the radiant energy density at the skin surface was determined by means of a radiation thermocouple. Near-infrared rays were produced by a 250-watt Mazda bulb, and far-infrared rays by a 450-watt carborundum heater. The energy absorption/volume of tissue was calculated at the end of one minute of heating. With both sources of heat, the skin absorbed the greatest amount of energy, and the muscle the least, as computed/volume of tissue. When the radiant flux density at the skin surface was almost identical, the energy absorption by the tissues was 22% greater with the carborundum unit than with the Mazda bulb. The distribution of the energy absorption was the same with both sources, thus indicating that the effectiveness of the carborundum heater was not due to marked cutaneous heating with relatively little deep heating. When the output of the carborundum unit was decreased by 43.5%, the energy absorption by the tissues was decreased 40.8%, thus indicating that when the same heater is used the energy absorbed is directly proportional to the energy radiated. Judged by the increase in temperature of tissues to a depth of 15 mm, this study showed that far-infrared radiation is more efficient than near-infrared radiation.

Tissue distribution, excretion and metabolic fate of chloramphenicol (chloromycetin). ANTHONY J. GLAZKO, LORETTA M. WOLF (by invitation) and WESLEY A. DILL (by invitation). *Research Labs of Parke, Davis and Company, Detroit, Michigan.* Chloramphenicol is an aromatic nitro compound for which chemical and microbiological assay procedures have been developed. The chemical method is based on reduction of the nitro group to a primary amine with metallic zinc or titanous chloride, followed by diazotization and coupling. It is suitable for determination of total drug in biological material, since it includes inactive degradation products as well as unchanged chloramphenicol. The drug is rapidly absorbed, with serum levels reaching a maximum in about 2 hours and falling gradually during the next 18 to 24 hours. Analysis of tissues in the rat and dog reveal high concentrations in the kidney and liver, with progressively lower concentrations in the lung, spleen, heart, muscle and brain. Unchanged chloramphenicol has been isolated from the urine of man and identified. About 5% of the administered dose is recovered in 24-hour urine specimens by microbiological assay, whereas 80 to 92% is recovered by chemical assay, indicating the presence of large amounts of inactive nitro compounds. A simple hydrolysis product of chloramphenicol

and a conjugate have been identified in urine. The conjugate was found to regain antibiotic activity following hydrolysis with a glucuronidase preparation. No increase in aryl amine excretion was observed in the urine of man or dog, but lower animals showed a significant rise. Incubation of chloramphenicol with rat liver slices *in vitro* resulted in loss of activity, with the formation of inactive nitro compounds and a small amount of aryl amine.

Site of action of soy bean trypsin inhibitor on the coagulation system of blood. MARY BETH GLENDENING and ERNEST W. PAGE. *Dept of Obstetrics and Gynecology, Univ of California Medical School, San Francisco, Calif.* Soy bean trypsin inhibitor (SBTI) markedly inhibits the rate of blood coagulation, and also protects animals against the toxic effects of intravenously administered thromboplastin. The findings of Tagnon (1946) and Macfarlane (1947) that the site of action is on some factor involved in the conversion of prothrombin to thrombin have been confirmed. The effect on thrombin is negligible. Initial experiments with plasma collected in silicone-coated apparatus indicated that the SBTI affected a plasma constituent as well as thromboplastin. A 2-stage technic with purified prothrombin as substrate and dilute beef serum as a source of accelerator globulin was utilized in order to identify this constituent. Thrombin formation ceases at approximately the same time regardless of the presence or absence of SBTI. The amount of thrombin formed, however, varies directly with the concentration of beef serum and inversely with the concentration of SBTI. Thromboplastin, freed of Ac-globulin is inhibited similarly but the amount of thrombin formed is much lower than when beef serum is present. In the absence of beef serum the phospholipid extracted from purified thromboplastin will not convert prothrombin to thrombin, although it becomes increasingly active with rising concentrations of beef serum. This suggests that an active constituent of beef serum, presumably Ac-globulin, can qualitatively substitute for a protein portion of thromboplastin. Present evidence supports the concept that both a phospholipid and a protein are essential for any appreciable conversion of prothrombin to thrombin, and that SBTI acts on both the protein moiety of the thromboplastin molecule and the serum Ac-globulin.

Effect of pteroylglutamic acid and 4-amino pteroylglutamic acid on the growth of sarcoma 180. A. GOLDIN (by invitation), B. GOLDBERG (by invitation), L. G. ORTEGA (by invitation) and L. B. SCHOENBACH. *Biology Sect., Medical Div., Army Chemical Center, Md., and the Dept of Preventive Medicine, The Johns Hopkins Univ. School of Medicine, Baltimore, Md.* The ability of folic acid (pteroylglutamic acid) to reverse the effect

of aminopterin (4-amino-pteroylglutamic acid) induced inhibition of the growth of sarcoma 180 was tested. Groups of mice bearing transplanted sarcoma 180 were treated with aminopterin plus a series of concentrations of folic acid. The weights of the tumors were compared with the tumors of mice which received aminopterin alone, folic acid alone, and with the tumors of control groups. A partial reversal of the aminopterin induced inhibition of tumor growth by administration of folic acid was a constant finding. The tumors of aminopterin treated mice averaged 35% of the controls. The tumors of mice treated with aminopterin, plus folic acid ranging in concentrations from 5-50 mg/kg, were 65-75% of the weight of the controls. Folic acid itself appeared to produce some inhibition of tumor growth when administered at higher concentrations. The reversal of aminopterin inhibition by folic acid is reflected in the histological appearance of the tumors. Tumors of mice treated with folic acid did not show the marked histological changes resulting from aminopterin inhibition, but approximated that of the control tumors.

The reflexology of the pudendal nerve field
 CHARLES H. GOOD (introduced by BERRY CAMPBELL) *Dept of Anatomy, Univ of Minnesota, Minneapolis, Minn*. Previous work on the reflexology of the spinal cord has dealt principally with the locomotor apparatus—the lumbo-sacral intumescence and the neuro-muscular apparatus of the hind limbs. Noteworthy are the fast components of the reflex in these locomotor fields and the absence of direct transmission of activity across the midline of the cord. The pudendal nerve mediates the sexual reflexes and other reflexes of the perineal-anal region. The effector structures are near the midline and frequently work in conjunction with their antimeres. Central latency is long, crossed return is the rule. Stimulation of the penis of cats results in an efferent discharge, crossed and uncrossed along the S₂ and S₃ ventral roots. The total latency to S₂ ventral root is 8.2 msec of which 2.2 msec is afferent conduction time. Crossed intersegmental as well as crossed segmental return is found. The forms of the reflexes are to be shown as well as their conditioned activity. *Parallel anatomical study of the fiber spectrum of the pudendal nerve and its roots is being made.*

Myocardial lactate and pyruvate metabolism in dogs under severe stress
 WALTER T. GOODALE and DONALD B. HACKEL (introduced by W. FLEISCHMANN) *Physiology Sect., Medical Div., Army Chemical Center, Maryland*. Development in this laboratory of a technique of coronary sinus catheterization (*Am J Physiol* 152: 340, 1948) has made possible the study of myocardial

metabolism in intact nembutalized and unanesthetized dogs. Removal of metabolites from coronary blood was assumed to represent myocardial utilization, which was then calculated from the product of two apparently independent variables: 1) Coronary blood flow, measured by the nitrous oxide method, and 2) Coronary arterio-venous difference, estimated from simultaneous femoral arterial and coronary venous blood samples. Severe stress from adrenalin infusion, shock, hemorrhage, severe anoxia, convulsions, and severe nutritional deficiency all caused a rise in arterial lactate levels above normal, but removal of lactate from coronary blood by the myocardium was greatly reduced, occasionally even to zero at arterial lactate levels of 40 to 60 mg %. Pyruvate removal was only moderately reduced. Although decreased lactate removal may indicate decreased myocardial lactate utilization, adrenalin release may be the common denominator in such situations of severe stress, leading to glycogenolysis within the heart (and liver), with endogenous production of lactate as an immediate emergency source of energy, with high tissue lactate levels produced endogenously, the myocardium should remove less lactate from coronary blood as the blood-to-tissue concentration gradient falls. Failure of the myocardium to remove lactate from coronary blood at elevated arterial lactate blood levels is apparently associated with a grave cardiac emergency, and often with a threat to survival.

Reduced synthesis of phosphocreatine in tissue homogenates from alloxan diabetic rats
 E. S. GORANSON (introduced by R. E. HAIST) *Dept of Physiology, Univ of Toronto, Toronto, Canada*. The phosphorylation of creatine, under aerobic conditions, has been studied in homogenates of various tissues of the rat, principally brain, incubated for short periods at 37°C. In the presence of added succinate, cytochrome C and adenosine triphosphate, the synthesis of phosphocreatine in preparations from alloxanized rats is significantly less than in those from normal animals. Insulin injected into the alloxanized rats prior to tissue sampling was found to promote an increased formation of phosphocreatine. Similar results have been obtained when malate and pyruvate are substituted for succinate in the homogenates. Insulin had no appreciable effect on the oxygen consumption in these experiments. The results indicate an action of insulin in increasing the efficiency of oxidative phosphorylations and the possible participation of insulin in reactions involving the tricarboxylic acid cycle.

Relation of the adrenal cortex to the increased macrophagic activity induced by starvation
 ALBERT S. GORDON and GRACE F. KATSH (by invitation) *Dept of Biology, Washington Square*

College of Arts and Science, New York Univ, New York City It has been reported (Gordon and Katsh, *Federation Proc* 7 42, 1948) that chronic inanition results in an increased phagocytic response of the macrophagic system towards colloidal substances, particularly thorium dioxide Experiments were designed to test the possibility that this starvation effect is mediated through the adrenal cortex Thirty male rats (150-200 gm) were subjected to chronic inanition (5 gm ration daily) for 25-30 days A body weight loss averaging 31% was experienced at the end of this time Of these 30 animals, 18 were adrenalectomized and 12 served as controls Each of 8 of the adrenalectomized animals was injected with a total of 20 cc of aqueous adrenal cortical extract (Upjohn) and 1 cc of lipoadrenal extract (Upjohn), distributed over a 3-day period before, and 30 hr after adrenal removal The other 10 adrenalectomized rats were injected with 21 cc of 1% saline, spaced in the same manner as above The 18 adrenalectomized animals (used 1 day after the operation), the 12 unoperated starved and 10 normal fed rats were injected intravenously with 0.3 cc thorotrast and killed approximately 20 hr later Mean values \pm standard errors for ThO_2 per gm dried spleen were 1) fed untreated, 58.2 ± 7.8 mg, 2) starved untreated, 175.5 ± 11.3 mg, 3) starved adrenalectomized given saline, 70.6 ± 7.1 mg, 4) starved adrenalectomized given adrenal cortical extract, 123.5 ± 13.4 mg

Histological studies of the spleens revealed that the increased numbers and marked activity of the macrophages, characteristic of the starved rat, were diminished by adrenalectomy and that adrenal cortical hormone, but not saline, was greatly effective in preventing this impaired condition in the adrenalectomized animal

Effect of insulin on the rate of oxygen consumption by frog muscle DESMOND R. H. GOURLEY (by invitation) and KENNETH C. FISHER *University of Toronto, Toronto, Canada* In the presence of citrate, succinate and acetate, insulin causes an increase in the rate of oxygen consumption by isolated intact frog muscle The effect of the citrate, succinate, and acetate appears to be correlated with the ability of these compounds to produce spontaneous contractions in the muscle

Experimental observations on neural mechanisms involved in itching DAVID T. GRAHAM and HELEN GOODELL (introduced by HAROLD G. WOLFF) *New York Hospital and the Depts of Neurology and Psychiatry, Cornell Univ Medical College, New York City* Itching was produced by application of cowhage to skin Two components could be distinguished in the resulting itch a superficial pricking and a deeper burning sensation Ischemia of the forearm, causing loss of pricking pain but

not of deep burning pain, abolished the superficial pricking component, but not the deeper burning component Procaine block of a cutaneous nerve, resulting in the abolition of burning pain from pin prick with retention of pricking pain in an area of incomplete anesthesia, prevented development of burning itch, but allowed pricking itch to develop It is suggested that the two qualities of itch are mediated respectively by the rapidly and slowly conducting pain fibers from the skin Pin pricks in an itching area, or in areas adjacent to it, or in the same dermatome as the itching area, even at a distance of 30 cm, abolished itching for 20 to 60 seconds In a zone of secondary hyperalgesia (developing after faradic stimulation over a cutaneous nerve), cowhage induced either pain or no sensation, but not itch The evidence cited above indicates that to understand the neural mechanisms of itching it is necessary to consider not only peripheral phenomena, but also processes in the central nervous system, presumably in the cord

Early stages of gastric surface epithelization RHODA GRANT (introduced by A. C. Ivy) *Dept of Clinical Science, Univ of Illinois College of Medicine, Chicago, Ill* Gastric surface epithelization uncomplicated by concurrent regeneration of glandular epithelium has been studied histologically (1-8) hr after a) mild irritants have removed the surface cells, b) minute superficial cuts Irritants were used 1) in cats with gastric fistula under acute conditions, or were fed 2) to cats by tube, and 3) to rats by tube Control cuts were biopsied immediately after infliction and compared with cuts allowed to heal for varying intervals up to 8 hr Serial sections were stained with hematoxylin and eosin or Mayer's mucicarmine

In the early stages of surface regeneration, the cells which restore the surface continuity in denuded areas include parietal cells These are most numerous in the walls of crypts reforming at the surface level, but occasionally may be seen on the free surfaces between regenerating crypts and in new surfaces forming in cuts at or below midgland level The majority of cells in the new surfaces stain faintly for mucus and may be from the bases of crypts not completely removed by the irritant, and/or mucous neck cells Evidence points, as in former experiments, to restoration of surface continuity and of the essential surface topography before proliferation replaces cells shifted to the surface

Effect of ethyl carbamate on temperature regulation RONALD GRANT and MARILYN E. ROBBINS (introduced by VICTOR E. HALL) *Dept of Physiology, Stanford Univ, Stanford University, Calif* Magoun (*Proc Soc Exp Biol Med* 37 711, 1938) showed that polypneic panting occurs at normal

or subnormal body temperatures in some cats anesthetized with urethane. We have investigated the possibility that urethane promotes heat loss by an action on the thermoregulatory centers. Rabbits anesthetized with urethane (1.0 to 1.5 gm/kg in 20% solution intraperitoneally) show a rapid drop of body temperature due to 1) decreased heat production, 2) vasodilation in the ears, 3) in a few cases, polypnea. After body temperature has fallen 1 to 3°C polypnea ceases, vasoconstriction occurs and the fall of temperature is retarded. Shivering may occur. Warming restores the vasodilation and incites polypnea usually at subnormal rectal temperatures. Despite this evidence that urethane lowers the temperature thresholds for activation of thermoregulatory mechanisms we have concluded that its action is probably not upon the primary centers of thermoregulation for the following reasons: 1) whereas vasodilation and polypnea are practically coincident in normal animals urethane usually slows respiration (rather than causing polypnea), while inciting vasodilation, and thresholds for activation are widely separate in warmed animals, 2) intravenous or subcutaneous injection rarely causes vasodilation or polypnea, 3) inhibition of heat loss mechanisms by bacterial pyrogens is unaffected by previous or subsequent intravenous injection of urethane, intraperitoneal injection may reverse the inhibition of vasodilation without affecting inhibition of polypnea.

Influence of repeated artificial pneumothorax on hematocrit values and plasma volume. WILSON C. GRANT (introduced by WALTER S. ROOT) *Dept of Physiology, College of Physicians and Surgeons, Columbia Univ., New York City*. Pneumothorax was maintained for 20 to 80 days in 10 unanesthetized dogs, 3 of which were splenectomized. One hundred to 500 cc of air were injected at intervals of from 2 to 5 days. The volume of the refill was always adjusted to maintain the desired elevated intrathoracic pressure (-2 mm Hg to +2 mm Hg). Pneumothorax was moderate and the animals remained in good condition. Measurements were made on jugular venous blood during a 2- to 3-weeks control period and at 2- to 3-day intervals in the pneumothorax period, at least 2 days following an insufflation. Hematocrit and O₂ capacity values increased 10 to 30% in 6 to 20 days. Plasma volume (T-1824) decreased 6 to 28% while the refractive index of plasma rose slightly. No significant increase in reticulocyte percentage was observed. Approximately the same response was observed in the splenectomized dogs. Blood values returned to those of control level within 1 to 2 weeks after cessation of pneumothorax. Anoxic anoxia was not an important factor, since normal values for arterial O₂ saturation were obtained in

18 out of 20 measurements on 5 dogs during the pneumothorax period. The animals responded to partial collapse by an increased pulmonary ventilation. The response of the blood to repeated pneumothorax is, therefore, a relative polycythemia which can be partially explained by plasma loss. No clear evidence of increased erythropoiesis was found. Hemoglobin production following pneumothorax is being studied by the Whipple-Robbscheit-Robbins technique on the chronically anemic dog.

Non-dependence of tubular sodium reabsorption upon glomerular function. D. M. GREEN and ALFRED FARAH (by invitation) *Depts of Medicine and Pharmacology, Univ of Washington, Seattle, Wash.* The amount of sodium reabsorbed by the tubular apparatus has been considered to represent a constant fraction of the amount filtered (Mokotoff *et al* (*Journal Clin Invest* 27 1, 1948)). Such a hypothesis would make the excretion of sodium primarily dependent upon glomerular function. However, the ability of many patients to maintain sodium balance despite markedly impaired filtration rates would appear more consistent with the supposition that the percentage of filtered sodium rejected by the tubular apparatus could be regulated independently of the filtration function. Evidence for the existence of such an independent regulation has been secured by measuring the percentage of filtered sodium excreted under different conditions of sodium load. Solutions of sodium chloride varying in concentration from 0.86 to 30% were infused into dogs at rates of 1 to 9 cc/min. Under such circumstances the test animals were found capable of increasing sodium excretion several 100-fold, to values which represented as much as 40% of the quantity of sodium filtered. These changes were not accompanied by consistent or proportionate alterations in glomerular filtration rate or renal plasma flow. Calculations based on the clearance data indicated that the rise in sodium excretion was due almost entirely to a decrease in the percentage of its tubular reabsorption.

Effects of adrenal resection on hypertension and diabetes. D. M. GREEN, J. N. NELSON (by invitation) and G. A. DODDS *Dept of Medicine, Univ of Washington School of Medicine, Seattle, Wash.* Bilateral adrenal resection was performed on a 29-year-old female suffering from hypertension and diabetes. Diabetes had been present since age 6. Daily insulin requirements were 70 to 85 u. Hospitalization for diabetic complications had occupied almost one quarter of the last 3 years. Hypertension, first recognized 8 years previously, had become severe and progressive. Transitory episodes of encephalopathy and myocardial failure characterized the last year. Preoperative blood

pressure averaged 260 mm Hg systolic, 140 mm diastolic. The heart was enlarged on X-ray. Renal function was approximately 40% of normal. Grade-4 retinitis was present. At operation, performed in 2 stages, not less than 98% of all adrenal tissue was removed. The immediate postoperative period was marked by paralytic ileus and transitory myocardial failure. Later complications included marked weakness, intermittent vomiting and one episode of frank crisis. The patient became ambulant during the 2nd week and was discharged on the 22nd postoperative day. Blood pressure fell to 110 mm Hg systolic, 80 mm diastolic 4 hr postoperatively. Elevated pressures were subsequently observed only during brief periods of desoxycorticosterone overdosage. X-ray 3 weeks postoperatively showed normal heart size. Cortical extract requirements initially exceeded 200 cc/day but decreased progressively during convalescence. Insulin needs roughly paralleled cortical extract dosage. Six weeks postoperatively the blood pressure was normal, the daily extract requirement was 15 cc and the insulin requirement 25 U.

Variation of choline acetylase content of brain in stressed and unstressed normal and adrenalectomized rats. RUVEN GREENBERG (introduced by F. A. HARTMAN) *Dept. of Physiology, Ohio State Univ., Columbus, Ohio*. In adrenal cortical insufficiency there is a generalized weakness whose mechanism is unclear. The nerve tissue conduction and maintenance is in some way involved. The choline acetylase content of acetone-dried brain powders made of pooled rat brains was measured by the rate at which the powders acetylated choline to form acetylcholine (method of Nachmansohn and Machado, 1943). The acetylations by brain extracts of adrenalectomized animals, stressed and unstressed, compared to sham-operated animals treated in the same way gave variable values in all the groups tested. The range of values between the adrenalectomized and sham groups and the stressed and unstressed groups overlapped. The effect of coenzyme (Lipmann, 1945) added to the acetylation substrate made for about 100% increase in synthesis. This increase did not vary significantly between the different preparations. The effect of adrenalectomy and stress, individually or combined, on brain tissue does not consistently change either the choline acetylase or coenzyme levels. These act on another part of the acetylcholine mechanism or on other mechanisms entirely.

Comparison of effects of a new ganglionic blocking agent and a new sympatholytic. K. S. GRIMSON and J. R. CHITTRUM (by invitation) and F. H. LOVINCIO *Dept. of Surgery, Duke University, Durham, N. C.* Similar studies have been performed testing 2,6 dimethyl diethyl piperidinium

Bromide (S. C. 1950) and 2-(N,p-tolyl-N-(m'-oxyphenyl)-aminomethyl)-imidazoline hydrochloride (C. 7337) in dogs and patients. In dogs effective doses of 1950 ranged from 5 to 10 mg/kg and of 7337 from 0.2 to 0.5 mg/kg. These amounts reduced blood pressures, prevented increase of pressure with occlusion of carotid arteries, prevented increase of blood pressure with anoxia, and reduced or blocked increase of blood pressure normally following stimulation of the central end of a divided vagus nerve. Pituitrin increased blood pressure after effective doses of either drug. Differences between the two drugs were apparent in other tests. Pulse rate decreased or did not change with 1950 and increased temporarily with 7337. Increase of blood pressure following adrenalin was accentuated after 1950 and blocked after 7337. With 1950 an increase of blood pressure occurred one or two minutes after discontinuing anoxia. This did not occur after 7337. Prostigmin following 1950 elevated blood pressure and restored responsiveness to vasomotor reflexes, but not following 7337. Chronic neurogenic hypertension in dogs was reduced by either drug. Increase of blood pressure with increased intracranial pressure was not prevented by effective doses of 1950, but was reduced or prevented by large doses of 7337. One to two mg/kg of either 1950 or 7337 injected intravenously in patients or 1 to 2 mg/kg of 7337 orally usually blocked increases of blood pressure with breath holding or with one hand in ice water, and warmed extremities. Results of additional clinical studies demonstrating differences of effects in patients will be presented.

Effect of spinal cord transection on blood acid-base balance during exercise. FRED S. GRODINS and DONALD P. MORGAN (by invitation) *Dept. of Physiology, Northwestern Univ. Medical School, Chicago, Ill.* Blood acid-base balance was studied in barbitalized dogs during electrically induced exercise of the hind limbs before and after section of the spinal cord at T10. The total CO_2 content and pH of arterial plasma were determined at rest and during a steady state of exercise, and the corresponding CO_2 tensions calculated from the Henderson-Hasselbalch equation. Percutaneous electrical stimulation with 60-cycle AC modulated at 100/min was used to produce oxygen consumptions of 2-7 times the resting value. In 11 experiments on 8 dogs, there was an average fall of 1.77 mm Hg in arterial pCO_2 during a steady state of exercise compared to the resting value. This mean difference was not significantly different from zero ($t = 1.65$, $P > 10\%$). In 14 experiments in 6 dogs after cord section, there was a mean increase of 3.94 mm Hg in arterial pCO_2 during a steady state of exercise which was highly significant ($t = 4.59$, $P < 1\%$). Oxygen consumptions during exercise

varied from 2.2 to 5.5 times the resting level in both groups. Plots of the acid-base pathways revealed essentially no change in the intact group for oxygen consumptions up to 2.5 times resting. Above this level, a metabolic acidosis pathway was followed. The sectioned group followed a pathway corresponding to a respiratory acidosis with a metabolic acidosis superimposed.

Distribution of microgram amounts of labeled thyroxine in the rat J. GROSS and C. P. LEBLOND (introduced by S. M. FRIEDMAN) *Dept of Anatomy, McGill Univ, Montreal, Canada*. Microgram amounts of labeled thyroxine were prepared by iodinating DL-diiodothyronine on a micro-scale or by extracting thyroxine from the plasma of rats previously given radio-iodine. The purity of the material was checked by isotope dilution. The distribution studies at 2, 24 and 72 hours after injection emphasized the predominant role of the liver in storing thyroxine and excreting it into the bile. Thyroxine is then mostly eliminated in the feces. In addition, large amounts are present in muscle and skin. These conclusions probably apply to the normal thyroid hormone because it was possible to demonstrate the presence of a thyroxine-like compound in thyroid tissue, plasma, liver and muscle under normal conditions.

Mucoproteins of gastric juice and mucus and the mechanism of their secretion ALLAN GROSSBERG (by invitation), S. A. KOMAROV and HARRY SHAY *Samuel S. Fels Research Inst, Temple Univ School of Medicine, Philadelphia, Pa*. Evidence has been obtained in support of earlier observations (Webster and Komarov, *J Biol Chem* 96:133, 1932) that at least two different mucoproteins are secreted by the gastric mucosa in the dog. One of the mucoproteins is characterized by the presence of both hexuronic acid and hexosamine, apparently in the proportions found in mucotin, sulfuric acid, and it is present both in alkaline mucus and in acid gastric juice; the second mucoprotein has hexosamine in its molecule but a much smaller proportion of hexuronic acid, if any at all. The prosthetic group of this latter mucoprotein may be identical with the 'neutral polysaccharide' described by Meyer, Smyth and Palmer (*J Biol Chem* 119:73, 1937). This mucoprotein is particularly characteristic of the alkaline mucus although it is also present in solution in acid gastric juice. Data were obtained on gastric secretions from dogs 1) in the resting state, 2) prior and subsequent to sham-feeding and 3) prior and subsequent to the injection of histamine. The analytical results indicate that 1) the secretion of the two mucoproteins is stimulated by sham-feeding but to different degrees, suggesting some difference in the secretory mechanisms involved, 2) the secretion of the two mucoproteins does not parallel that

of either pepsin or acid, 3) the effect on secretion with increased nervous stimulation is greatest for pepsin and the least for the hexuronic acid-free mucoprotein, with an intermediate effect for the other mucoprotein, 4) histamine does not stimulate the secretion of either mucoprotein.

Experimental production of hemorrhages in dogs with the use of aminopterin and their control with toluidine blue BURTON J. GROSSMAN, MARGARET H. SANDERSON, and WILLADENE EGNER (introduced by J. Garrott Allen) *Dept of Surgery, Univ of Chicago, Chicago, Ill*. The incidence of hemorrhage in patients with acute leukemia appears to be increased during the early period of aminopterin (4-aminopteroyl glutamic acid) therapy. The bleeding tendency is not present when the patient is in complete remission. Studies of the effect of aminopterin on the clotting mechanism in the normal dog were undertaken. These included the whole blood clotting time, prothrombin time, platelet count, observation for lysis of clot, the amount of protamine required to allow standard heparinized samples to clot and gross observations for the absence or presence of bleeding. Ten dogs, weighing 8 to 10 kg, were given a daily dose of 0.5 mg of aminopterin. Bleeding from the mouth and gastrointestinal tract occurred in all animals between the 8th and 11th day. At this time the platelet count was rarely below 150,000. The clotting times were normal or slightly prolonged. The prothrombin activity was unchanged except just before death. Increased lysis was not observed. The protamine requirement of standard heparinized blood was increased and this increase was out of proportion to the slight increase in the whole blood clotting time. Aminopterin was discontinued in 2 dogs and bleeding stopped after 4 days. Two dogs were given a single intravenous dose of toluidine blue (5.0 mg/kg of body weight) and bleeding stopped within 6 hr. Six dogs were continued on aminopterin and bleeding was controlled with a single injection of toluidine blue. The protamine requirement returned to normal. These animals died within 3 days without external evidence of hemorrhage, although 2 showed a hemorrhagic gastritis.

Clinical and physiological studies on thiomerin—a subcutaneously injectable mercurial diuretic J. GROSSMAN (by invitation), R. E. WESTON, I. S. EDELMAN (by invitation) and L. LEITER (by invitation) *Medical Division, Montefiore Hospital, New York City*. Approximately 200 subcutaneous injections of Thiomerin (Di-sodium-N-(γ -carboxymethylmercaptomercuri- β -methoxy) propyl camphoramate) in more than 40 patients produced changes in body weight and daily urinary volume and chloride excretion comparable to those following the injections of Mercuzanthin and Mercu-

hydrin. No systemic reaction or unusual local discomfort was observed. Only two reactions occurred at the site of injection, one consisted of ecchymosis in a patient with a bleeding diathesis and the other, of necrosis and sloughing of skin when Thiomerin was administered into an area of dependent edema. Both patients tolerated subsequent injections without ill-effect. In addition, simultaneous renal clearances of mannitol, PAH, sodium, chloride and uric acid, prior to and following intravenous Thiomerin or Mercuzanthin administration were determined. Except for the transient renal hemodynamic response to the Theophylline fraction of the Mercuzanthin, no significant differences between the diuretic effects of the two drugs were noted. In collateral studies on other tubular functions, both mercurials were found to have qualitatively and quantitatively similar depressive actions (i.e. decreased glucose T_x and renal PAH extraction). A comparison between the effects of intravenous and subcutaneous administration of Thiomerin in the same patients revealed no significant differences except in the time of response.

Action of neurohumoral agents in the human skin. M. I. GROSSMAN, HENRY D. JANOWITZ (by invitation) and R. R. SONNENSCHN (by invitation). *Dept. of Clinical Science, Univ. of Ill. College of Medicine, Chicago, Ill.* Physiological activities known to be mediated by neurohumoral agents are sweating by acetylcholine, piloerection by sympathin, and constriction of small blood vessels by sympathin. Intradermal injection of neurohumoral agents, in addition to stimulation of the effector organs just listed, stimulates certain nerve endings. These include a) sensory nerve endings for pain and itch which respond to histamine, b) motor nerves to the sweat glands and piloerector muscles which respond to acetylcholine and to nicotine, and c) certain dorsal root ramifying systems of nerves involved in vasodilation, hyperalgesia and itchy skin, all subject to stimulation by histamine. The effects under b) and c) can be classified as axon reflexes and the drugs listed with them are capable of initiating the reflex. The neurohumoral effector agent of these axon reflexes is known in the case of b) but not of c). The nicotinic effects can be blocked by tetraethylammonium (TEA). TEA also blocks the initiation of the axon reflex itchy skin surrounding a histamine wheal but it does not interfere with the local sensation of spontaneous itch. Pontocaine causes local anesthesia concomitantly with surrounding flare and hyperalgesia, suggesting that it selectively anesthetizes pain nerve endings but stimulates, directly or indirectly, nerves concerned in establishment of hyperalgesia and flare. The results with TEA in itchy skin and pontocaine in hyperalgesia

each demonstrates dissociation between the local primary sensation and the altered state of sensibility surrounding the injection site, thus supporting the hypothesis that the nerve fibers responsible for the altered state are not the same ones which convey the impulses for the spontaneous sensation.

Pressure changes in the pulmonary artery and aorta before and after ligation of the patent ductus arteriosus. ROBERT F. GROVER (by invitation), HENRY SWAN II (by invitation) and CLARENCE A. MAASKE. *Depts. of Physiology and Pharmacology, and of Surgery, Univ. of Colorado Medical Center, Denver, Colo.* Measurements of pulmonary artery and aortic blood pressures by puncture of the vessel at operation were made by means of resistance type strain gauges in 7 patients just before and after ligation of patent ductus arteriosus. Cardiac catheterization was employed in some of these patients and comparisons between pulmonary arterial blood pressure before and after opening the thorax were made. Immediately following ligation of the patent ductus arteriosus, the mean pulmonary artery pressure fell an average of 11 mm Hg for all patients. Excepting one patient, pulmonary artery pulse pressure was unchanged or only slightly increased. Following ligation of the duct, mean aortic pressure rose an average of 9.5 mm Hg, with an associated decrease in pulse pressure and an elevation of diastolic pressure. The contours of the pulse waves obtained while the ductus was patent were distorted in both the pulmonary artery and the aorta, probably resulting from pressure irregularities accompanying the abnormal blood flow. Ligation of the ductus generally gave pulse waves which approached the normal by reduction of these distortions. Good correlation was seen between the pulmonary artery pressures obtained by catheterization with the chest closed and those obtained by needle puncture with the chest open. More extensive comparisons of the normal pressures under these two conditions have been conducted on dogs.

Excitability changes in dorsal roots produced by electrotonic effects from adjacent afferent activity. HARRY GRUNDFEST and JONATHAN MAGNES (by invitation). *Dept. of Neurology, College of Physicians & Surgeons, Columbia Univ., New York City.* Electrical activity produced in the spinal cord of cats by stimulating a dorsal root induces electrotonic potentials in an adjacent, inactive root. According to Lloyd and McIntyre (*Federation Proc.* 7: 74, 1948), the first three phases, beginning negatively, are referable to the intramedullary afferent volley. Following, and overlapped by the terminating negative cord potential, they found a positive phase which is "an electrotonic extension of the potential of terminating collaterals" of the active root, with 3 msec

"half-life" This sequence of electrotonic potentials in the inactive root should cause modifications of excitability Stimulating electrodes on a root, close to the cord, tested the temporal changes of excitability during cord activity produced by conditioning stimulation of another root (cats, nembutal anaesthesia) Excitability was heightened during the first half msec after the conditioning volley Depression followed, lasting about 3 msec (maximum between 1.5 and 2.5 msec) Though of shorter duration, this depression is consonant with the existence of the positive electrotonic phase of Lloyd and McIntyre, and further demonstrates its reality The briefer duration may be ascribed to the rapid decay of the magnitude of the electrotonic potential in the extramedullary root These excitability changes have been observed in approximately 50% of the root pairs tested Variability is to be expected since the effect depends upon appropriate spatial relations between the intramedullary terminations of active and inactive fibers

Action of antitrypsin and antifibrinolysin on the first phase of the blood clotting reaction M MASON GUEST and THOMAS E NELSON (by invitation) *Dept of Physiology and Pharmacology, Wayne Univ College of Medicine, Detroit, Mich* The proteolytic enzyme, trypsin, is inactivated by either soy bean trypsin inhibitor or beef plasma antifibrinolysin (antiplasmin) Similarly, fibrinolysin (plasmin) is inactivated by both trypsin inhibitor and antifibrinolysin However, it has been found that only one of these inhibitors modifies the first phase of the blood clotting reaction Soy bean antitrypsin (crystalline material prepared by M Kunitz or the E C Loomis preparation) inhibits the first stage of the clotting reaction but antifibrinolysin (E C Loomis preparation) has no demonstrable effect upon the rate or character of clot formation The failure of antifibrinolysin to alter the clotting reaction suggests that this substance is different from soy bean antitrypsin and that proteolytic activity involving fibrinolysin is not a part of the clotting mechanism Investigation of the mode of action of antitrypsin in inhibiting the first phase of the clotting reaction has failed to reveal whether this substance acts as an antithromboplastin, an antiprothrombin, an anti-accelerator globulin or whether it acts by blocking the reaction of these substances Unless an inhibitor of thrombin formation can be shown to react at a measurable rate with one of the essential components of the first phase of the clotting reaction, reducing the potency of this component as a participant in the thrombin formation reaction, it does not appear possible to determine the point of action of such an interfering substance with methods available at present

A chromogenic steroid occurring in the urine of

males after muscular exertion H S GUTERMAN and L M KRAUS (introduced by R Levine) *Dept of Metabolic and Endocrine Research, Medical Research Inst, Michael Reese Hospital, Chicago, Ill* In performing pregnandiol determinations on urine samples from normal adult males according to the method previously published from this laboratory (*J Lab & Clin Med* 33 356, 1948), we found unusually large amounts of 'pregnandiol' present in some of the specimens Study of the chromogen revealed that on extraction it is present in the neutral non-ketonic steroid fraction when separated with Girard's reagent T Precipitation with digitonin indicated that a β -hydroxyl group might be present in the 3 position Since the Zimmermann reaction produced no color with the material after digitonin precipitation, it is believed that the presence of ketonic groups in position 3, 17 and 20 can be ruled out Bromine water was decolorized by an alcoholic solution of the material, and thus the presence of at least one double bond was indicated Thinking that the product might be 5 β androstenediol (3 β -17 α), we applied Bostock's fluorescent test (*Nature* 162 577, 1948) The material under study gave a green-blue fluorescence instead of the violet shade emitted by crystalline androstenediol Further studies are being conducted to characterize this urinary excretion product To date, the chromogen has been found only in males It appears to be of significance physiologically since it occurs in the urine in rather large amounts following muscular exertion

Lipide studies on whole anterior lobe, nuclei, and cytoplasmic particulates of lamb pituitary glands M H HACK (introduced by HUBERT R CATCHPOLE) *Dept of Pathology, Univ of Illinois, College of Medicine, Chicago, Ill* Lyophilized whole anterior pituitary of lamb and isolated cell fractions were assayed for free and protein-bound phosphatides according to the procedure described earlier (*J Biol Chem* 169 137, 1947) All cell fractions were obtained from homogenates prepared by Claude's method The large granule fraction was clearly separable into a buff-colored upper layer (mitochondria) and a white lower layer (eosinophilic granules) The identity of all fractions was confirmed histologically A new "acetal" was shown, by means of fuchsin sulfurous acid, to be present in the lipides extracted from the intact tissue and from each of the cell fractions by acetone, chloroform-methanol (1:1), and by benzene Histochemical confirmation was effected by means of the same technique applied to frozen sections of fresh tissue Acetal phosphatide was barely detectable The distribution of lecithin, cephalin, and sphingomyelin, expressed in μ M/gm dry weight, and the relative concentration of "acetal" were

	LECITHIN	CEPHALIN	SPHINGOMYELIN	'ACT. TAL.'
Whole tissue	75	58	17	+
Mitochondria	117	87	46	++
Eosinophilic granules	62	54	29	+
Microsomes	162	156	40	++++
Nuclei	43	38	0	+

One-third to one half of both lecithin and cephalin were present as lipoprotein. The sphingomyelin was largely free except in the microsome fraction. As in the case of cell fractions of rat liver, each fraction is here shown to be distinct in its phosphatide composition.

Myocardial metabolism in thiamine-deficient dogs studied by intravenous catheterization of the coronary sinus. D. B. HACKEL, W. T. GOODALE, R. P. JOHNSON (introduced by W. FLEISCHMANN). *Physiology Section, Medical Division, Army Chemical Center, Md.* Studies of the myocardial utilization of lactate and pyruvate were made in intact normal dogs and in dogs with acute and chronic thiamine deficiency, using the technique developed by Goodale et al. (*Am J Physiol* 152:340, 1948) for catheterizing the coronary sinus. Mean arterial pyruvate levels of 1.71 ± 0.13 mg % with coronary A-V differences of 0.76 ± 0.1 mg %, and lactate levels of 12.2 ± 0.96 mg % with coronary A-V differences of 5.3 ± 0.5 mg % were obtained in a series of normal nembutalized dogs.

Thiamine deficiency was produced by feeding a diet of canned dog food which had been autoclaved at 15 lb pressure for 12 hours to destroy thiamine, and supplemented by other synthetic vitamins of the B complex as well as Vitamin C. Anorexia and weight loss began in about 3 weeks, going on to convulsions with opisthotonus in 6 weeks. Complete relief of symptoms was brought about by the intravenous administration of 2 mg of crystalline thiamine. Chronic thiamine deficiency was produced by supplementing the above diet with daily intramuscular injection of 50-100 γ of crystalline thiamine. Small additions to, or subtractions from, the daily dose of thiamine were reflected in prompt weight changes. Acute deficiency resulted in decreased pyruvate utilization by the heart, with arterial levels up to 5.9 mg %, and almost no lactate utilization, despite arterial levels up to 60 mg %. Determinations done in the course of chronic deficiency revealed an uptake by the heart of both lactate and pyruvate which was below the expected normal range.

Effects of shock (low arterial blood pressure) on the pulmonary vessel pressures. FRANCIS J. HADDY (by invitation), GILBERT S. CAMPBELL (by invitation) and M. B. VISSCHER. *Dept of Physiology, Univ of Minnesota, Minneapolis, Minn.* Studies have been made on 64 dogs anesthetized

with nembutal, 30 mg/kg, on pulmonary artery and venous pressures measured with a Statham strain-gauge attached to flexible catheters. The average mean pulmonary artery and venous blood pressure measured relative to the intrathoracic pressure in 57 animals with peripheral arterial blood pressures above 100 mm Hg was 17.8 and 8.5 mm Hg respectively. In 7 animals with blood pressures below 100 mm Hg the pressures were 14.0 and 2.3 mm Hg respectively. Separating the group of 57 animals into those with peripheral arterial blood pressures between 100 and 150 mm Hg and those with pressures above 150 mm Hg one finds the pulmonary arterial and venous pressures in the former 40 animals to be 16.7 and 7.6 mm Hg respectively and in the latter 17 animals to be 18.5 and 9.7 mm Hg respectively. From these data there appears to be a positive correlation between the peripheral arterial blood pressure and the pulmonary pressures. From the same group of animals there was found to be no significant difference in the pulmonary pressures in males or females. When broken down into animals with body weights below 20 kg and those above 20 kg, there was noted no significant difference in pulmonary arterial or venous pressures in relation to weight. It is likely that a decreased cardiac output in shock is at least partly responsible for the observed effects.

Light flux constancy at the intensity lumin of the eye for total receptor cross-sectional areas. CHARLES HAIG. *Dept of Physiology and Biochemistry, New York Medical College, New York, N. Y.* By methods previously described (*Federation Proc* 7:47, 1948) it has been demonstrated that for homogeneous rod populations, i.e., for specific retinal regions, the product of the threshold intensity I and the retinal (stimulus) area A is constant for areas ranging from 0.04 degree subtense to 1.0 degree subtense. For homogeneous cone populations IA is constant for areas up to 0.5 degree subtense. Calculations have been made for a single eye to determine whether the luminous flux is constant for rod and cone cross-sectional areas in non-homogeneous retinal regions, e.g., the fovea as compared with peripheral regions. The data of Østerberg (1935) and Polyak (1941) on rod and cone diameters and population densities in various retinal regions provide the means for computing A in terms of total rod or cone cross sectional area in a given retinal (stimulus) area. For rods it is found that IA so computed is constant from 2 degrees out to 6 degrees from the foveal center. From 10 out to 18 degrees IA is again constant at twice the value found closer to the center. For the inner cone segments it is found that IA is constant from the foveal center out to 10 degrees in the periphery, beyond which it rises to a value twice as great at 18 degrees in the periphery. These calculations for

one eye indicate that measurements on several eyes will reveal that the luminous flux actually incident upon the receptor elements is constant for the entire central area

Oxygen consumption of liver slices from febrile and normal rabbits V E HALL, J FISHGOLD (by invitation) and R GRANT (by invitation) *Dept of Physiology, Stanford Univ, Stanford, Calif* Observations published in the literature suggest that during the early stage of fever the temperature in or near the liver rises earlier and in greater degree than the rectal temperature This has been interpreted as showing that an increase in liver metabolism contributes significantly to the febrile temperature rise However, measurement *in vitro* of the O_2 consumption of liver slices taken from rabbits 15 minutes after the injection of typhoid-paratyphoid vaccine (a time when the liver temperature was said to have been rising) yielded a mean Q_{O_2} value of 3.71 as compared with a mean of 4.32 for untreated controls The difference is statistically significant and shows that the liver metabolism under the conditions employed is depressed in tissue taken from febrile animals If the heat production of the liver is increased early in the fever produced by bacterial pyrogen, it is attributable to factors inoperative when the liver metabolism is studied *in vitro*

Studies of a case of congenital heart disease with cyanotic episodes W F HAMILTON, J A WINSLOW (by invitation) and W F HAMILTON, JR (by invitation) *Depts of Physiology, Medicine and Roentgenology, Univ of Georgia School of Medicine, Augusta, Ga* The patient, an 18-year-old girl had been through grammar school and was in fair health though she had been subject to spells of cyanosis and fainting Clinical impression was Tetralogy of Fallot During two cardiac catheterizations she became intensely cyanotic and irrational but not unconscious Pressures in vein and auricle were normal Rt ventricle 100/0, Aorta 65/30, Femoral 85/40, Pul art 5/0 These data confirm patency of the interventricular septum and pulmonary stenosis Blood in right ventricle, pulmonary artery and femoral artery were 10-20% saturated with A-V difference between 0 and 1% Oxygen consumption varied around 200 cc/min though for 2 minutes it apparently ceased When not cyanotic O_2 consumption was steady at 150 cc/min with blood pressure 140/80 and arterial O_2 saturation of 70-80% Cardiac index at this time was 2.04 L and circulation time 14 sec by dye injection method Cyanotic episodes resulted from activity, emotion, or vasodilator drugs and were accompanied by lowered arterial pressure, oxygen saturation, and O_2 uptake

Various explanations may be considered The favored one is that the functionally single ventricle

pushes blood out into the two circulations as governed by their respective resistances In this case the pulmonary resistance seems to be very high and a lowering of the systemic resistance causes increasing amounts of the ventricular output to pass out of the aorta and less or even no blood to perfuse the lungs This caused temporary reduction or even cessation of oxygen uptake and varying degrees of arterial unsaturation

Measurement of cardiac volume in the dog from X-ray shadow area W F HAMILTON, JR (by invitation) and PHILIP DOW *Depts of Physiology and Roentgenology, Univ of Georgia School of Medicine, Augusta, Ga* An effort has been made to standardize a cardiac-volume method for use during experiments on circulatory dynamics After sacrifice experiments, P-A roentgenograms were taken of the closed chests of 53 supine dogs The chests were opened immediately, the great vessels were tied off, and actual cardiac volumes were measured by immersion These volumes were compared with the areas of corresponding shadows on the films (corrected for tube distance)

No formula found for dog or human hearts approximated the relationships obtained, so a new formula was empirically derived The best fit was given by an equation of the form $Vol = a \left[\left(\frac{s}{l} \right)^{\frac{1}{b}} \times area \right]^b$, in which s is the shorter and l the longer dimension of the shadow Parallel projection of a prolate spheroid should give $a = 0.75$ and $b = 1.5$ The oversimplifications in this hypothesis are indicated by the empirical results $a = 0.4$ and $b = 1.7$ This formula predicts the volume of dog hearts larger than 100 cc with an average error of 5% (maximum 13%) Predictions for smaller hearts are consistently too high or too low (average error 22%) Most of these, however, were from puppies and do not fall in the range of animals ordinarily used in the experiments Supplementary projections were not employed to increase accuracy, since such would not be feasible during experiments for which the method is devised

Effects of di-isopropyl fluorophosphate (DFP) on electroencephalogram and cholinesterase activity J L HAMPSON (by invitation), C F ESSIG (by invitation), ALICE WILLIS (by invitation), and H E HIMWICH *Medical Division, Army Chemical Center, Md* Di-isopropyl fluorophosphate (DFP) was injected into one common carotid artery of curarized rabbits receiving artificial respiration A needle electrode was inserted through the skull over each cerebral hemisphere The electroencephalographic changes were observed with the Grass apparatus and cholinesterase activities of various parts of the brain were subsequently determined by the method of Michel

The cerebral parts included the cortex, midbrain, medulla and cerebellum. The electroencephalographic changes were placed in five categories of increasing severity: 1) No permanent change from the control; 2) Early permanent, alterations including slight increases in regularity, amplitude and rate; 3) Intermediate, phase 1, marked changes limited however chiefly to the right hemisphere; 4) Intermediate, phase 2, marked changes (short of grand mal) appearing in both cerebral hemispheres; 5) Continued grand mal-like activity on both sides.

In general, with succeeding categories, cholinesterase activity decreased more profoundly until the fourth category was obtained. Statistical analyses of cholinesterase activities revealed however no differences between no permanent and early permanent. But between early permanent and intermediate phase 1 all differences were significant while between intermediate phase 2 and grand mal no significant differences were noted. Though a rough correlation between cholinesterase activities and abnormalities of the electroencephalogram was observed the extent of the fall in the cholinesterase activity is not sufficient to explain the degree of the abnormality in the electroencephalogram.

Hemoglobin iron as a stimulus for the production of ferritin. JOHN K. HAMPTON, JR. (introduced by H. S. MAYERSON) *Dept. of Physiology, School of Medicine Tulane Univ., New Orleans, La.* Studies by Granick and others have shown that iron is stored chiefly in the liver, spleen and bone marrow as the protein-iron compound, ferritin. Evidence is also available to indicate the presence of ferritin in crystallizable amounts in the kidneys of the cat, dog, and human. Its presence in horse kidney has been demonstrated in minute amounts. The present experiments extend these findings to the mouse and rabbit kidney. It has proved difficult to crystallize ferritin from the kidney by the usual CdSO_4 method from animals on the usual laboratory regime or after minimal doses of hemoglobin. However, when increasing amounts of hemoglobin are injected intraperitoneally, correspondingly larger amounts of ferritin appear in the mouse and rabbit kidneys. With optimal doses of hemoglobin iron, all animals show the presence of ferritin in their kidneys. More ferritin is found in the liver than in the kidney when small doses of Hb are used, but when larger (and optimal) doses are used, the amounts of ferritin in the liver and kidney tend to be similar. Experiments in progress are designed to investigate these relationships in the human kidney.

Effect of stimulation of sympathetic nerves on capillary filtration. ESTHER HARDENBERGH and JAMES V. MALONEY, JR. (introduced by WILLIAM

H. FORBES) *Dept. of Physiology, Harvard School of Public Health, Boston, Mass.* Variations in lymph flow and composition have been used in studying the effects of stimulation of sympathetic nerves on capillary filtration in the head and hind limb regions of the dog. After cannulation of the regional lymphatic vessels, control collections were made during standardized passive movement of the part. The effectiveness of sympathetic nerve stimulation was indicated by skin temperature changes. The quantity of lymph was increased 50-100% over control values during stimulation, while the protein concentration was somewhat reduced. In the hind paw most of the increase occurred in the first 10 minutes of stimulation. Such an increase appears surprising since the filtration pressure should be decreased by the intense precapillary vasoconstriction produced by the stimulation. Several explanations have been considered: 1) a lymph reservoir which is somehow 'squeezed out' is rendered unlikely by the fact that there is no post-stimulation decrease in flow; 2) increased post-capillary resistance is rendered unlikely by the fact that lymph flow is increased even when capillary pressures have already been raised by venous obstruction; 3) gross changes in capillary bed due to closing of arterio-venous anastomoses does not affect the result, since the increase in lymph flow occurs when the paw is immersed in a water bath at 15 degrees Centigrade. There appears to be an increase in capillary filtration during stimulation of sympathetic nerves, but the exact mechanism is not established.

Renal excretion of creatinine in man. KENDRICK HARE, HAROLD GOLDSTEIN (by invitation), HENRY L. BARNETT (by invitation), HELEN McNAMARA (by invitation), and RUTH S. HARE (by invitation) *Dept. of Pediatrics, The New York Hospital and Cornell Univ. Medical College, New York City.* Using the adsorption-elution method of Hare and Hare for creatinine determinations, we have compared the renal plasma clearance of endogenous creatinine with simultaneous inulin clearances in 24 observations on 22 normal subjects ranging from 1 week to 40 years of age. Each observation included 3-9 clearance periods of 10-20 minutes duration. The average creatinine/inulin clearance ratio was 1.03 with a range of 0.82-1.26. The clearance of exogenous creatinine, given by mouth or by vein, was similarly studied in 9 experiments on 6 normal subjects for a total of 76 periods. The average creatinine/inulin clearance ratio was 1.08 with a range of 0.97-1.20. In chronic renal insufficiency the clearance of both endogenous and exogenous creatinine is elevated above the inulin clearance to give creatinine/inulin clearance ratios of 1.29-2.64.

Determination of creatinine in blood and urine

RUTH S HARE (by invitation) and Kendrick Hare *Dept of Pediatrics, The New York Hospital and Cornell Univ Medical College, New York City* Pure creatinine solutions of 0.05-0.40 mg % develop maximal color with alkaline picrate within 20 minutes and remain stable, tungstic, picric, trichloroacetic acid or cadmium, zinc or iron filtrates of serum or plasma continue to develop color, as measured at 500 μ in a Beckman spectrophotometer, for 2 hours or more. The interference of the non-creatinine chromogens can be avoided by adsorbing the creatinine on Lloyd's reagent and eluting with alkaline picrate solution (Borsook, *J Biol Chem* 110:481, 1935). This procedure gives 98-101% recovery of creatinine added to serum or urine, synthetic creatinine, containing radioactive carbon, is completely adsorbed by Lloyd's reagent from urine and from plasma filtrates. Approximately 80% of the material in normal human serum giving the Jaffe reaction is creatinine, 90-100% of the urinary chromogen is creatinine. The serum creatinine falls from 0.8 mg % in the first week of life to 0.3 mg % at one month, begins to rise at two years and reaches the adult level of 0.8 mg % at 15-18 years. In chronic renal insufficiency, the serum creatinine constitutes 90% or more of the total chromogen.

Effects of lanatoside C upon ectopic ventricular

activity following coronary occlusion A SIDNEY HARRIS and ROBERT H KOKERNOT (by invitation) *Baylor Univ College of Medicine, Houston, Texas* The threshold intravenous dose of lanatoside C for the production of ventricular ectopic discharges in unoperated dogs anesthetized with morphine and light barbitol sodium was found to be about 0.125 mg/kg when administered in two equal parts separated by one hour. The anterior descending arteries were ligated near their origins. The ectopic rhythms produced by coronary occlusion were present during the period from about 8 hours to 3 days following the operation. Lanatoside C in varied doses was given at intervals ranging from 10 hours to 13 days after occlusion. When given during the first 4 days, the ectopic threshold dose, either in the presence or absence of a preexisting ectopic rhythm, was found to be lowered to 0.1 mg/kg or slightly less, i.e., about 70 to 80% of the control threshold. After 4 days the ectopic threshold was not lower than the preoperative control except in four dogs which at the beginning of the test exhibited electrocardiographic patterns interpreted as aberrant conduction. Tests with 0.06 mg/kg produced no ectopic discharges at any time. Evidence from other sources indicates that this dose is more than sufficient to increase work output in a failing heart, therefore these findings may indicate that danger associated

with ventricular ectopic activity following coronary occlusion is not increased by conservative use of lanatoside C. The dose required to produce retching and vomiting was consistently lower than that which produced ectopic beats in unanesthetized dogs.

Late development of ectopic ventricular activity following coronary occlusion

A SIDNEY HARRIS and ROBERT H KOKERNOT (by invitation) *Dept of Physiology, Baylor Univ College of Medicine, Houston, Texas* The anterior descending arteries of the hearts of 25 dogs were ligated proximal to any large branch. The dogs were anesthetized with morphine and a minimal dose of barbitol sodium (180 mg/kg). Under such anesthesia the hearts are fully susceptible to the development of ventricular ectopic systoles. To prevent the occurrence of rapid ectopic discharges and death via ventricular fibrillation within the first 10 minutes following occlusion, the occlusion was made in 2 stages. Partial occlusion was followed 30 minutes later by total obstruction. No animals so operated were lost during the early period in which, following one-stage occlusion, the usual mortality is near 50%. Frequent electrocardiographic observations and records were made. Ventricular ectopic systoles began to appear in significant numbers after periods of 6 to 12 hours in the different experiments. Eight hours was the most common time for the beginning of the rise of ectopic activity beyond an occasional premature beat. At the end of 12 hours ectopic activity was prominent in every animal studied through the period except one that maintained a sinus rhythm. One animal had a moderate ectopic frequency from the time of occlusion. Typically, a maximal ventricular tachycardia was reached between 10 and 21 hours, and ectopic activity persisted from 2 to 4 days. Large highly necrotic gross infarcts were found in almost all hearts upon sacrifice (10 to 22 days).

The uptake of radiocalcium by the skeleton

HAROLD E HARRISON and HELEN C HARRISON (introduced by NATHAN W SHOCK) *Dept of Pediatrics, The Johns Hopkins Univ Medical School, and Pediatric Division, Baltimore City Hospitals, Baltimore, Md* The equilibrium between skeletal calcium and calcium of the body fluids was studied by determinations of the uptake of Ca^{45} by the bone of animals given $\text{Ca}^{45}\text{Cl}_2$ solution. Groups of rachitic rats, rachitic rats treated with vitamin D., and control rats were used. During the experimental period, some of the animals received a high calcium diet and others, a diet low in calcium. The animals were sacrificed at intervals from 2 hours to 2 weeks following administration of Ca^{45} . Measurements of the radioactivity of the calcium of blood serum and the skeleton were made, and the

ratio of Ca^{45} to Ca^{40} expressed in terms of counts per second per mgm calcium. Equilibrium between body fluid calcium and bone calcium occurs rapidly and in some instances is reached by 72 hours. Epiphyseal bone calcium attains equilibrium with serum calcium more rapidly than that of diaphyseal bone, and the rate of equilibrium is not related to the rate of bone salt deposition. The uptake of Ca^{45} by the skeleton is not a measure of new bone salt addition. Following introduction of Ca^{45} into the body fluids exchange of Ca^{40} of the skeleton for Ca^{45} of the body fluids occurs until the ratio of these isotopes is the same in both phases.

Effect of cinchophen on the secretory activity of Brunner's glands. K. HARTIALA (by invitation) and M. I. GROSSMAN, *Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago, Ill.* Experiments were carried out to determine whether cinchophen when given in doses known to produce ulcers in the dogs has any effect on the Brunner's gland secretion. The juice was obtained either from pouches or everted flaps made from the first part of the duodenum. The original blood supply and nerve connections were maintained. 200 mg of cinchophen per kg of body weight was given orally daily for 4 days to 6 dogs. It was noted that the basal secretion was markedly affected after the cinchophen administration. The alterations took place usually after the second day and in some cases even in the first day after the ingestion of drug. The total output of juice diminished to less than a half of the volume of that of the controls. Both the alkali content and the pH were decreased although the great decrease in the total alkali output was mostly a result of the decreased amount of juice secreted. The mucous content was not analysed but in the cases most affected by cinchophen the juice became thinner and was waterish. There was also a decrease in the volume of juice secreted in response to a standard test meal (Pard, milk, white bread).

Inhibition of activity of visual receptors by illuminating nearby retinal areas in the *Limulus* eye. H. K. HARTLINE, *Dept. of Biophysics, Johns Hopkins Univ., Baltimore, Md.* and the *Johnson Research Foundation, Univ. of Pennsylvania, Philadelphia, Penna.* A discharge of impulses in a given single optic nerve fiber from the eye of *Limulus* can be elicited only by the illumination of some one particular facet of the eye, containing the receptor that gives rise to that nerve fiber. It has been found that illumination of neighboring areas of the eye, while unable to initiate activity in a given fiber, can nevertheless affect the responses to illumination of its receptor. A steady discharge of impulses was obtained by a small spot of light confined to the appropriate facet, illuminating nearby facets then caused a

slowing of the discharge. The frequency dropped markedly at first (after a latency of 2-4 sec.), then rose somewhat, on turning off the adjacent illumination, the frequency increased and within 4 sec. had recovered its original value. This inhibitory effect was greater the higher the intensity and the greater the area illuminated, it decreased with increasing distance between the particular facet and the neighboring area illuminated. The role of this effect in enhancing visual contrast is obvious: brightly illuminated areas inhibit the activity from dimly lighted regions more than the latter inhibit the activity from the former. In *Limulus*, axons of the receptor cells enter a non-ganglionic plexus before leaving the eye via the optic nerve, by recording from fibers between the receptors and the plexus, it could be shown that the inhibitory effect of adjacent illumination depended on the integrity of the nervous connection between the receptor and the plexus.

Effects of experimental anaemia on the circulation in dogs. J. D. HATCHER (by invitation), F. SUNAHARA (by invitation), J. C. VAN NOORDWIJK (by invitation) and O. G. EDHOLM, *Dept. of Physiology, Univ. of Western Ontario, London, Canada.* The cardiac output, peripheral blood flow, and blood volume have been measured before, during and after the development of anaemia by small daily haemorrhages. While the haemoglobin level is falling, there is a slight decrease in cardiac output and right auricular pressure. This is considered to correspond to the hypodynamic phase of Howarth and Sharpey-Schafer. When the haemoglobin level falls to 50% or less of the initial value, the cardiac output and the right auricular pressure increase abruptly. At this stage, the daily haemorrhages are stopped, the cardiac output rises to reach a peak value averaging 160% of the pre-haemorrhage outputs. The greatest cardiac output is obtained approximately 2-4 days after the cessation of haemorrhage, at a time when the haemoglobin value is still at its lowest level. The hyperdynamic phase persists for 2-5 days and then the cardiac output declines reaching a normal level at a time when the haemoglobin level has recovered to 75-500% of the pre-haemorrhage value. Oxygen consumption is increased significantly during the hyperdynamic phase. The saturation of the mixed venous blood diminishes during the hypodynamic phase and rises in the hyperdynamic phase. The total blood volume decreases approximately 25% during the period of haemorrhage, while the plasma volume remains relatively unchanged. The plasma volume and total blood volume rise during the hyperdynamic phase, but the overall changes are not marked.

Consciousness and reflex potentialities of dogs during immersion hypothermia. H. O. HARTNICK

and A H HEGNAUER *Dept of Physiology, Boston Univ School of Medicine, Boston, Mass* Random observations on CNS activity on more than 150 dogs during immersion hypothermia are worthy of record The choice of pre-cooling anesthetic is of paramount importance Light ether or cyclopropane, withdrawn periodically, reveals with considerable accuracy the rectal temperature (R T) at which consciousness is abolished by cold narcosis This varies from 22.5° to 26° R T (24.5° to 28° cerebral temperature) The 22.5° observation was made during rewarming, at which point the dog righted his head and attempted standing Failure to stand was due to intense shivering and limb spasticity In one experiment after withdrawal of cyclopropane the dog strained at his bonds at R T 20.5 and again at 15.8 (cerebral temperatures 23.5° and 20.5° resp) Spontaneous reflex activity was noted as follows shivering, which under pentothal-Na is rarely observed below 21°, may under light ether be evident at 16° or 17°, vocalization (whimpering, whining, moaning) to 17°, barking in one case at 20°, spontaneous jaw, head or limb movements to 17° (exceptional case 15.8°), spontaneous respiration in one preparation at 11.8° (2/min), in 2 preparations below 13.6°, and a total of 12 dogs breathed at R T 16° or lower (cerebral temperature 20° or less) After pentothal alone consciousness during rewarming is never observed at rectal temperatures below the thirties, although the last injection may have been made some 2 or 3 hours earlier Furthermore, the temperatures at which the above reflexes disappear are all higher than those recorded

Ascorbic acid metabolism in patients with certain gingival disturbances RAYMOND HAYES (by invitation), WALTER M BOOKER and MARIANNA B SEWELL (by invitation) *Dept of Oral Medicine, College of Dentistry, and Department of Pharmacology, College of Medicine, Howard Univ, Washington, D C* Fasting plasma ascorbic acid in patients suffering with various types of gingivitis was found to be significantly lower than in a control normal group of patients The cell ascorbic acid levels of the blood in the same patients was equal to or in some instances greater than that of the control groups By and large, only when there is deficiency in both plasma and cell ascorbic acid were clinical signs of gingivitis striking or even present When ascorbic acid is administered (orally or intravenously) to patients and to normal subjects, the plasma level rises faster than the cell level, suggesting something of a barrier between the plasma and the cells Many of the patients excrete ascorbic acid in the urine in greater quantity and at faster rate than the normal subjects Efforts are in progress to compare the rate of urinary excretion with the clinical state of the

patient regarding gingivitis There are presented some outstanding examples of extremely low kidney threshold regarding ascorbic acid excretion among some of the patients The low kidney threshold to ascorbic acid apparently bears no relationship to the general nutritional state of the patient in terms of plasma proteins and blood sugar Diminution of serum chlorides upon the intravenous or oral administration of 100 to 300 mg of ascorbic acid is seen in all patients and in normal subjects

Effect of pituitary adrenocorticotropin on the plasma hypertensinogen concentration of dogs F W HAYNES, P H FORSHAM (by invitation), G W THORN and L DEXTER (by invitation) *Dept of Medicine, Harvard Medical School, and the Medical Clinic, Peter Bent Brigham Hospital, Boston, Mass* The effect of purified hog anterior pituitary adrenocorticotropin (ACTH, Armour) on plasma hypertensinogen was studied in trained, unanesthetized dogs ACTH, without appreciable contamination with thyrotropic, gonadotropic and growth hormones, but containing posterior pituitary activity up to 0.08 units per mg, was used in daily doses equivalent to 20 to 80 mg of Armour standard (LA-1-A) Hypertensinogen was determined by incubation of plasma with an excess of hog renin and cat assay of the hypertensin formed Data are based on the mean of at least 4 satisfactory assays on each plasma sample In 5 experiments on normal dogs receiving ACTH, either intramuscularly in divided doses over a 3-day period or intravenously as an infusion over several hours, the hypertensinogen concentration increased for several days and then returned to its previous level The control hypertensinogen levels of these dogs (1.7-3.1 cat units per cc of plasma) were increased by 0.6 to 2.9 cat units after ACTH Adrenocortical activation was suggested by a significant fall in eosinophils during ACTH administration ACTH failed to influence the hypertensinogen concentration in an adrenalectomized dog Hypertensinogen and eosinophil levels failed to show a significant change in control experiments designed to test the effect of sampling and of posterior pituitary contamination in a normal dog

Corticosteroid release from the isolated adrenal gland OSCAR HECHTER *Worcester Foundation for Exptl Biology, Shrewsbury, Mass* Isolated beef and sheep adrenal glands were perfused with homologous citrated blood Following the addition of adrenocorticotrophic hormone (ACTH) it was consistently observed that the venous blood contains an increased amount of formaldehydegenic substances (F S) in the neutral chloroform fraction designed to concentrate adrenocortical steroids These F S may be obtained from perfusates by extraction of the acidified blood with chloroform or

chloroform-ethanol mixtures or by chloroform extraction of dialysates of blood. With both methods, it has been found that there is either slight or no release of F S from glands perfused in the absence of ACTH. In 5 experiments with beef glands, following the addition of ACTH (20 to 200 μg per cc) there is an average release of F S corresponding to 26 μg (Range 10–42) equivalents of 11-desoxycorticosterone per minute per gland (weights 15–20 gm). In some glands, increased F S output was maintained for as long as 4 hours. In 2 experiments with sheep glands the release of F S following ACTH was of the same order of magnitude per gm gland. Assay of fractions, from four different beef adrenal persates for glycolytic activity, using the method of Olson *et al* (*Endocrinology* 35: 430, 1944) revealed two to be active, 50 and 100 μg F S equivalent to 50 and 60 μg Compd E Kendall respectively. The other two extracts were inactive at a level of 100 and 400 μg F S. In 4 experiments, despite wide variations in dosage, the intrarterial injection of epinephrine consistently failed to release F S from perfused glands. The significance of these *in vitro* findings will be discussed.

Temperature gradients in dogs in immersion hypothermia. A. H. HEGNAUER, *Dept of Physiology, Boston Univ School of Medicine, Boston, Mass.* The procedure followed in this laboratory for producing rapid, drastic hypothermia in dogs (after preliminary anesthetization and required surgery—animals in supine position on dog board) is immersion to the neck in an iced bath at 2°–4° C. With the head end of the board a few inches higher than the foot the portion of the animal unimmersed includes the head, the ventral third of the thorax and all but the nape of the neck. The proportion of exposed to submerged body is approximately that obtaining when a man floats on water in a 'Mae West' life vest. Under these conditions there occur not only core to surface, but also cerebral (C T) to rectal temperature (R T) gradients. Cerebral, mediastinal (M T), right heart blood (B T) and rectal temperature as well as muscle and skin temperature (or some combination of these) is recorded continuously via Speedomax (Leeds & Northrup). Plotting R T linearly as abscissa and deviations therefrom as ordinate, curves are obtained from which the following conclusions may be drawn: 1) C T is higher than R T, and progressively so with decreasing R T. 2) M T, initially slightly below rectal, is equal to the latter at R T 36° and subsequently parallels the curve for C T, about 1° lower. 3) Right heart blood (B T) is initially $\frac{1}{2}$ ° less than R T, falls further on immersion and then crosses R T when latter reaches 28°, and thence parallels C T and M T. When R T reaches 12° the C T, M T, and B T are 18°, 17°, and 16° respectively. Skin temperature from submerged area rapidly approaches bath temperature. Muscle temperature (hind leg) cools more slowly than skin, but much more rapidly than R T.

Pulse rate and blood pressure of dogs in immersion hypothermia. A. H. HEGNAUER and H. O. HATERIUS, *Dept of Physiology, Boston Univ School of Medicine, Boston, Mass.* Average pulse rate (P R) and blood pressure of 18 lightly anesthetized dogs (sodium pentothal) obtained during cooling in an iced bath (2°–4° C) were plotted against the right heart blood temperature (B T). A thermocouple was placed in right heart with continuous recording on Speedomax in degrees centigrade. The pre-immersion P R was 155. Disregarding the initial abrupt increase and decrease in rate from moment of immersion to B T 36° the rate follows a smooth regression curve to B T 15° at which point the P R is 15. Between B T 36° and 26° the apparent $Q_{10} = 2.1$, and increases to 5.5 between 25° and 15°. Plotting $\log P R$ vs $1/T^\circ$ abs revealed no sharp breaks. Tentatively as a working hypothesis a regression curve ($Q_{10} = 2.5$) was constructed, passing through the origin of the experimental curve (at P R 155). The computed curve again coincides with the P R curve at B T 19°, when the two follow a similar course. Between B T 39° and 19° the P R curves lies well above the computed curve, and it is suggested the elevation is a measure of sympathetic activity at these temperatures.

Action of heparin on protoplasmic gelation. L. V. HELLBRUNN, *Dept of Zoology, Univ of Pennsylvania, Philadelphia, Penna.* Earlier studies from this laboratory have indicated that the gelation of protoplasm is similar to the clotting of blood. Before a cell divides, its protoplasm gels and the appearance of the mitotic spindle is preceded by the so-called mitotic gelation. In the egg of the worm *Chaetopterus*, this mitotic gelation is prevented by immersion of the eggs in dilute solutions of heparin. Suppression of mitotic gelation results in an inhibition of cleavage. Similar results were obtained with frog eggs. The initiation of mitosis in these eggs tends to be prevented by the presence of heparin. There is an indication, therefore, that in living cells the colloidal behavior of the protoplasm is to some extent at least determined by the equilibrium between thrombin-like substances and heparin or heparin-like substances. Actually, in the *Chaetopterus* egg there is direct evidence for the presence of either heparin or some substance which stains like it.

The role of the Sertoli cell in the testis-pituitary axis in men. CARL G. HELLER, WILLIAM O. MADDOCK (by invitation), EDWIN C. JUNGCE (by invitation), and WARREN O. NELSON, *Depts of Medicine and Physiology, Univ of Oregon Med*

School, Portland, Oregon, and Dept of Anatomy, Univ of Iowa Med School, Iowa City, Ia The supporting cell of Sertoli is claimed to elaborate a non-androgenic hormone whose role is to inhibit the secretion of pituitary gonadotrophin. If this is correct, no alterations in gonadotrophic output should occur in instances in which Sertoli cells remain intact and germinal cells disappear. Testicular biopsies in men with azoospermia or oligospermia have revealed 34 cases in which at least some of the seminiferous tubules were devoid of all germinal elements except the Sertoli cell. Such tubules constituted 10% to 100% of the total seminiferous tubules and were associated with either hyalinization of the basement membrane or with tubules having germinal cells present, or both. Despite the intact Sertoli cells, elevation above normal in the gonadotrophin titers was regularly encountered in cases having less than 70% of active tubules. A roughly direct proportionality exists between the height of the titers and the degree of spermatogenic failure. This suggests that the Sertoli cell does not enter into testis-pituitary regulation, but suggests that during active spermatogenesis, gonadotrophins are inactivated in the presence of incomplete or failing spermatogenesis, a smaller amount of gonadotrophin is utilized, allowing for a rise in titer.

Adrenal cortical function and the effect of adrenocorticotropin in myasthenia gravis LEON HELLMAN (introduced by R. E. WESTON) *Medical Division, Montefiore Hospital, New York City* Hypertrophy of the lymphoid tissues and the thymus occurs in many patients with myasthenia gravis and in patients and animals with adrenal cortical hypofunction. Atrophy of the thymus can be induced in animals by the administration of adrenocorticotropin (ACTH). Since surgical removal of the usually enlarged thymus appears to benefit patients with myasthenia gravis, ACTH was administered to patients with myasthenia gravis in an attempt to 'hormonally' remove their thymuses. Several adrenal cortical functions were studied in these patients. The subjects were 5 patients with classical myasthenia gravis, responsive to prostigmine, none of whom had demonstrable thymic tumors. Injection of 35 mg ACTH (Armour) produced the responses characteristic of normal adrenal function. Circulating eosinophils fell over 60%, circulating lymphocytes fell over 40%, and uric acid-creatinine ratio increased over 70%. Sodium restriction to 100 mg daily for 10 days did not produce adverse effects or a tendency to lose sodium. 17-ketosteroids were normal in 3 of the patients tested. 0.2 mg epinephrine produced a 60% fall in circulating eosinophils. Administration of 125 mg ACTH daily for 4 days to 3 patients produced the changes associated with adrenal

cortical hyperfunction. If anything, the patients' clinical courses were adversely affected. This may be related to the potassium diuresis or the altered intracellular-extracellular Na-K ratio produced by ACTH. Administration of 500 mg synthetic Compound A (supplied by Merck and Company) to 2 patients produced a temporary dramatic restoration of muscular function.

Effect of adrenocorticotropin in human chronic lymphatic leukemia LEON HELLMAN (introduced by R. E. WESTON) *Medical Division, Montefiore Hospital, New York City* The administration of adrenocorticotropin (ACTH) produced a decrease in circulating lymphocytes and eosinophils in the human and adrenal cortical extracts are said to produce a lymphopenia and regression of lymphoid tissue in mice with spontaneous lymphatic leukemia. Mice with marrow aspiration were injected with 25-125 mg ACTH (Armour). The initial peripheral white count ranged from 3000-400,000 WBC, the majority being in the upper range, and the lymphocytes were usually over 90% of the total WBC. After ACTH, no leukemic patient showed a significant decrease in circulating lymphocytes, but all except 2 showed a fall in circulating eosinophils of over 60%. Initial eosinophil counts tended to be low. Total WBC remained unchanged while the uric acid-creatinine ratio was usually increased over 60%. No changes were observed in lymph nodes removed from two patients before and after the ACTH. In contrast, 25 normal controls given simultaneous injections of equal amounts of the same ACTH, showed a fall of over 40% in circulating lymphocytes, a decrease of over 60% in circulating eosinophils, an increase in total WBC of 50-120%, and an absolute polymorphonuclear leukocytosis with a shift to the left. 25 cc lipo-adrenal cortex extract (supplied by the Upjohn Company) produced changes in normals and leukemics similar to ACTH. The effect of ACTH on the peripheral blood may be useful as a biologic test for lymphoid leukemia and has been used successfully in two patients with aplastic anemia. It is suggested that mouse lymphoid leukemia differs significantly from the human variety.

The concurrent feeding of amino acids RICHARD HENDERSON (by invitation) and ROBERT S. HARRIS *Nutritional Biochemistry Labs, Massachusetts Inst of Technology, Cambridge, Mass* Groups of weanling rats were fed a basal diet containing casein (30), corn starch (55.4), hydrogenated fat (10), tryptophan (0.17), histidine (0.13), salt mixture (4) and vitamin mixtures (0.36). Control groups received the basal diet + 1% lysine. Test groups received the basal diet and the basal diet + 2% lysine at alternate feedings. The lysine replaced equal weights of corn starch in the diet. Groups were fed (1) one hour and fasted 7

hours, (2) one hour and fasted 5 hours and (3) one hour and fasted 3 hours throughout successive experiments lasting 27 days. A time-interval feeder was devised for the automatic opening and closing of the feeding jars. Significant differences were noted in nitrogen retention and in bodyweight gain, weight gain per gram of diet eaten, weight gain per gram of lysine eaten. These differences were greater as the feeding intervals lengthened. Thus a delay of three hours or longer, in the feeding of lysine as a supplement to a lysine-low diet interfered with metabolism. Lysine must be fed concurrently with other amino acids in the diet for most efficient utilization by the rat.

Factors determining cerebral oxygen supply during positive acceleration J. P. HENRY, O. GAUER (by invitation), E. E. MARTIN (by invitation), S. S. KETTY (by invitation) and K. KRAMER (by invitation). *Aero Medical Lab., Air Materiel Command, Wright-Patterson Air Force Base, Dayton, Ohio*. Extraordinarily low mean cephalic arterial pressures of less than 20-30 mm Hg (blackout) can be endured during positive accelerations lasting as long as 3 minutes without loss of consciousness. When compared with the effects of a comparable drop in pressure during hemorrhagic shock, there is an unexpected retention of mental performance and orientation. In order more clearly to define the mechanisms involved, radial arterial and cerebral venous pressures, and arterial and cerebral venous oxygen saturations were measured in man during acceleration. These parameters were measured two at a time using electrically operated gauges for the pressures and a Kramer glass cuvette oximeter for the oxygen saturations. The fall in arterial pressure at head level induced by the acceleration was closely followed by a drop in jugular venous pressure. In spite of black-out (4.5 g) one subject still had 60 mm Hg mean pressure differential across the brain. The venous pressure became less negative as arterial pressure rose during the cardiovascular response to the sharp fall in arterial pressure induced in the carotid sinus by the acceleration. Arterial oxygen saturation also started to fall approximately 7 seconds after acceleration commenced and by the end of the 15 second run at 4.5 g it had attained approximately 90%. Further, after a 5-7 second delay the venous oxygen saturation fell and showed fluctuations paralleling the arterial and venous pressure changes. Typical changes in venous oxygen content were from 10.5 vols % resting to 7.5 vols % at blackout. Thus arterial as well as venous oxygen content should be known to determine the oxygen supply of the brain during prolonged acceleration. The extent of the fall in cerebral venous oxygen content at blackout points to an inadequacy of cerebral oxygen supply.

Some responses of man to internal thermal stimulation AUSTIN HENSCHEL, HENRY LONG-STREET TAYLOR and ANCEL KEYS. *Laby of Physiological Hygiene, Univ. of Minnesota, Minneapolis, Minn.* The gastric motility, blood sugar concentration, blood pressure, pulse rate and finger skin temperature responses to internal thermal stimulation were measured on a total of 16 young men who were free from signs of gastrointestinal and cardiovascular abnormalities. The thermal stimulus was a standard ice cream mixture to which 15% barium sulphate was added. Either 400 or 100 gm portions of the test meal was eaten in exactly 5 minutes, the test meal temperature was -8°C , 26°C or 65°C . Gastric motility as measured by gastric emptying time was not altered by the temperature of the test meal. The blood sugar concentration increased a maximum of 22 mg % 30 minutes after the -80°C test meal, the maximum after the 65°C test meal was 12 mg %. Finger skin temperature decreased an average of 3.25°C at 10 minutes after the -8°C test meal and was not back to normal at 50 minutes, the 26°C and 65°C test meals had no effect. An increase of 14.8 mm Hg systolic and 17.6 mm Hg diastolic blood pressure and 20.2 beats per minute pulse rate occurred during ingestion of the 400 gram test meal at -8°C . The values were normal within 2 minutes after completion of the meal. There was no blood pressure, pulse rate or skin temperature response to the 100 gram test meal served at -8°C .

Thermistors as applied to temperature measurements in animals J. F. HERRICK and E. GLARBORG. *The Mayo Foundation, Rochester, Minn.* The thermistor (thermally sensitive resistor) has proven a convenient, simple, rugged and accurate method for measuring the temperature of various animal tissues in our laboratory. This type of thermometer introduces a new order of sensitivity so far as resistance thermometers are concerned. We are using thermistors for measuring temperatures as low as -40°C in certain investigations on frost-bite and also temperatures as high as animal tissues can be heated without irreversible changes being produced. The thermistor is particularly suitable for measuring temperature changes in bone and bone marrow. Rectal temperatures of small animals can be recorded continuously, quite conveniently. The thermistor can be introduced easily into arteries, veins, muscle and subcutaneous tissue and left in place throughout the day when studying changes in temperature caused by various methods of heating or cooling. We have been using the thermistor for measuring changes in skin temperatures of the dog for almost two years and find it a very convenient method. All temperatures may be recorded on moving photographic film or by

direct observation Temperatures may also be observed at a considerable distance from the site of measurement The various types of thermistors, the necessary equipment for measuring temperatures and the method of calibration will be described, and representative data discussed

Mechanism and management of edema GEORGE R. HERRMANN, JOHN W. CHRISS, E. H. SCHWAB and PAUL M. SIMS *Univ of Texas Medical School, Galveston, Texas* The newer conceptions of cardiac output and renal blood flow in the genesis of sodium retention and edema formation are discussed The influence of mercurials on sodium output is stressed Thiomerin—an organic mercurial with a sulfhydryl radical and a new diuretic with low toxicity—is discussed Satisfactory diuresis has been obtained following both subcutaneous and intravenous injections Thiomerin given intravenously has also been studied for any significant electrocardiographic changes

Myocardial insufficiency and its management with especial reference to a new and safe mercurial diuretic GEORGE R. HERRMANN, JOHN W. CHRISS, E. H. SCHWAB, MILTON R. HEJTMANCIK and PAUL M. SIMS *Univ of Texas Medical School, Galveston, Texas* Sodium retention is the most important factor in edema formation in congestive heart failure Decreased cardiac output results in diminished renal circulation, with 90-fold sodium reabsorption Sodium, with its retained water added to the extracellular fluids, produces edema Organic mercurial decrease tubular reabsorption of electrolytes and water, producing diuresis A 70-fold sodium excretion accompanies mercurial diuresis Intravenous mercurial diuretics have resulted in sudden death, with ventricular fibrillation demonstrated in 2 cases, and 20% of patients show cardiac mechanism disorders In attempting to counteract this myocardial toxicity, a sulfhydryl group was substituted for theophylline This mercapto, thiomerin, is less toxic than any other mercurial diuretic Thiomerin is well tolerated subcutaneously, and produces satisfactory diuresis Intravenous use to compare its effects on the heart with that of other mercurials has resulted in a minimum of even slight reactions This drug is potent, less irritating, and practical to use

An estimation of the regional distribution of the cutaneous heat losses ALRICK B. HERTZMAN and WALTER C. RANDALL *Dept of Physiology, St Louis Univ School of Medicine, St Louis, Mo*

We have explored the possibility of calculating the total heat losses from the surface of the body and their regional distribution from the estimations of cutaneous blood flows (photoelectrically recorded skin pulses) and the A-V temperature differences (oral temperature minus skin temperature) in the various skin regions Total heat losses

thus estimated approximate those which are calculated from O_2 consumptions and changes in average body temperature The corresponding fractions of the total heat losses as contributed by the various skin regions of the resting nude subject are approximately trunk 25, leg 19.5, arm 14.2, face 15.5, hand 15.2, foot 10.7% These fractions vary from subject to subject, with the metabolic rate and environmental conditions The various skin regions may be grouped (in resting nude subjects) into two divisions one group (face, palm, plantar surface and digit pads) characterized by high rates of blood flow (above $0.06 \text{ cc/cm}^2/\text{min}$) and high rates of heat loss (above $0.2 \text{ calories/cm}^2/\text{min}$), the other group (trunk, leg, arm and dorsal surfaces of hand and foot) characterized by low rates of blood flow (below $0.03 \text{ cc/cm}^2/\text{min}$) and low rates of heat loss (below $0.08 \text{ calories/cm}^2/\text{min}$) The value of the fraction, blood flow divided by the difference in temperatures of skin and air, shows a similar regional distribution, suggesting that differences in evaporative rates may set flow levels in the corresponding skin regions

Determination of acetylcholine in cell-free enzyme systems by a colorimetric method SLOMO HESTRIN and EMILY F. HEDAL (introduced by DAVID NACHMANSOHN) *Dept of Neurology, College of Physicians & Surgeons, Columbia Univ, New York City* The development of a chemical micro-method for acetylcholine has permitted assay of this ester in the presence of choline and acetate in large excess The method utilizes the fact that esters are rapidly converted at alkaline pH by hydroxylamine into corresponding hydroxamic acids The latter may be determined colorimetrically as in the procedure for acyl assay which has been described by Lipmann and Tuttle

Results obtained with the use of this method in experiments on synthesis of acetylcholine by acetylcholine-esterase and choline acetylase will be discussed

The antagonism of cinchona alkaloids to the circulatory effects of intravenous epinephrin EDWIN P. HIATT *Dept of Physiology, Univ of North Carolina, Chapel Hill, N. C.* The effect of standard intravenous doses of epinephrin (0.5 cc of $1:10,000$) on arterial blood pressures of unanesthetized dogs was recorded photographically using a membrane manometer The dogs were then given several oral doses of quinine or quinidine sulfate (15 mg/kg at intervals of several hrs) attaining a sustained plasma concentration of 5 to 10 mg/l These drugs abolished both the pressor effect and the changes in rate which usually follow epinephrin injection Lower concentrations of the alkaloids had less effect and larger doses of epinephrin overcame the inhibitory effect of the alkaloids These results confirm the effects reported

repeatedly since 1919 by investigators using intravenous injections of the cinchona alkaloids without measurement of plasma alkaloid concentrations. The reciprocal effect was shown by Dreisback and Hanzlik (*Jour Pharm & Exp Therap* 83 167, 1945) and others, using epinephrin to counteract the depressor effect of intravenously injected cinchona alkaloids. We have previously demonstrated that these drugs cause renal vasodilatation and decrease the blood pressure of dogs with experimental neurogenic hypertension. Perhaps these effects are due to an inhibitory effect on adrenergic sympathetic endings. Since it has been demonstrated that these alkaloids can inhibit certain parasympathetic endings as well as motor fibers to skeletal muscles (*Jour Pharm & Exp Therap* 85 55, 1945), it may be these agents can inhibit all motor nerve endings.

Effect of prostigmine and urecholine on human gastric motility N C HIGHTOWER, JR (by invitation), C F CODE, F T MAHER (by invitation) and C G MORLOCK (by invitation) *Section on Physiology, Mayo Foundation and Division of Medicine, Mayo Clinic, Rochester, Minn*. Gastric motility was recorded by means of a system consisting of an undistended balloon containing approximately 28 cc of water and an air cushion of 122 cc between the water-filled balloon and a glass-spoon pressure recorder. The volume pressure coefficient of the system was 0.13. The pressures developed in the balloon were recorded photographically, 1 cm of H₂O pressure producing 1 mm of deflection in the record. Studies were made in normal human beings during fasting, and after the intramuscular injection of 0.5 mg of prostigmine and after the oral administration of 5 mg of urecholine. A quantitative analysis of the photographic records has been attempted, and the results of this analysis have been summarized statistically. The data show that the rate of type II contractions (propulsive type) never exceeded the rate of the smaller type I contractions ('20 second rhythm' of Carlson), and that the administration of the drugs in question did not alter this relationship. Neither drug changed significantly the total amount of motility activity in the stomach, nor did they affect the incidence of the various types of motility. The drugs produced a slight increase in the mean height of the type II contractions, but the significance of this change is questioned. The study supports the hypothesis that the small type I contractions represent a basic fundamental rhythmic sequence of motility activity in the stomach.

Effect of tridione on convulsions caused by diisopropyl fluorophosphate (DFP) H E HIMWICH, C F ESSIG (by invitation), and J L HAMPSON (by invitation) *Army Chemical Center, Medical Division, Edgewood, Md*. In a series of more than

100 experiments diisopropyl fluorophosphate (DFP) was injected into one common carotid artery of curarized rabbits under artificial respiration. With the small amounts of atropine required to protect the heart from succumbing to excessive acetylcholine, it was possible to give DFP in dosage large enough to produce convulsions and still maintain life. Changes were noted in the electroencephalogram which in their most severe form resembled the condition of status epilepticus. Since the subsequent analysis of the brain tissues disclosed a great fall in the action of cholinesterase, the enzyme which hydrolyzes acetylcholine, the changes in the brain waves were presumably produced by excessive acetylcholine. In 10 experiments the intravenous administration of tridione corrected the grand mal type of electroencephalogram in doses of 0.1 to 0.3 gm/kg and restored the electroencephalogram to normal with doses of 0.2 to 0.4 gm/kg. In other experiments, in which tridione was injected before DFP, tridione exhibited a preventive action for approximately 4 hours. Though the grand mal electrical pattern was prevented the normal electroencephalogram was not maintained and increased activity was observed. The administration of tridione in no way influenced the profound fall in cholinesterase activity. It would seem therefore that tridione counteracts the effects of excessive concentrations of acetylcholine in the brain.

Cholinergic nature of the vestibular receptor mechanism, forced circling movements (motion picture). H E HIMWICH, C F ESSIG (by invitation), J L HAMPSON (by invitation), P D BALES (by invitation) and A M FREEDMAN (by invitation) *Medical Division, Army Chemical Center, Md*. Diisopropyl fluorophosphate (DFP) was injected into the common carotid artery of animals belonging to four species: Rabbits, cats, dogs and monkeys. Though the dosage required varied with the species, the results were similar. The head turned to the side opposite to that of injection and compulsive circling movements were made to the side opposite, hence the name adverse syndrome. Labyrinthectomy or section of the eighth nerve prevented the development of the syndrome when the injection was made on the operated side but not when the DFP was introduced into the other common carotid artery. Either atropine or scopolamine cured the syndrome.

Ketosis in diabetic humans in response to the stress of interpersonal adjustment. LAWRENCE E HINKLE, JR and GEORGE T CONGER (introduced by STEWART WOLF) *Dept. of Medicine and Psychiatry of the New York Hospital and Cornell Univ. Medical College, New York City*. Eleven diabetic individuals were subjected to experimental study. Increased glycosuria and ketosis

were observed repeatedly during conflicts associated with fear, depression, frustrated anger and, as far as could be ascertained in the absence of significant changes in diet, insulin or activity. Accordingly, 2 of these subjects were studied in the hospital where diet, insulin and activity were carefully controlled. A marked increase in glycosuria in one, and clinically typical keto-acidosis in the other, were induced when the individuals were subjected to significant threats to their emotional security. Three of the subjects were studied in short-term experiments under sodium amytal narcosis. In all a significant rise in the concentration of ketone bodies in the peripheral blood occurred during a discussion of their personal conflicts. Following reassurance, in each case the ketones fell to lower levels. Ketone measurements were made by the micro-method of Greenberg and Lester. Blood samples were also obtained from a catheter introduced directly into the hepatic vein. Significant elevations of ketone content during tension and conflict with return to control levels during reassurance were demonstrated.

Acute renal cortical ischemia produced by stimulation of the pressor area of the cerebral cortex. E. C. HOFF, J. F. KELL, JR. (by invitation), NELSON HASTINGS (by invitation), E. H. GRAY (by invitation) and D. M. SHOLES (by invitation). *Neurological Science Laby of the Medical College of Virginia, Richmond, Va.* Green and Hoff (*Am J Physiol* 118: 641, 1937) reported in cats and monkeys that limb volume usually increased while kidney volume diminished during pressor responses evoked by electrical stimulation of the cerebral cortex. Denervation of the kidney abolished this renal vasoconstriction so that the kidney passively dilated during the mean blood pressure rise. In the present experiments, acute blood pressure rises, lasting 15 to 90 seconds, were evoked by stimulation, unilaterally, of foci on gyrus preceus, gyrus sigmoideus, and orbital and mesial surfaces of the cat's cerebral cortex. In each experiment, one side only of the cerebral cortex was stimulated repeatedly for 1 to 2 hours using an inductorium or square-wave generator. Finally, during the height of a maintained pressor response, 30 to 50 cc of India Ink solution were injected into the right common carotid artery towards the aorta. There was pronounced vasoconstriction in the cortex of both kidneys as evidenced by virtual exclusion of the ink from the renal cortical vascular bed. This was in sharp contrast to findings in unstimulated control animals, in which both kidneys were deeply injected throughout. It is concluded that bilateral renal cortical ischemia results from excitation of the pressor area of the cerebral cortex, and that this vasoconstriction is part of the generalized vaso-motor response underlying the acute experimental

blood pressure rise. Possibly certain abnormal cerebral activity in man may result in persistent or recurrent renal cortical ischemia, and this mechanism may lie at the basis of essential hypertension.

Nature of apneusis and the pontine influence on respiration. HEBBEL E. HOFF and C. G. BRECKENRIDGE (by invitation). *Dept of Physiology, Baylor Univ. College of Medicine, Houston, Texas.* Dogs decerebrated by transection after occlusion of the carotids and the basilar artery at the lower border of the pons universally exhibit apneustic breathing, after transection encroaching on the pons and vagotomy. Apneustic breathing, consisting of alternate periods of inspiratory spasm and expiration at slow rates (1 or 2/min or less), is in the dog capable of maintaining life for several hours. When the carotids are occluded only during transection, pontine section and vagotomy produce apneusis in the majority of experiments, but certain animals show 'normal' respiration. Animals showing apneustic breathing resume 'normal' breathing terminally as the preparation deteriorates. When a further section encroaching on the medulla is made in 'apneustic' animals, the phases of inspiratory spasm are converted into periods of phasic 'normal' inspiration and expiration akin to Biot's breathing, often reverting to 'normal,' or 'normal' breathing appears immediately. Intravenous injection of cyanide produces apneusis or prolongs and intensifies it. When the carotid bodies and sinuses are denervated, apneusis does not appear after pontine decerebration and vagotomy, 'normal' or Biot's breathing alone occurs.

It is concluded that a) the medullary respiratory center can maintain periodic respiration in the absence of the pons and the vagi, b) apneusis represents an exaggerated inspiratory response to anoxemic stimulation via the carotid bodies 'occluding' the normal respiratory action of the medullary center, and c) such enhancement probably represents unchecked activity of the pontine portion of the midbrain facilitatory center.

Failure of topical calcium and magnesium to prevent eugenol-induced desquamation of gastric mucous epithelium. FRANKLIN HOLLANDER and SHIRLEY D. KRAUS (by invitation). *Gastroenterology Research Laby, Mount Sinai Hospital, New York City.* Previous studies have demonstrated that the mucigogue action of topical stimuli on the gastric mucosa of Heidenhain pouch dogs is usually accompanied by extensive desquamation of the surface epithelium, probably because of a loosening of the cement substance. A similar, though less marked, decrease in cellular cohesion may also underlie the invasive character of cancerous tissue, and evidence is accumulating in the literature that Ca- and Mg-ions are of considerable importance for maintaining the integrity of the cell-

binding mechanism in such tissue—as well as in several varieties of normal tissue from lower forms of life. Therefore, the present study was designed to determine whether the shedding of gastric mucous epithelium, induced by 1% aqueous eugenol alone, can be influenced markedly by the addition to this mucicogue-desquamating agent of CaCl_2 (0.02, 0.04, and 0.06%), alone or with MgCl_2 (0.01 and 0.03%). NaHCO_3 was added to adjust the pH to about 7. Mucus specimens, collected in 4 half-hour intervals following stimulation, were examined for viscosity, opacity, and the presence of cells (microscopically). No essential difference in these characteristics was observed between these experiments and controls without inorganic salts. Further experimentation is required to show whether the desquamating effect of eugenol emulsion is entirely independent of Ca- and Mg-ions, even though these may be part of the cement substance, or whether this effect is so powerful, even with 1% concentration, that it completely overcomes the influence of those ions under the conditions of these experiments.

Effect of sympathetic blocking procedures and of sympathectomy on peripheral blood flow. S. W. HOOBLER, J. W. AVERA, W. J. LITTLE, M. M. PEET and R. C. BASSETT (introduced by A. H. SMITH). *Depts. of Medicine and Surgery, Univ. of Michigan Hospital, Ann Arbor, Mich.* Certain sympathetic blocking techniques all of which abolished toe-thigh temperature gradients were studied by means of the venous occlusion plethysmograph. Resting blood flow in the foot averaged 0.7 cc/100 cc limb volume/minute (range 0.5–1.0). Average maximum flow after reflex body heating was 4.5 cc (range 2–7), after 500 mg tetraethylammonium chloride intravenously. 7.6 cc (2.8–10.3), after unilateral paravertebral block (L1–L3). 18 cc (11–26), after caudal anesthesia (1.5% metycaine to D3) or spinal anesthesia. 34 cc (16–60), within 12 hours after lumbar sympathectomy in 3 cases. 30.1 cc (12–36). In the latter instances the early post-operative result corresponded well with pre-operative paravertebral block of spinal anesthesia. The disparity between increase in digital temperature and increase in blood flow as determined in the plethysmograph was emphasized by an observation under caudal anesthesia utilizing 0.75% metycaine. In this hypertensive patient skin temperature gradient was abolished and anesthesia to pin prick as well as anhydrosis occurred to a level of D2. The blood pressure did not fall nor was there a measurable increase in blood flow in the foot. It may be concluded that complete block of the appropriate sympathetic ganglia or spinal vasomotor outflow can produce very great increases in foot blood flow, but that total block can not be assumed from

abolition of skin temperature gradient or of sudomotor activity alone.

After 3 out of 4 sympathectomies, plethysmographic measurements showed a reduction to pre-operative levels in 3 to 7 days. This was maintained thereafter for periods up to 15 months despite continued elevation of the digital temperature and anhydrosis.

Blood temperature in the heart and blood vessels of the dog. STEVEN M. HORVATH, E. L. FOLTZ (by invitation), A. RUBIN (by invitation) and B. K. HURT (by invitation). *Dept. of Physical Medicine, Graduate School of Medicine and the Dept. of Pharmacology, School of Medicine, Univ. of Pennsylvania, Philadelphia, Penna.* Temperatures of the blood, tissues, skin and rectum in anesthetized dogs were determined. Courmand type catheters with thermocouples at the tip were placed roentgenoscopically in the coronary sinus and the right ventricle or pulmonary artery. Smaller catheters were similarly inserted into the femoral artery and vein. The emf developed by the thermocouple was measured with a type K-2 Leeds and Northrup potentiometer. Since morphine-nembutal anesthesia has a tendency to lower body temperature, it was possible to study the temperature gradients at different rectal temperatures. The table below presents the mean temperatures and gradients obtained in 19 experiments on 11 animals. The rectal temperature was used as the point of reference in determining the gradients.

LOCATION OF CATHETER	$^{\circ}\text{F}$	$A(T - R)$
Rectum	99.1	
Rt. ventricle	98.7	-0.44
Pulmonary artery	98.7	-0.40
Coronary sinus	98.9	-0.20
Femoral artery	98.3	-0.84
Femoral vein	96.6	-2.51
Thigh muscle	97.6	-1.47
Thigh, subcutaneous	96.5	-2.64
Thigh, skin	92.1	-7.04

The work of the heart was altered variously with intravenous injections of epinephrine, aminophylline and methedrine and with inhalations of amyl nitrite. The temperature of the blood in the right heart during the action of these drugs was determined and the data will be discussed.

Determination of filtration rate and renal plasma flow in dogs without analysis of urine. C. RILEY HOUCK. *Division of Physiology, Univ. of Tennessee, Memphis, Tenn.* The renal plasma clearances of sodium p-aminohippurate (PAH) and mannitol were determined simultaneously by 1) the plasma slope method without urine analysis, and 2) the conventional urine collection method, and the values compared. According to Newman,

Bordley, and Winternitz (*Bull Johns Hopkins Hosp* 75 253, 1944), the renal clearance (C) of a substance is the product of its volume of distribution in the body (Vc) and the rate of disappearance from plasma (S). Under nembutal anesthesia, 4 dogs were injected intravenously with 5 gm of mannitol and 750 mg of PAH through a calibrated syringe. After 40 minutes, 5 10-minute blood samples were drawn, during which time urine was collected through an indwelling bladder catheter. In order to test for extra-renal loss of these substances, the dogs were subsequently bilaterally nephrectomized and 5 more 10-minute blood samples drawn. The urine clearance/slope clearance ratios for the 4 dogs were for mannitol 0.940, 0.923, 0.956, and 0.781 (av 0.90), and for PAH, (no determination on first dog), 1.052, 0.956, and 0.872 (av 0.96), respectively. In every case, extra-renal disappearance of PAH and mannitol was zero. Except for one dog, it is apparent that the slope clearance of either PAH or mannitol can be substituted for the respective urine clearance. Consequently, it may be concluded tentatively that glomerular filtration rate and renal plasma flow can be determined in dogs by the analysis of plasma alone without the collection and analysis of urine.

Disappearance of mannitol and PAH from plasma of bilaterally nephrectomized dogs
C RILEY HOUCK *Division of Physiology, Univ of Tennessee, Memphis, Tenn*. For mannitol and sodium p-aminohippurate (PAH), the volume of distribution (Vc), the rate of disappearances from plasma (slope or proportion of Vc cleared of each substance per minute), and the clearance (extra-renal) (C) were determined in 16 bilaterally nephrectomized dogs under nembutal anesthesia according to the procedure of Newman, Bordley, and Winternitz (*Bull Johns Hopkins Hosp* 75 253, 1944). Immediately after nephrectomy, 65 mg of PAH (16 dogs) and/or 1700 mg of mannitol (8 dogs) were injected rapidly intravenously from a calibrated syringe. After 40 minutes, from 5 to 8 venous blood samples were drawn at 10-minute intervals and analyzed. For mannitol, $V_c = 246$ cc/kg, $S = 0.0785\%/min$, $C (= V_c S) = 0.168$ cc/min/kg. For PAH, $V_c = 280$ cc/kg, $S = 0.239\%/min$, $C = 0.796$ cc/min/kg. It is apparent that the volumes of distribution of PAH and mannitol are very similar. Furthermore, the extra-renal loss of PAH from plasma is greater than the loss of mannitol. When compared to the total urine (renal + extra-renal) clearances in normal dogs (Houck, *Federation Proc* 7 339, 1948), the mannitol clearance in these nephrectomized dogs (extra-renal clearance) is only 4%, and the PAH extra-renal clearance only 6% of the respective total urine clearance. Consequently, in calculating the clearance of PAH and mannitol in intact normal

animals, by use of the slope method, the extra-renal clearances can be ignored because the extra-renal loss is negligible.

Vascular perfusion of the prostate gland of the dog
PERRY B HUDSON (by invitation), W W S BUTLER III (by invitation), HERBERT BRENDLER (by invitation) and W W SCOTT *James Buchanan Brady Urological Inst, Johns Hopkins Hospital, Baltimore, Md*. For the first time a reliable surgical technique has been devised which allows perfusion of the prostate gland through its normal arterial and venous channels. This preparation is accomplished by ligation of all branches of the abdominal aorta and inferior vena cava except for the arteries and veins on each side which constitute the vascular supply to the prostate gland. The autonomic nerve supply may be preserved. The urethra is preserved permitting cannulation for collection of prostatic fluid during perfusion of the gland. This preparation is proving useful in the study of the metabolism of the dog prostate gland, offering certain advantages over purely *in vitro* techniques.

Active salt transport and its regulation in the isolated frog skin
ERNST G HUF (introduced by ERNST FISCHER) *Baruch Center of Physical Medicine and Dept of Physiology, Medical College of Virginia, Richmond, Va*. Freshly isolated frog skin actively transports salt from its natural outside to its natural inside (accumulation effect). Experiments have been carried out using 'inside out' skin bags, formed of the skin of the hind limbs. The bags were filled with 5 ml of Ringer solution and immersed in 2.5 l of Ringer solution of the same concentration, this was varied in different experiments from 4 times up to 20 times its normal concentration. After 12 hours, less fluid of a lowered salt concentration was left in the bag. The weight of the filled bag at the end of the experiment was always lower than the weight at the beginning, indicating a transport of fluid through the skin. An increase in weight of the bag was observed in experiments where the Ringer solution was poisoned with NaCN (0.01 M). In this case the amount and the concentration of the Ringer solution placed in the bag was not diminished appreciably at the end of the 12-hour period. The accumulation effect is greatest when the skin is exposed to diluted Ringer solution and decreases with increasing concentration of the Ringer solution. Apparently the concentration of the salt solution regulates the mechanism of accumulation of salt. The endocrine and the nervous system seem to have little regulatory influence.

Toxins of *Streptococcus pyogenes* and cell respiration
F R HUNTER (introduced by A K PARPART) *Dept of Zoological Sciences, Univ of Oklahoma, Norman, Okla*. The effect of toxins of *Streptococcus pyogenes scarlatinae* on the rate of

O₂ consumption of several types of cells was studied. Almost complete inhibition was found using chicken erythrocytes, dogfish (*Mustelus canis*) erythrocytes, *Asterias* eggs and fertilized *Arbacia* eggs, the rate of respiration in the last instance being essentially the same as that of unfertilized eggs. Since it has previously been suggested that unfertilized *Arbacia* eggs respire through a non-ferrous, autooxidizable carrier while fertilized eggs have an active cytochrome system, the present data would suggest that the scarlatinal toxin reacts in some way with the cytochrome system. Confirmation of this suggestion was obtained from experiments involving chicken erythrocytes and the respiratory carriers, pyocyanine and methylene blue. The rate of O₂ consumption of control chicken erythrocytes was increased slightly by the addition of pyocyanine or methylene blue while the rate of respiration of erythrocytes which had been inhibited by the toxin was accelerated to the same level as the controls by the addition of these carriers. These experiments demonstrate that the toxins of *Str. pyogenes* inhibit the respiration of several different types of cells by reacting with the cytochrome system.

Electrical and functional activity of spinal motor neurons. JOHN HUNTER, RICHARD LILLIE (by invitation), and ROBERT GESELL. *Dept of Physiology, Univ of Michigan, Ann Arbor, Mich.* The ventral root of the phrenic nerve of the dog exhibits two characteristic types of electrical activity. 1) slow periodic waves which increase and decrease in negativity with inspiration and expiration respectively, and 2) quick potential changes superimposed upon the inspiratory fraction of the slow waves. The slow potentials diminish in magnitude with distance from the cord and are thus concluded to spread from the cord by electrotonus. The quick potentials suffer no decrement and are concluded to represent the inspiratory action potentials. Continuing unbroken electrotonic activity in the phrenic root indicates that the dendrites and cell body are always negative with respect to the neuraxon and that neurocellular currents consequently circulate between dendrites and neuraxon via internal and external circuits. Waving and waning of this continuing current produces periodic changes of intensity of the slow electrotonic component in the phrenic root. Rise of negativity of the slow component is attended by increasing numbers of action potentials. Sporadically and artificially increased depth of inspiration are attended by increased magnitude of the electrotonic component and by correspondingly increased numbers of action potentials. It is postulated that the current circulating between the dendrites and neuraxon generates nerve impulses at or in the proximity of the axon hillock. The current is therefore designated

'generating current'. It is suggested that our concept of specialization of function of the neuron questions the need of retaining the theory of synaptic transmission of nerve impulses and subsequent conduction along dendrites and cell body.

Proprioception and convulsions. J. HYDE, J. GAY and E. GELHORN. *Laby of Neurophysiology, Dept of Physiology, Univ of Minnesota, Minneapolis, Minn.* The influence of proprioceptive impulses on cortically induced afterdischarge and on chemically induced convulsions has been studied on cats and monkeys (*Macacus Rhesus*) in dial anesthesia. Fixation of a joint so as to prevent shortening of a muscle during contraction leads to an increased afterdischarge as well as increased amplitude of EMG in response to a standard cortical stimulus. In contrast to this proprioceptive facilitation of afterdischarge, removal of proprioception by appropriate posterior root section decreases afterdischarge and in some cases qualitatively changes it from a tonic to a clonic form. Afferent proprioceptive impulses induced by stimulation of the peripheral end of the motor root S₁ have been seen to alter convulsive spikes induced by topical application of strychnine on the cortex. The effect is largely confined to the contralateral sensori-motor cortex and is greater in the hindleg than in the foreleg and face area. It consists in either increase or decrease in frequency of the strychnine potentials. Finally, convulsive movements induced by applying strychnine to the exposed dorsal columns of the cord are reduced or eliminated by deafferentation although not significantly altered by removal of cutaneous impulses. The experiments furnish evidence for the fact that convulsive potentials and movements are greatly influenced by proprioceptive impulses.

Determination of cardiac output by an improved electrical conductivity method. RAYMOND C. INGRAHAM. *Dept of Physiology, Univ of Illinois, College of Medicine, Chicago, Ill.* Basically the present method follows H. L. White's recent modification rather closely. A small quantity of hypertonic salt solution is injected as rapidly as possible into the right atrium. Conductivity changes are recorded in blood flowing through a specially designed conductivity cannula tied in a peripheral artery. The output of the bridge, of which the conductivity cannula constitutes one arm, is amplified, rectified and then recorded by the deflection of a suitable sensitive reflecting galvanometer. The degree of bridge imbalance is optically recorded and later translated to concentration changes of sodium chloride by the construction of a calibration curve. The improvements in the present method are essentially in the design of all constituent parts so that it accomplishes the desired purpose to a degree consistent with modern

electronic methods. Comparison of cardiac output as determined by this method in the intact animal with the direct Fick and dye injection method has yielded results that compare very favorably. Determinations were made simultaneously. It was found that the improved design required the use of a smaller amount of a more dilute solution of sodium chloride than previous applications of this method have required. This minimizes the error due to salt loss in passage through the lungs. Recirculation can be recognized, if it occurs, and corrections made. This method thus allows for continuous recording of cardiac output data and also simultaneous optical recordings of pressure variations in any accessible portion of the cardio-vascular system.

Dynamics of evaporative heat loss for a rapidly cooling human body TOHRU INOUE (by invitation), NATHANIEL GLICKMAN, and ROBERT W. KEETON. *Dept of Medicine, Univ of Illinois, Chicago, Ill.* The dynamics of evaporative heat loss for a rapidly cooling human body in a 'comfortable' environment after having been exposed to a hot environment was studied on 6 healthy young men. The subjects were clothed in 90% cotton union suits containing variable amounts of moisture accumulated as a result of 1 or 2-hour exposures to a hot environment. The hot room was maintained at 37°C with a water vapor pressure of 41.1×10^3 dynes/cm² and the 'comfortable' room at 24°C with water vapor pressures of 9.1×10^3 , 18.2×10^3 , and 24.3×10^3 dynes/cm² with air velocity minimal and constant. Observations included weight loss and skin temperatures obtained at short intervals for 1 hour. The equation governing evaporative heat loss was theoretically derived for the dynamic state. Hitherto, this was applied only on subjects in approximate equilibrium with the environment with the introduction of wettedness as a calculated factor to account for an indeterminate variable. Under dynamic conditions, available moisture in the union suit and on the skin was found to adequately express wettedness. The inclusion in the equation of the amount of available moisture yielded a linear correlation for each of the 36 tests with an average correlation coefficient of 0.992. Thus, for subjects under rapidly changing physiological conditions, the amount of evaporative heat loss was proportional to the gradient of water vapor pressure between the skin and the ambient environment, amount of available moisture on the skin and in the union suit, surface area and time.

Developmental relationships between electrical activity and histogenesis of the cerebellum JOHN JACOBS and RAY S. SNIDER (introduced by H. W. MAGOUN). *Depts of Pathology and Anatomy, Northwestern Univ Medical School, Chicago, Ill.*

Correlation studies have been made between the first appearance of the fast intrinsic spontaneous electrical activity in the cerebellum of the newborn and the histogenesis of the cerebellar cortex. Anesthetized or acutely decerebrated newborn guinea pigs and rats between 1 and 28 days, as well as rat fetuses, were used. A complete comparative neuro-electrogenetic study on reptiles, avians and mammals, including primates, is in progress. Electrical activity was visualized and photographed from a cathode ray tube after amplification through a Grass Model III amplifier. Both bipolar and monopolar silver wire and cotton wick electrodes were used. For histogenetic studies silver, Nissl, Golgi and Feulgen (for thymonucleic acid) stains were used on Purkinje, granule and basket cells. Typical cerebellar electrical activity of 10-60 microvolts with a frequency of 150-250/sec first appears about the 12th day postpartum in the rat and is present in the newborn guinea pig. Several histogenetic changes take place in the cerebellum of the rat about the 12th day: 1) connections of the granule cell glomerulus are established, 2) nuclear chromatin of the Purkinje cell first appears Feulgen positive, 3) Purkinje cell nucleolus shows a trace of thymonucleic acid, 4) cytoplasm of the Purkinje cell is Feulgen negative but gives a positive Nissl reaction as early as 10 days. Granule cells are Feulgen positive as early as 9 days. Data are not complete on basket cells.

Antihemolytic action of a hemolytic agent M. H. JACOBS, MARIAN WILLIS LEFEVRE (by invitation) and CAROLYN STOUT (by invitation). *Dept of Physiology, Univ of Pennsylvania, Philadelphia, Penna.* Sodium oleate shows a notoriously complex relation between concentration and hemolytic activity and according to circumstances increasing its concentration may either increase or decrease the rate of hemolysis. This and other complications are due, at least in part, to the co-existence of a hemolytic and an antihemolytic effect of the oleate. The hemolytic effect has the general characteristics of an induced cation permeability, as shown by shrinkage of the cells in isosmotic sucrose, protection by low concentrations of sucrose, rapid return of volume after shrinkage by concentrated NaCl and swelling in alkaline solutions. This effect, which increases with concentration, is more or less disguised by the antihemolytic effect, which also increases with concentration and seems to be due to the prevention by oleate at the cell surface either of swelling of the cells or escape of hemoglobin, or both. The antihemolytic effect is well shown in photographically recorded hemolysis curves. If during hemolysis by weak oleate, more oleate is suddenly added the process may instantly, though temporarily, be stopped. Osmotic hemolysis may similarly be

checked. Conversely, removal of protective oleate by appropriate dilution with isotonic NaCl causes immediate hemolysis, provided that the initial exposure has been sufficient to render the cells cation permeable. A more striking way of showing the same thing is by the addition of washed normal erythrocytes to a suspension of other erythrocytes that have been altered but simultaneously protected by oleate, under properly chosen conditions hemolysis of the treated cells then immediately occurs.

Influence of niacin on the excretion of ketone bodies in normal fasted rats R. G. JANES and J. BRADY (by invitation) *Dept. of Anatomy, State Univ. of Iowa, Iowa City, Ia.* In previous experiments we have shown that relatively large amounts of niacin will produce a ketonuria in severely diabetic male rats. In the present study it has been found that the daily administration of 35 mg of niacin to normal female rats was ineffective in altering acetone body excretion. However, if female rats were given daily intraperitoneal injections of niacin during a 5-day fasting period, a marked ketonuria occurred. Control fasted rats receiving injections of physiological saline failed to show a pathological level of ketone body excretion. Niacin has been given to rats at levels of 5, 20, 35 or 50 mg per day. All of these animals showed greater acetone body excretion levels than control rats but the 35- and 50-mg dosages appeared to produce a more marked ketogenic response although some variation was seen in each group.

Regulation of food intake in normal and esophagostomized dogs HENRY D. JANOWITZ (by invitation) and M. I. GROSSMAN *Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago, Ill.* Factors concerned in regulating intake of food were investigated in dogs by noting 1) effects of intragastric feeding on normal feeding in dogs with gastric fistulas, 2) effects of real and sham feeding on eating time in dogs with esophagostomies and 3) effects of oral supplementary prefeeding on food intake in intact animals.

Under standardized conditions, intragastric feeding of 50% or more of the average daily meal had an immediate significant but not equivalent depressing effect on oral food intake. If time was allowed for gastric evacuation (4 hours) the feeding had no such inhibitory effect. Division of the exteriorized esophagus in otherwise normal dogs led to immediate significant prolongation of eating time. Satiety conferred by sham feeding was of short duration, usually less than two hours. Beyond this point, increasing food deprivation or lengthening intervals between sham feeding did not increase the duration of sham feeding. Intragastric feeding and absorption of such feeding (up to 4 hours) had no inhibitory effect on sham feeding time. Sham

feeding time was shortened but not reduced to normal by simultaneous sham and intragastric feeding. Gastric distention by balloon reduced sham feeding time only when obvious nausea was induced. The oral prefeeding of a fraction of the daily meal reduced food intake by a corresponding amount in intact animals.

These studies disclose two sets of factors which under these standardized conditions influence regulation of intake of food: 1) oral factors ('gustatory satiety') and 2) gastric distention factors, which to be effective must act simultaneously with the oral factors.

Action of Phenylindanedione on prothrombin time L. B. JAKES, E. TAYLOR (by invitation) and E. LEPP (by invitation) *Dept. of Physiology, Univ. of Saskatchewan, Saskatoon, Sask., Canada.* Meunier, Mentzer and Molho (*Compt. rend. Acad. sc.* 224: 1666, 1947) have reported that 2-phenyl indane dione-1,3 (PID) is a more rapidly acting prothrombopenic agent than dicumarol. The effect of PID on prothrombin time was tested in dogs and rabbits. Single doses showed a rapid but slight effect on prothrombin time. Repeated administration of the drug at frequent intervals caused a marked effect on the prothrombin time. 8.3 mg/kg every 8 hours gave a prothrombin time of infinity, 1 mg/kg/8 hours doubled the prothrombin time. Withdrawal of PID caused the prothrombin time to return to normal within 36 hours. Vitamin K had no effect on the action of PID.

The prolonged prothrombin time following administration of PID was accompanied by a normal concentration of prothrombin in the blood but there occurred a very marked diminution in Factor V activity (Owren). The concentration of Factor V itself was not reduced, suggesting the presence of an inhibitor of Factor V. PID itself had little effect on the components of the clotting system. Large doses of the drug over a long period of time plus other factors produced a hemorrhagic condition similar to that of dicumarol overdosage. Withdrawal of the drug rapidly reversed this condition. No toxic effects were observed with 8.3 mg/kg given every 8 hours for 48 days to dogs. The PID was excreted by the kidneys.

Studies on 'dumping syndrome' in the dog following anastomotic operations and vagotomy N. C. JEFFERSON (by invitation), C. W. PHILLIPS (by invitation), R. LEVINE and H. NECHLES *Dept. of Gastrointestinal Research, Medical Research Inst., Michael Reese Hospital, Chicago, Ill.* Following a study on patients suffering from the so-called dumping syndrome after subtotal gastrectomy (*Gastroenterol.* Feb. 1949), the present study on dogs was done in order to obtain more controlled conditions. Intravenous and oral glucose and fructose tolerance tests were performed before and

after operation, and in some tests emptying time was determined with the barium meal. The following anastomotic operations were done: Polya, Hofmeister, gastro-enterostomy, pyloro-plasty, entero-enterostomy and additional vagotomies. The following results were obtained: in no normal control dog did hypoglycemia develop with any of the tolerance tests. In none of the operated animals did the tolerance test evoke hypoglycemia. Subsequent to anastomotic operations the oral glucose tolerance curve showed a persistent increase in height and duration. In most animals in which bilateral transthoracic vagotomy was performed with or subsequent to anastomotic operations, the oral glucose tolerance curve showed an additional increase in blood sugar levels. Oral glucose tolerance tests on 2 dogs with subtotal gastrectomy held in the upright position during the test revealed increased height of the glucose tolerance curve. In 2 animals with anastomotic operations in whom following vagotomy an increased glucose tolerance curve occurred, atrophy of the pancreas was found at autopsy.

Further studies on electrical analogs of the circulatory system KENNETH E. JOCHIM, *Dept of Physiology, Univ of Kansas, Lawrence, Kansas*. In a previous report simplified electrical analogs of the circulatory system were described, and results of an analytical and experimental study of their behavior were presented. These circuits consisted of single or multiple mesh networks which were energized with half sine wave current pulses, and in which the distensibility of the arterial system was represented by one or more parallel condensers.

This study was extended by introducing two additional complications which simulated more closely the conditions in the circulatory system: 1) the networks were energized with current pulses of various wave-forms, and for each one it was shown, experimentally and analytically, that the ratio of pulse volume, or maximum arterial uptake, to stroke volume (using hemodynamic terminology) fell between 0.50 and 0.56 over a wide range of values of the circuit parameters. A graphical method was evolved for computing this ratio for any input wave-form, whether its equation is known or not. 2) It has been shown by Hamilton and by the author that the distensibility curves of rubber tubes and arterial segments depend on the rate of change and direction of change of applied tension. This type of distensibility variation was simulated electrically and its effect on the pulse volume/stroke volume ratio was shown, analytically and experimentally, to be insignificant within certain limits.

Cause of injury in rapid deceleration MILTON JOFFE (by invitation) and FRED A. HITCHCOCK, *Dept of Physiology, Ohio State Univ, Columbus,*

Ohio. Experiments have been carried out on cadavers and anesthetized dogs to determine the effects of rapid deceleration. Acceleration was accomplished by falling weights attached by a steel cable to the lower extremity of the test body. Rapid deceleration was produced by a safety harness attached around the waist and connected by means of a tail line to the cross member of a T-shaped channel iron frame. The G forces produced were recorded photographically from vacuum tube type accelerometers. The decelerative forces were less than those expected from calculation, which was probably due to the lack of rigidity in the tube attachments. It was observed that there was no damping effect of tissues on the transmission of forces from the belt to the heart.

Seven dogs were subjected to multiple decelerations. Serial electrocardiograms were made on all dogs over a period ranging up to 9 days or until death. Autopsies were performed at death. Results of serial electrocardiograms on dogs showed inversions or other abnormalities of the T-wave in one or more of the 3 standard leads. X-ray heart shadow in one case, autopsy in all 7 cases, showed a dilatation of the heart with thinning of right ventricular wall. Four of 7 dogs also showed pulmonary, hepatic or renal vascular engorgement. The findings pointed to a condition of right heart failure—the traumatic heart injury syndrome.

Influence of hydrostatic pressure and of urethan on the thermal inactivation rate of bacteriophage FRANK H. JOHNSON and RUTH A. C. FOSTER (by invitation), *Biological Lab, Princeton Univ, Princeton, N. J.* The activity of *Escherichia coli* bacteriophages T-1, T-2, T-5 and T-7 is destroyed at 60 to 68°C in nutrient broth, pH 6.6, at rates which change with time during the exposure to heat. Addition of 0.005 M MgCl₂ retards the rate of destruction of infective centers of T-1, T-2, T-5, but not of T-7, and results in a first order rate of destruction of T-5. Urethan in concentrations of 0.05 to 0.5 M accelerates the rate of thermal destruction of all 4 of these bacteriophages. Increased hydrostatic pressure, up to 10,000 lb p s i, retards the rate of thermal inactivation of T-1, T-2, and T-5, but slightly accelerates that of T-7. In presence of urethan, pressure opposes the rate of destruction of T-5 but has little effect on that of T-7.

An intact mammalian muscle preparation for use in studies on respiratory metabolism SHIRLEY A. JOHNSON (by invitation) and KENNETH C. FISHER, *Univ of Toronto, Toronto, Canada*. An examination of the properties of the isolated rat diaphragm lead to the conclusion that this preparation is far from representing normal resting muscle. We have therefore given consideration to other, possibly more normal, preparations. It has been found that the

extensor digitorum longus from the lower leg of 50 to 70-gm rats is a very satisfactory preparation. At 30°C it always remains excitable for at least 6-8 hours, and in pure oxygen the rate of diffusion of oxygen into the preparation is sufficient to maintain the normal rates of consumption. Though this muscle appears to be more satisfactory for use than is the diaphragm, even it is probably not strictly resting tissue.

Suppression of urinary chorionic gonadotrophin, symptoms and pulmonary metastases in chorion-epithelioma by diethylstilbestrol EDWIN C JUNGCK (by invitation), WILLIAM O MADDOCK (by invitation), CLIFFORD L FEARL (by invitation), and CARL G HELLER *Depts of Physiology, Gynecology and Medicine, Univ of Oregon Med School, Portland, Oregon*. Large doses of stilbestrol are effective in suppressing anterior pituitary gonadotrophin secretion in post-menopausal and castrated women. Therefore, stilbestrol was administered to a 28-year-old woman with pulmonary metastases from a uterine chorionepithelioma, in hope of similarly depressing the gonadotrophins of chorionic origin, and with the vague hope that suppression of function might be accompanied by morphological regression of the malignant cells. At the time the primary tumor was removed (total utero-salpingo-oophorectomy), no pulmonary metastases were visualized. Four months later, multiple pulmonary lesions characteristic of metastatic chorionepithelioma were noted along with weight loss, asthenia and hemoptysis. Six months after operation, urinary excretion of chorionic gonadotrophin was 3200 IU/24 hours. After 4 weeks of daily intramuscular injections of 10 mg of stilbestrol in oil, chorionic gonadotrophin excretion dropped to 640 IU, symptoms disappeared and pulmonary metastases were regressing. In 9 weeks, hormone titers were 6-16 IU/24 hours and remained at that level as long as daily injections were continued, radiological evidence of pulmonary metastases was no longer present. Stilbestrol, 10 mg in water suspension, was as effective as the oil preparation when given intramuscularly. After 6½ months, 25 mg stilbestrol daily by mouth was substituted for the injections. A slight rise in titer was observed, which disappeared when the dose was increased to 50 mg daily by mouth.

The above findings could be due to coincidence, however, they suggest that 1) stilbestrol may inhibit chorionic as well as pituitary gonadotrophins and 2) morphological and functional regression may accompany each other when stilbestrol is administered in chorionepithelioma.

Site of action of myanesin (tolserol) in the central nervous system B R KAADA (introduced by JOHN F FULTON) *Laby of Physiology, Yale Univ School of Medicine, New Haven, Conn*

Following the announcement of the muscular relaxation effect of Myanesin (3-ortho-tolonyl-1,2-Propanediol) by Berger and Bradley in 1946 there has been marked interest in the potential value of this drug as an adjuvant to anesthesia and in the treatment of neuro-muscular disorders such as rigidity, spasticity, muscle spasm and involuntary movements. The site of action of this drug, particularly in clinical doses (20-30 mg/kg), is as yet unknown. With such doses of Myanesin it has been confirmed that hyperirritable spinal reflexes (in our study experimentally produced in cats and monkeys under light chloralose anesthesia, by injection of prostigmine and strychnine) can be reduced, but are without any effect on normal reflexes. In the present study it has been shown that at higher dosage (40-50 mg/kg, i.v.) the multi-neuron reflex discharges are depressed before the two-neuron ones, and correspondingly flexion and crossed extension reflexes are depressed earlier than the knee jerk. In spinal animals strychnine increases predominantly the multineuron reflex discharge, which can be reduced to normal promptly with Myanesin. Complete inhibition of strychnine convulsions has also been obtained from electrical stimulation of the bulbo-reticular suppressor region and facilitation of subconvulsive doses from the facilitatory region. The multi-neuron discharges can be inhibited or facilitated at lower stimulus intensities than the two-neuron reflex discharge from these regions. The threshold for obtaining suppression or facilitation of spinal reflexes from stimulation of the bulbar suppressor and facilitatory centers was measured before and after the administration of clinical doses of Myanesin. The threshold was found decreased for the suppressor and increased for the facilitatory stimuli, indicating that Myanesin in some way affects the balance between inhibitory and facilitatory impulses playing upon the spinal neurons in favor of the former. The possibility that Myanesin at least partly may act via or on the same structures as the suppressor or facilitatory systems is suggested.

Respiratory and vascular responses in monkeys from temporal pole, insula, orbital surface and cingulate B R KAADA, K H PRIBRAM and J A EPSTEIN (introduced by JOHN F FULTON) *Laby of Physiology, Yale Univ School of Medicine, New Haven, Conn*. The results of electrical stimulation of the cortex of the temporal and frontal lobes and the insula of monkey with recording of vascular and respiratory responses are reported. Square wave pulses of low intensity (3-8 volts) and of varying frequency and pulse duration were used. From the temporal pole blood pressure alterations and respiratory inhibition were obtained.

The optimal parameters of stimulation were found to be a frequency of about 40/sec and long pulse durations (10 msec). With these optimal parameters similar vascular and respiratory responses were obtained from a continuous cortical region extending through the anterior insula, the posterior orbital surface of the frontal lobe, the subcallosal region to the rostral limbic gyrus and also including basal olfactory structures (uncus, limen insulae, anterior perforated space). The lateral surface of the frontal lobe including the depths of the arcuate and principal sulci were explored, but only inconsistent respiratory changes were obtained from the cortex dorsal to the superior limb of the arcuate sulcus and ventral to its inferior limb. The continuous responsive region is covered either by allocortex or by agranular or sparsely granular isocortex showing some characteristics of the nearness of allocortex. The relevant connections of these areas are discussed.

Radioactive diiodotyrosine in studies of thyroidal and tissue organic iodine GOPAL KARANDIKAR (introduced by WILLIAM T. SALTER) *Dept of Pharmacology, Yale Univ School of Medicine, New Haven, Conn.* All mammalian tissues contain organic iodine (so-called 'thyrenzyme'), except when hypothyroidism follows thyroidectomy or prolonged treatment with a goitrogen. When radioactive iodide is administered, the thyroid gland forms radioactive diiodotyrosine, later the organic iodine in peripheral tissues increases while the radioactive iodide declines. After the administration of radioactive diiodotyrosine intraperitoneally to rats (both normal and goitrous), part of the ensuing changes in iodine metabolism is due to iodide originating from decomposed diiodotyrosine. This feature is dependent quantitatively upon the dosage employed, because in larger dosage relatively less diiodotyrosine is de-iodinated. Both thyroid gland and peripheral tissue share in the newly acquired iodine. In less than an hour after diiodotyrosine is injected the total iodine in peripheral tissues increases. Besides iodide, diiodotyrosine contributes to the increase, but in the early stages its association with protein fractions is largely by adsorption. Eventually some of the newly acquired iodine is incorporated in the tissue protein (e.g., muscle, liver), although the distribution is not uniform throughout the several muscle protein fractions. In the building up of the genuine ('thyrenzyme') peripheral protein-bound iodine the thyroid gland plays an important role, which is influenced by goitrogenic substances.

Bile action on the volume and content of gastric juice in triple histamine experiments JERZY KAULBERSZ and RYSZARD BILSKI *Dept of Physiology, Jagellonian University, Cracow, Poland.* This study is a continuation of previous work done

at Wayne Univ College of Medicine by one of us (J. K.), which revealed in single histamine experiments (*Federation Proc* 1:45, 1942) that bile increases the secretion from the Pavlov pouches and gastric fistulas, while it seemed to have little if any effect on the secretion from the Heidenhain pouches. Beamer, Friedman, Thomas and Rehfuess (*Federation Proc* 3:3, 1944) noted that bile introduced into the stomachs of dogs with fistula results in a secretion of gastric juice, and instilled directly into the intestine it increases the gastric secretory response to proteoses.

In the present investigation a triple histamine method on dogs with Heidenhain pouches was employed, with one bile plus histamine and two histamine control tests on each day of the experiment. The total output of HCl and peptic power was determined. When histamine alone was administered 3 times a day in 24 experiments the average mEq of the first and third periods were identical, those of the second period 5% lower. Bile solution (5-10% ox bile) was introduced through a gastric tube in 54 triple histamine experiments, 18 times in each period. It evoked an increase of the volume of secretion from the Heidenhain pouches, affecting only slightly the acidity and the peptic power. The average mEq were 16-27% higher than those of the controls. Bile also exerts in dogs with Heidenhain pouches a slight stimulatory effect on the gastric secretion after histamine, which may not be easy to detect in single histamine experiments because of the daily variations in the HCl output. In cases of hypersecretion, the regurgitation of bile into the stomach may present a source of continuous stimulation.

Functional disturbances and their compensation following circumscribed lesions of the vestibular nuclei S. R. KEMBERLING (by invitation) and E. A. SPIEGEL *Depts of Exptl Neurology and of Pediatrics, Temple Univ School of Medicine and Hospital, Philadelphia, Penna.* Circumscribed lesions of the left vestibular nuclei (VN) were produced in 46 cats, in 29 the symptomatology was studied up to 5 months. Definite vestibular symptoms developed in 5 cats without abnormal head posture. In 4 others torticollis remained the only symptom except for slight homolateral hypotonia demonstrable by measurements only. The localizing value of this dissociation remains to be analyzed histologically. Postrotatory nystagmus tested in 12 cats showed no disturbance in one, reduction to left in 5, transitory loss to left and replacement by a few jerks to right in 2 (appearance of latent spontaneous nystagmus to right?). Definite compensatory phenomena as observed following labyrinthectomy (Ruttin) were absent. In 4 cases induced nystagmus to left was increased (effect of

latent tendency toward spontaneous nystagmus to left?) Residual slight reduction of extensor tone could be demonstrated only by measurements in 3 cats. Despite incomplete recovery of tone, decerebration could produce definite extensor rigidity of the forelimbs. Following decerebration there was in 3 cats no or only minimal difference of posture of the forelegs with slightly diminished extensor tone demonstrable by measurements only. In one cat measurements failed to reveal differences of left and rightsided extensor tone. In one instance maximum extensor tone (elbow) was higher on the operated side and twice extension was more pronounced in the left than the right hindleg (apparently overcompensation). The experiments point to the importance of the reticulate substance besides the V N for maintenance of tonus (Spiegel and Bernis, *Arbeiten Neurol. Inst. Vienna*, 1925).

Effect of oxygen administration on the survival of dogs explosively decompressed to 30 mm Hg. JOHN P. KEMPH (by invitation) and F. A. HITCHCOCK, *Dept. of Physiology, Ohio State University, Columbus, Ohio*. It has previously been reported from this laboratory that dogs survive exposure to a barometric pressure of 30 mm Hg for not more than 2 minutes. A series of experiments has been carried out in which oxygen was administered by insufflation to dogs explosively decompressed to this pressure. These dogs usually survived exposures of 3 minutes to this pressure. Insufflation was begun immediately after explosive decompression and continued until after the animal was returned to atmospheric pressure. Immediately upon recompression there was a bradycardia. The heart rate then gradually increased to a tachycardia, followed by a gradual decrease to a normal rate. Spontaneous respiration began about 60 seconds after insufflation was stopped. Occasionally animals survived 2 such explosions. In the animals which did not survive 3-minute exposures, several spasmodic ventricular beats were noted and autopsy occasionally showed the auricles to be contracting rhythmically. It is believed that this procedure is beneficial in two ways: 1) the pO_2 of alveolar air is increased and 2) the intrapulmonary pressure is maintained at a level sufficient to prevent the rapid evaporation of body fluids and to delay the formation of intravascular bubbles.

Influence of hesperidin methyl chalcone on the action of cardiac, striated and smooth muscles. W. JOHN KENFIELD and DAVID F. BOHR (introduced by ROBERT GESEIL), *Dept. of Physiology, Univ. of Michigan, Ann Arbor, Mich.* Observations have been made to determine the effects of hesperidin methyl chalcone (HMC) on the action of various isolated muscle tissues. One of the primary goals of this study has been to determine if the drug

has any activity as a general protoplasmic toxin. The influence of HMC on the isolated sartorius muscle of the frog was studied by comparing the shape of its fatigue curves obtained in the presence of HMC with the shape of control fatigue curves. The presence of 0.2 mg HMC per cc in the Ringer's bath did not change the shape of the fatigue curves. A study was made of the amplitude of contraction of the turtle's ventricle perfused with Ringer's solution containing various concentrations of HMC. No decrease in amplitude of contraction was observed with concentrations varying from 0.02 mg per cc to 0.2 mg per cc. The influence of HMC on the activity of smooth muscle was studied by observing its effects on isolated longitudinal muscle of the rabbit duodenum. In concentrations varying from 0.01 to 2.0 mg/cc of Tyrode's solution, HMC caused a decrease in both muscle tone and amplitude of intrinsic rhythmic contractions. These changes were completely reversible. Although definite changes in the physiological activity of smooth muscle were observed, no evidence was obtained to indicate that HMC has a toxic effect on isolated tissues.

Factors influencing the demonstration of renal tubular potassium secretion in the normal dog. THOMAS J. KENNEDY, JR., JAMES G. HILTON, and ROBERT W. BERLINER (introduced by JAMES A. SHANNON), *Research Service, First (Columbia Univ.) Division, Goldwater Memorial Hospital, and the Dept. of Medicine, College of Physicians and Surgeons, Columbia University, New York City*. Evidence of renal tubular secretion of potassium has been previously presented (Berliner and Kennedy, *Proc. Soc. Exptl. Biol. & Med.* 67:542, 1948; Mudge, Foulks and Gilman, *Ibid.*, p. 545). Experiments involving infusion of K salts have been done on 10 normal dogs. Excreted potassium was compared with filtered potassium, estimated as the product of plasma K and glomerular filtration rate. The latter was measured as the clearance of creatinine, inulin, or thiosulfate which, measured simultaneously, give identical results under the circumstances of these studies. Direct evidence of secretion, manifested by an excess of excreted over filtered K, has been obtained in all 10 dogs. In only one of 8 dogs could this be demonstrated without previous oral administration of K salts. Even in K-tolerant dogs there are considerable individual differences in the magnitude of the excess of excreted over filtered K. The ratio of excreted to filtered is a function of the anion with which K is infused—non-reabsorbable anions such as thiosulfate or ferrocyanide give considerably higher ratios than chloride. Within the range of plasma K safely attainable, there is no evidence of depression of the ratio of excreted to filtered as the rate of infusion of K is increased.

Sub-estrous response to dietary restriction and reversal in castrate C3H mice JOSEPH T. KING, CARMEN B. CASAS (by invitation) and M. B. VISSCHER *Dept of Physiology, Univ of Minnesota, Minneapolis, Minn* The sub-estrous response seen in certain strains of ovariectomized mice when fed *ad libitum* can be inhibited by restricting the caloric intake to about 66% of normal (Casas, King and Visscher, *Federation Proc*, 6: 87, 1947). Groups of 10 or more C3H mice, ovariectomized at 19-21 days of age, have been studied to determine the estrous response to full feeding after periods of restriction from 1 to 6 months; other groups were reversed from full feeding to restriction at periods up to 4 months. Control groups always full fed or always restricted were also studied. Restriction was to about 66% of the calories eaten by controls fed *ad libitum*; the absolute amounts of protein, salts and vitamins were essentially the same as taken by the control group. Smears were taken by the lavage method. In general restricted mice developed dense, mixed smears in 4-12 weeks after reversal to *ad libitum* feeding; there is considerable individual variation in the time required to develop a positive smear. Mice placed on restriction after full feeding show a positive smear for 2-5 months. If the period of full feeding is not longer than 1 month the mice usually remain in anestrus. Related weight changes are discussed.

Mixing rate within the mobile phosphate pool of cows MAX KLEIBER, A. H. SMITH (by invitation), N. P. RALSTON (by invitation) and D. E. JASPER (by invitation) *College of Agriculture, Univ of California, Davis, Calif* Intravenous injection of P^{32} into cows allowed the measurement of turnover rates for various processes of phosphate transfer. These rates characterize the mobility of the phosphate pool. The turnover time for mixing the mobile phosphate in blood with that of the extracellular tissue fluid of the cow is of the order of 10 minutes.

Procedure for the *in vitro* study of hormonal influences in nitrogen metabolism DANIEL L. KLINE (introduced by JANE A. RUSSELL) *Dept of Physiological Chemistry, Yale Univ School of Medicine, New Haven, Conn* Rat diaphragm and liver slices were incubated in 4 cc of Krebs-Ringer-bicarbonate solution at 38°C and the nitrogen which appeared in the medium was determined at 15 and 195 minutes. To obtain results which were consistent and biologically meaningful, it was necessary to centrifuge from the medium fragments which separated from the tissues during incubation. The N which appeared in the medium during the first 15 minutes was non-metabolic in origin, arising chiefly as debris from slicing. This was shown by the failure of ionic, osmotic and temperature changes to affect the 15-minute value, whereas

cutting the tissue into more pieces of the same thickness increased this value. The output of N during a period of 3 hours after an initial 15-minute equilibration period is believed to be metabolic in origin since the rate of addition of N to the medium during this time was lessened by lowering the temperature of incubation and was increased by the exclusion of O_2 and the addition of iodoacetate to the medium.

For normal animals the addition of total N to the medium between 15 minutes and 195 minutes was 0.31 ± 0.01 mg/100 mg of wet diaphragm and 0.35 ± 0.02 mg/100 mg of wet liver slices. The amino N increment in the medium was 52 ± 3 mcgm/100 mg of diaphragm and 44 ± 2 mcgm/100 mg of liver. In hypophysectomized rats the increments of total N were 0.18 ± 0.02 mg/100 mg of diaphragm and 0.24 ± 0.03 mg/100 mg of liver. The amino N increments were 29 ± 2 mcgm/100 mg of diaphragm and 28 ± 2 mcgm/100 mg of liver. These values are significantly different from those obtained in the normal series.

Isolation of legcoproporphyrin HENRICH KLÜVER *Division of the Biological Sciences, Univ of Chicago, Chicago, Ill* In a study of the prophyrins in the root nodules of legumes we have found that the nodules of *Phaseolus vulgaris* (var Red Kidney bean) contain a porphyrin which appears to be characteristically different from previously described naturally occurring porphyrins. The absorption and fluorescence spectra of the free porphyrin and of its methyl ester as well as of various metal complexes are practically identical with those of coproporphyrin. As regards solubility, the legcoporphyrin behaves like the Waldenström type of uroporphyrin. It can be transferred from aqueous extracts to ethyl acetate at a pH of 3.2 to 3.5. The legcoporphyrin obtained from the nodules of plants 4-5 weeks old is chromatographically separable into two porphyrins on a $CaCO_3$ column. Different extraction procedures and rechromatography do not affect this separation into two porphyrins. It has not been possible to distinguish the two components spectroscopically. Our attempts at crystallizing the legcoporphyrin ester or its components have failed although we have succeeded in crystallizing the esters of protoporphyrin and mesoporphyrin derived from the heme compounds in the same nodules. Microscopic and microspectroscopic examinations of sections through root nodules under ultraviolet clearly indicate that the legcoporphyrin is confined to the cytoplasm of the infected cells. Future studies of symbiotic nitrogen fixation must consider not only the presence of leghemoglobin in the infected cells, but also the presence of such substances as legcoporphyrin. It is of further interest that the organs and body fluids of

porphyria patients may contain a porphyrin which seems to possess the characteristics of leucoporphyrin

Oximetric method for determination of the oxygen saturation of mixed venous blood in man JULIAN KNUTSON (by invitation), BOWEN E TAYLOR (by invitation), and EARL H WOOD *Section on Physiology, Mayo Foundation, Univ of Minnesota, Rochester, Minn* Arterial oxygen saturation was recorded continuously and simultaneously in 6 subjects by both the modified ear-piece oximeter and a whole-blood oximeter attached to an indwelling radial artery needle. The instrumental time lag was 2.5 and 2 seconds for the ear-piece and whole-blood oximeter, respectively. Both oximeters were calibrated against Van Slyke analyses of arterial samples obtained when each subject breathed air, 14% oxygen and 100% oxygen. The Matthes procedure as modified by Elam and co-workers was used. This consists of deep expiration followed first by a deep inspiration of helium and, second, expiration to mid-position followed by about 30 seconds of breath-holding.

After the inspiration of helium, the initial decrease in arterial saturation (circulation time) occurred at 4.5 (3-7) seconds at the ear and at 4.6 (4-6) seconds at the wrist. Saturation decreased 17 (13-23) % in 10 (6-15) seconds and then reached a plateau in 3 of the subjects, or decreased gradually in the remainder until 4.8 (3-7) seconds (ear) and 7.5 (4-10) seconds (wrist) after the inspiration of air. The average duration and saturation at the arterial plateau was 4 (3-5) seconds and 78 (77-79) % at the ear and 4 (3-6) seconds and 75 (75-79) % at the wrist. In the 3 subjects in whom an arterial plateau was obtained, the cardiac index calculated by the Fick principle (assuming the plateau value equalled the saturation of mixed venous blood) was 3.1 (2.6-3.5) L/M²/min. The contour of the changes in arterial oxygen saturation recorded photoelectrically at the ear and directly on the arterial blood at the wrist were similar in every instance, indicating that the ear oximeter can be used successfully for determination of the arterial saturation plateau by the Matthes procedure.

Function of the ear in health and disease (motion picture) H. G. KOBRAK, JOSEPH E. HIND (by invitation) and ROBERT B. MILLER (by invitation) *Univ of Chicago, Chicago, Ill*. The purpose of the film is to demonstrate the response of the ear to sound in health and disease. A method was developed at The University of Chicago by which it was possible to photograph the acoustic vibrations of the ear during the process of hearing. The method consists in proper optical magnification combined with stroboscopic illumination. The first part demonstrates the normal ear. Ear drum vibrations in response to sound stimulation are

shown. Movements of ossicular chain and the vibrations of the round window membrane are demonstrated. The phase difference between the movements of the stapes in the oval window and the round window membrane is visualized. Diseases of the middle ear are demonstrated on fresh temporal bones. Pathological function due to inflammatory adhesions and due to stapes fixation are demonstrated. Hearing curves of certain types of deafness are shown and the response of the ear demonstrated. By using electronic filters, the sound perception of a diseased ear is given in the sound track similar to the actual hearing defect. Therefore, the audience is able to see the pathologic function on the screen and simultaneously hear the distorted acoustic signal which the patient perceives. The action of hearing aids and intensity changes due to the action of the hearing aid is demonstrated. The acoustical vibrations of a surgical 'fenestra' in the lateral semicircular canal are demonstrated. In the final scene, the response of the ossicular chain to a piece of orchestral music is given.

Some factors influencing the contractile process SAUL R. KOREY (introduced by DAVID NACHMANSOHN) *Dept of Neurology, College of Physicians and Surgeons, Columbia Univ, New York City*. Investigations have been initiated to test factors which influence the contractile mechanism of the muscle fiber in contrast to those acting on the conductive mechanism. A most suitable material for studies on the contractile system are the muscle fibers prepared according to the method of Szent-Gyorgyi. In these fibers, kept in glycerol, conduction is abolished whereas contraction can be produced by ATP but not by electrical stimulation. Among a great number of chemical compounds tested, none were found to be able to produce contraction. This specificity of ATP supports Szent-Gyorgyi's assumption that the effect observed is basically identical with the physiological mechanism of contraction. Among the compounds which had no effect were adrenalin, acetylcholine and eserine. The activity of several enzymes in these fibers was studied, especially that of ATPase. Their relation to the contractile process will be discussed. The effect of some enzyme inhibitors was also investigated. Inhibitors of sulfhydryl groups abolish the effect of ATP. The action of these inhibitors was found to be reversible. The possible interpretation of this observation will be discussed.

Skin resistance patterns associated with visceral disease IRVING M. KORR *Kirkville College of Osteopathy and Surgery, Kirkville, Mo*. This abstract reports the results of a preliminary investigation of the part that visceral disease and irritations may play in determining the electrical

skin resistance patterns previously described (*Federation Proc* 7 67, 1948, and this issue) Two classes of patients having visceral disease have been explored with the dermohmmeter (Jasper) and found to have low-resistance areas (LRA) which not only showed segmental relation to the viscus involved, but were fairly consistent for a given disease entity and were related to the referred pain pattern Patients who had had myocardial infarcts had LRA over 2 or more of the upper 4 thoracic vertebrae, near the sternum at corresponding rib levels, and over the medial edges of one or both scapulae in the corresponding dermatomes In one subject, repeatedly examined over a period of months, such areas were first observed 3 weeks prior to a coronary occlusion Patients with duodenal cap ulcer had areas of markedly lowered resistance over the spinal muscles, on the right side, opposite vertebrae T-5 to T-8, over corresponding ribs on the anterior chest wall and to the right of the umbilicus In both diseases LRA appeared to coincide with or overlie the most painful or tender parts of the reference zone

Experimental alterations in segmental sympathetic (sweat gland) activity through myofascial and postural disturbances IRVIN M KORR, *Kirkville College of Osteopathy and Surgery, Kirkville, Mo* It was previously shown (*Federation Proc* 7 67, 1948) that patterns of electrical skin resistance over the trunk varied from individual to individual, but that areas of reduced resistance (hyperactive sweat glands), especially in or near the midline, remained relatively constant in each individual for weeks or months, the low-resistance areas (LRA) often showed a dermatomal distribution, were correlated in some subjects with reduced segmental motor reflex thresholds, overlay or adjoined areas of deep hyperesthesia, frequently showed 'trigger zone' characteristics Subsequent investigations have been directed at the factors related to or contributing to segmental sweat gland hyperactivity Dermohmmeter exploration of many subjects has shown the following 1) LRA occurred in segments in which there were known myofascial tenderness or rigidity, abnormal intervertebral relations, vertebral pathology, or other somatic or visceral irritations in the corresponding spinal segments 2) Clinical improvement was accompanied by elevation of the skin resistance and shrinking of LRA 3) Injection of 0.3 ml 6% NaCl into erector spinae or intercostal muscle caused appearance of LRA throughout the corresponding dermatomes (spine to sternum) which outlasted the referred pain by many hours 4) Experimental postural imbalance induced by wearing a 'lift' under one heel for several hours produced new LRA in the segments

in which pain and tenderness appeared, enlarged pre-existing LRA in other, often distant, segments and further reduced their resistance These observations support the hypothesis that myofascial and postural disturbances are accompanied by and may induce or maintain segmental hyperactivity in the sympathetic nervous system

Further studies on the effect of 'neurotripsy' on the partially denervated muscle of the dog A J KOSMAN and J N FREDERICK (introduced by J S GRAY) *Dept of Physiology, Northwestern Univ Medical School, Chicago, Ill* In a previous communication (*Proc Soc Exptl Biol & Med*, in press) we had reported that 'neurotripsy' (traumatic massage of a muscle) was ineffective in arresting the loss of strength and mass of the partially denervated anterior tibial muscle of the dog The present investigation was undertaken in order to determine the effect of the 'neurotripsy' procedure upon muscles subjected to a more extensive denervation and observed for a longer period of time Partial denervation of the anterior tibial muscles of 8 dogs was accomplished by the removal of the motor roots of L₅ and L₆ bilaterally Closed manual 'neurotripsy' was performed on the anterior tibial muscle of one side 3 months following the initial lesion and the animals were then sacrificed 4 months following the 'neurotripsy' At the time of death the mean tension and weight of the treated muscles were 2500 gm and 7.29 gm, respectively, and of the untreated muscles, 2250 gm and 6.77 gm respectively These differences are not statistically significant

The spleen as a blood reservoir during hypoxia KURT KRAMER and ULRICH C LUTZ (introduced by HARRY F ADLER) *Dept of Physiology, USAF School of Aviation Medicine, Randolph Field, Texas* The response of the spleen to different types of hypoxia was studied in 20 dogs under light nembutal anesthesia Continuous recordings were taken photoelectrically of arterial blood O₂ saturation and hemoglobin content The spleen was exposed without interfering with vascular and nervous attachments and its weight recorded with an electric balance Blood pressure tracings were taken by strain gage manometer In acute hypoxia induced by breathing pure nitrogen the spleen did not lose weight until the arterial O₂ saturation was approaching zero After that the organ contracted visibly and lost considerable weight Simultaneously the systemic hemoglobin content increased During recovery breathing air the spleen regained its initial size in about 20 minutes In less abrupt hypoxia using the rebreathing method two phases of splenic activity were observed Slow contraction commenced when the arterial O₂ saturation dropped below 40% Shortly before failure of respiration and after the asphyxial rise in blood pressure

the spleen discharged a large amount of blood as evidenced by a drop in weight of approximately 150 gm and a rise in circulating hemoglobin of 3 gm %. The inverse relationship between spleen weight and systemic hemoglobin was also observed during subsequent recovery when the excess hemoglobin disappeared while the spleen was refilling. In blood samples taken from the splenic vein during contraction hemoglobin concentration was 30-40 % higher than in arterial blood. Control animals in which the splenic vessels were ligated before the hypoxic episode showed no change of hemoglobin concentration in the general circulation.

Dark adaptation in lesions of the optic pathways HOWARD P. KRIEGER (by invitation) and MORRIS B. BENDER *Psychophysiological Lab., Dept. of Neurology, New York Univ. College of Medicine, New York City*. A study of the visual threshold for a luminous target, presented in a dark room, was made in patients with lesions of the optic pathways. These patients did not have disease of the retina. Preliminary perimetric studies, made under 7-foot candle illumination, revealed consistent defects in the fields of vision. Three patients had homonymous hemianopsias, a fourth an almost complete double hemianopsia and a fifth a temporal hemianopsia of the left eye. The machine used for testing was Wald's modification of the Hecht-Schlaer adaptometer. It was so altered that the source of light which acted as the target could be placed at any angle along any meridian in the subject's field of vision. All measurements were made on one eye at a time and in a dark room. Examination under these conditions disclosed that the luminous target was perceived by the patient in the apparently blind half-field. However, the final threshold in this region was about 500 times higher than in the corresponding normal region. Other significant findings were a great fluctuation in the threshold of the ability to see the target and a marked lag in the rate of dark adaptation. The patient with bilateral homonymous field defects showed similar changes in both fields of vision. The patient with the unilateral temporal field defect showed a change in the dark adaptation of the seemingly preserved temporal half. Parallel changes were found in other visual and sensory functions. In summary it was found 1) that patients with lesions of the optic pathways were able to see a luminous target, presented in the dark, in their fields of vision which were apparently blind upon perimetric examination and 2) that fields, apparently normal upon perimetric examination, may have changes in their dark adaptation process.

Control of early hypertension with dihydrogenated ergot derivatives and the physiological significance W. G. KUBICEK, F. J. KOTTKE AND

MILDRED E. OLSON (by invitation) *Dept. of Physical Medicine, Univ. of Minnesota, Minneapolis, Minn.* A significant reduction in arterial blood pressure was produced in young hypertensive patients following administration of dihydroergocornine methanesulfonate (DHO 180). A decrease of 10 to 20 mm Hg in diastolic pressure persisted for the duration of the drug therapy (2-3 weeks) and gradually increased to the control level after removal of the drug. Since DHO 180 is known to have sympathicolytic and adrenolytic actions the above observations indicate that sympathetic nervous system activity may contribute to the establishment of early hypertension. Renal clearance tests revealed normal kidney function in these patients.

Adsorption and fluorescence characteristics of crustacean sinus gland extracts ELOISE KUNTZ (introduced by IRWIN W. SIZER) *Arnold Biological Lab., Brown Univ., Providence, R. I.* The sinus glands of crustacea secrete one or more hormones which affect chromatophore activity, molting, oxygen consumption, phosphate metabolism, etc. Abramowitz (*J. Biol. Chem.* 132: 501, 1940) obtained a partially purified preparation which had the properties of a basic amine. This paper includes two methods of separation based on cation adsorption column techniques. An active principle is adsorbed by the sodium form of Amberlite IRC-50 at pH 5. It can be eluted with 0.1 N HCl. Another effective adsorbent is Folin-permutit which has been prewashed with 0.1% acetic acid. Elution is accomplished with 10% KCl. The strong fluorescence exhibited by the hormone in the oxidized form (maximum fluorescence with exciting light of 3450 Å) has been used to estimate the relative concentration of minute quantities of the purified sinus gland extract and can also be used to follow purification procedures. There is a linear relationship between concentration and intensity of fluorescence. The hormone is easily oxidized and reduced by mild redox systems. Glutathione oxidizes the reduced form. This oxidation is readily reversed by addition of sodium hydrosulfite to the hormone-glutathione mixture. Sodium hydrosulfite alone is ineffective in acid or neutral solutions but can reduce the oxidized form of the hormone in alkaline medium.

Mass spectrographic studies on expired and alveolar air GEORGE H. KING III (by invitation) and FRED A. HITCHCOCK *Dept. of Physiology, Ohio State University, Columbus, Ohio*. Studies made with the mass spectrograph developed in this laboratory for the continuous recording of the partial pressures of O₂ and CO₂ and already reported have shown that the pO₂, the pCO₂ and the RQ of alveolar air are all a function of the duration of exhalation. This work has been continued

and extended. In recent experiments maximal exhalations made in three seconds have been compared with similar exhalations lasting for 10 seconds or longer. In the longer exhalations the final pO_2 is always lower and the pCO_2 higher than in the shorter exhalations. The difference is often 10 mm or more. In the longer exhalations the curves of both the O_2 and the CO_2 approach a plateau although they do not completely level off. Calculations of the R/Q at one second intervals confirm the previous report of Hitchcock and Stacy that the quotient during the first second is high and tends to decrease as the exhalation progresses. The value of the R/Q toward the end of a maximal exhalation but not necessarily at the extreme end shows the best agreement with the R/Q of exhaled air.

Transition of fibrinogen to fibrin KOLOMAN LAKI (introduced by WILLIAM J. BOWEN) *Inst of Biochemistry, Budapest Laby of Physical Biology, Exptl Biology and Medicine Inst National Insts of Health, Bethesda, Md*. The transition of fibrinogen to fibrin, brought about by the action of thrombin, proceeds in 2 stages. Thrombin acting enzymatically is involved only in the first step, and its role is to modify the fibrinogen molecules. In the second step, the altered fibrinogen molecules polymerize to the fibrin clot. Iodination of fibrinogen results in preparations that do not polymerize any more but still respond to the action of thrombin with a shift in the isoelectric point. This shift very likely means that some acidic groups of the fibrinogen molecule are split off by thrombin. A difference between the acid-base titration curve of fibrinogen and that of fibrin shows that some groups of the fibrinogen molecules with a pK of about 9 are involved in the polymerization process. These could be either $-NH_2$ groups or the phenolic group of tyrosine. The polymerization is not simply an end-to-end association of the altered fibrinogen molecules. First these molecules polymerize to a unit with a particle weight of about 1.5 million and then these units, probably connected by electrostatic forces, build up the primary fibrils. In this respect, the polymerization of fibrinogen seems to be strikingly similar to the polymerization of actin, a protein of muscle fibril.

Attempt at direct measurement of values required for calculating the pulmonary diffusion coefficient for oxygen CHRISTIAN J. LAMBERTSEN, JOHN K. CLARK (by invitation), DOMINGO M. AVIADO, JR. (by invitation), ROBERT G. PONTIUS (by invitation), LARI S. BARKER (by invitation) and JOHN MOYER (by invitation) *Laby of Pharmacology, Univ of Pennsylvania, Philadelphia, Penna*. Simultaneous, direct measurements were made of alveolar, arterial blood and

mixed venous blood gas tensions and rate of O_2 consumption in a group of normal human subjects in an attempt to obtain data required for calculating of the pulmonary diffusion coefficient for oxygen. Subjects were studied while breathing air, 8% and 100% oxygen. The data obtained illustrated the important practical and theoretical difficulties to be expected in attempting direct measurement of the pulmonary diffusion coefficient for oxygen. The data provides values for alveolar and arterial O_2 tensions during the breathing of air, 8% and 100% oxygen, and a comparison of the several methods of alveolar gas tension determination employed. The removal of nitrogen from the lungs by 100% oxygen inhalation suggested the feasibility of using the end expiratory Haldane-Priestly sampling method to obtain a close approximation to mean alveolar pO_2 in subjects breathing pure oxygen since, under these conditions, all alveoli should contain nearly equal and constant O_2 tensions. The difference in pO_2 between alveolar and arterial samples in 5 subjects breathing 100% oxygen ranged from 4 to 15 mm Hg, indicating that a maximum of about 1% of the cardiac output has not been exposed to oxygen in the alveoli. The use of air, low oxygen and high oxygen inhalation may permit estimation of the relative importance of poorly ventilating alveoli and addition to pulmonary venous blood of blood which does not pass through alveolar capillaries.

The law relating blood flow to perfusion pressure HAROLD LAMPORT *Laby of Physiology, Yale Univ School of Medicine, New Haven, Conn*. Blood flow (I) in the hindlimb of a dog varies with the n th power of perfusion pressure (P). When vascular tone rises, n increases and the constant (C) in the formula, $I = CP^n$, shifts in a manner which needs to be correlated with the corresponding changes in vessel dimensions. This logarithmic pressure flow curve characterizes pseudoplastic fluids. When slippage in the fluid does not occur (a discontinuous change in its flow character), the law of flow in a capillary of length L and radius R for pseudoplastic fluids is $I = \frac{\pi R^3 m}{n+3} \left(\frac{RP}{2L} \right)^n$, where m and n are constants for the fluid in the tube. Perhaps blood, at high flow rates, should be considered to 'slip', the central streaming red cells with their longer dimension oriented axially being distinct from the surrounding plasma sheath. However, a gradual change in the flow character of the blood from vessel wall to axis seems more likely. Since the pressure-flow relation of blood is fairly well described by the same logarithmic expression which is effective for pseudoplastic fluids without slippage (oil-water emulsions analogous to blood are an example), the development for the glass capillary has been applied to the blood vascular system.

After allowing for the effects of branching of vessels, etc., an equation results which tested satisfactorily in a set of four pressure-flow curves on the same preparation under different degrees of vasoconstriction

Tetraethylammonium and gastric motility of the dog ARDELLE LANE (by invitation), C R ROBERTSON (by invitation), and M I GROSSMAN *Dept of Clinical Science, Univ of Illinois College of Medicine, Chicago, Ill* Gastric motility in the human is inhibited by tetraethylammonium (TEA). As the gastric secretory response to histamine is decreased by TEA in the human and not in the dog, this study was undertaken to determine if this species difference exists in regard to the effect of TEA on gastric motility. A series of x-ray studies on dogs given a barium meal was made with and without TEA (10 mg/kg I.V.). Films were taken immediately after the meal and in the case of TEA administration immediately after the drug was given and at half hour intervals for a period of 2 hours and at hourly intervals until the stomach was empty. A series of x-ray studies on dogs directly after a barium meal and simultaneous administration of atropine and epinephrine (1 mg of each) were taken as a control procedure for comparison with the effects of TEA. Gastric motility tracings before and after intravenous administration of TEA were made in gastric fistula dogs by the balloon water manometer method. In the x-ray studies gastric tone and emptying time were not appreciably changed by the administration of TEA. In the experiments in which atropine and epinephrine were given gastric tone fell as shown by x-ray. The kymographic records of gastric motility showed individual variation through motility was either unaltered or slightly inhibited for a few minutes. These studies indicate that in the dog gastric motility does not respond as in the human to the administration of TEA.

Electrocardiographic findings in anthracosilicosis LEONARD P. LANG (by invitation) and HURLEY L. MOTLEY *Dept of Medicine, Jefferson Medical College and Cardio-Respiratory Lab, Barton Memorial Division of Jefferson Hospital, Philadelphia, Penna* The incidence of right heart strain as manifest by electrocardiographic changes was correlated with physiological studies of pulmonary function, chest roentgenograms and fluoroscopic examination in over 150 patients with respiratory complaints and a history of prolonged exposure to dust inside coal mines (20-45 years). Post-mortem examinations of miners have revealed that there is a high incidence of right ventricular hypertrophy and that many eventually die in cardiac failure. The physiological studies used for comparison with the electrocardiographic findings included maximal breathing capacity, total lung

volume, the degree of emphysema as measured by expressing the volume of residual air as percentage of total lung volume, and the mean partial pressure of O_2 in arterial blood (Riley method). Cardiac fluoroscopy was of little value in the study of these miners because the dense fibrous tissue masses in the hilar areas obscured the cardiac borders. Fixed rotation of the heart and fibrous adhesions complicated the interpretation of an electrocardiographic diagnosis of right ventricle hypertrophy. No apparent correlation was noted between the x-ray stage of silicosis as classified from the roentgenogram and the electrocardiographic changes. However, a relationship was observed between the incidence of right heart strain and the degree of emphysema based on quantitative measurements of residual air. In general the lower the resting mean arterial pO_2 , the higher the incidence of right heart strain, however, short induced periods of acute hypoxia produced minimal electrocardiographic changes.

The effect of handometer exercise upon normal grip strength ELEANOR M. LARSEN (introduced by W. J. MEEK) *Dept of Physiology, Univ of Wisconsin Medical School, Madison, Wis* The usual grip strength of normal individuals is determined largely by their occupation. If normal grip strength may be increased by Handometer exercise, the hand strength of individuals weakened by injury or disease might be expected to improve by employing the same method. The purpose of this investigation was to determine the effect of Handometer exercise upon normal grip strength. The usual hand strength of 14 young adult women, right-handed with one exception, was measured by a grip dynamometer before and after 20 bouts of work performed bi-weekly. The exercise apparatus was the Handometer which consists essentially of a sphygmomanometer-type hand bulb attached to an adjustable air-pressure system gauge. The work of compressing the bulb against a constant resistance of 30 lb pressure was continued at the rate of 60 contractions per minute until the needle could no longer be held constant. The number of contractions were counted and recorded for each hand. The results for every subject consist of 3 grip dynamometer readings for each hand pre- and post-exercise, and 20 sets of Handometer data for each hand. The grip dynamometer data were analyzed statistically. The results indicate that the usual right hand grip strength is stronger than the left, and that the Handometer exercise increased the grip strength in both right and left hands, with a slightly greater increase in the strength of the left hand.

Renal mechanism of albumin excretion in patients with nephrotic syndrome HENRI D. LAUSON, FRANCIS P. CHIVARD (by invitation), and

HOWARD A. EDWIN (by invitation), *Hospital of The Rockefeller Inst. for Medical Research, New York City*. Intravenous administration of Red Cross human plasma albumin to nephrotic children 3 to 5 years old has made possible a study of the mechanism of albumin excretion. The amounts given varied from 18 to 37 gm. Albumin concentrations in plasma and urine were estimated by Chow's immunological method (*J. Biol. Chem.*, 104: 457, 1935). The plasma concentrations varied from 0.2 to 0.9 gm. per 100 ml. before infusion, increased by 1 to 2 gm. after infusion, and then fell during the next 24 to 48 hours, during which repeated clearances of albumin, and in some cases, insulin and endogenous creatinine were measured, together with plasma volume. The plasma volume varied in the same direction as the albumin concentration. In about half the experiments albumin clearance was approximately constant with all concentrations of plasma albumin. In the other experiments, however, during the first few hours after the albumin infusion, when plasma volume and albumin concentration were near the maximum, the albumin clearance rose 50 to 100%. Creatinine clearances did not show proportional increases, suggesting that the rise in albumin clearance was due to some cause other than those which control the glomerular filtration of water and electrolytes. The data are consistent with the interpretation that the cause of nephrotic albuminuria is increased glomerular permeability to albumin, almost all of the filtered albumin being excreted. The rise of albumin clearance is correlated with plasma volume expansion, and it may be that this expansion mediates a further increase in glomerular permeability.

The elasticity of the rat lung. RICHARD W. LAWTON AND ALLEN L. KING (introduced by JAMES D. HARDY). *Dept. of Pathology, Cornell Univ. Medical College, New York City, and Dept. of Physics, Dartmouth College, Hanover, N. H.* Changes are observed in elasticity of rat lungs with age. Retardation of growth by restriction of diet also alters the observed elastic characteristics of the lungs (Bixton, Harnen and Pierlup, *J. Gerontology* 4: 105, 1946). Chronic pneumonia and bronchiectasis are eventually found in a high percentage of rats of advanced age. A mathematical analysis of elastic properties of rat lungs was undertaken, utilizing the data of the above authors, in order to evaluate the relation of the elastic changes to aging and the disease process. A single alveolus is generalized as a hollow shell whose wall is composed of a mesh of rubber like molecules arranged as irregular closed rings. The whole lung is considered as a multiple of the single alveolus. The equation based on the current statistical mechanical theories of high polymers, $p - p_0 = A(c_0/r)^2 [1 + \beta(r/c_0) - (c_0/r)^2 (1 + \{\beta(c_0/r)\}^2)]$,

In the equation p and c_0 are the initial and p and r the distended pressure and radius respectively. The constant A is proportional to the number of elastic chains participating in the distension. The parameter β characterizes the elastic chains in the manner similar to Young's modulus. The notation $1/r^3$ is the inverse Langevin function. The changes in the constants of the equation are correlated with the growth curve and the incidence of disease in the rats.

Biological studies with 4-methylestrone. J. H. LAWSON and MARY LEO CLARY (by invitation). *Rutgers Univ., New Brunswick, N. J., and Ciba Pharmaceutical Products, Summit, N. J.* Introduction of a methyl group into the aromatic ring of estradiol is reported to abolish its activity. Tests involving the effect of methylating estrone are here presented. 4-methylestrone failed to stimulate uterine weight or histology in immature rats 18 hours after the single subcutaneous injection of as much as 2.0 mg. or after 10 daily injections of 0.25 mg. Furthermore, 1.0 mg. of 4-methylestrone did not alter the uterine stimulating action of concomitantly injected estradiol benzoate (2 μ g.) when both steroids acted over 18 hours. The estrogenic action of 0.01 mg. of estradiol benzoate injected for 10 days was not influenced by the simultaneous administration of 0.25 mg. of 4-methylestrone in immature female rats. A single injection of 1.0 mg. did not influence seminal vesicle weight of immature male rats in 72 hours nor did it interfere with the action of 0.25 mg. of testosterone propionate. Administration of 0.5 mg. of 4-methylestrone daily for 10 days to 22 day old male rats failed to influence body weight increase or to alter the weights of the pituitary, adrenal, thymus, kidney, testis, seminal vesicle or ventral prostate. The poundotrophic hormone content of the pituitary was normal. Thus the estrogenic action of estrone is greatly reduced or abolished by the introduction of a methyl group.

Plasma and liver protein concentrations in adrenalectomized and in thiouracil-fed adrenalectomized rats. J. H. LAWSON. *Bureau of Biological Research and Dept. of Zoology, Rutgers Univ., New Brunswick, N. J.* Adrenalectomized adult male rats maintained for 20 days on 1% NaCl and pair fed normals have comparable plasma protein levels although plasma albumin concentrations are subnormal, a reflection of subnormal food intake. Deoxycorticosterone acetate (Percorlon, Ciba) (DCA) at 0.5 mg. daily permitted normal food intake and growth in adrenalectomized rats. Pair fed controls exhibited normal plasma protein concentrations but the operated rats' plasma albumin level was not improved above that of a salt maintained rat due to either hemodilution or hormone deficiency. Hypothyroidism increases plasma pro-

bulin levels and thiouracil increases liver protein. Hypothyroidism in adrenalectomized rats maintained with NaCl or with DCA was studied by feeding 0.25% thiouracil for 20 days and comparing results with pair fed adrenalectomized and normal rats. Food intake was reduced by thiouracil with body weight loss resulting. Total plasma protein and plasma globulin levels increased following thiouracil. NPN was also higher whereas hematocrit decreased. Thyroid and liver weights were significantly above those of controls and analysis of the liver revealed a normal water content but a significant increase in total and relative amounts of liver protein in thiouracil fed rats. Adrenalectomized control rats maintained liver protein as well as pair fed normal rats. Thus, adrenalectomy does not alter the action of thiouracil on plasma and liver proteins.

Predisposition to vasodepressor properties of morphine induced by hemorrhagic hypotension. RICHARD E. LEE (by invitation) and BENJAMIN W. ZWEIFACH, *Dept. of Pharmacology, Syracuse Medical College, Syracuse, N. Y. and Dept. of Medicine, New York Hospital, New York City.* Several investigations concerning the action of morphine in hemorrhagic shock have not shown any consistent notable vasodepressor action. The agent was administered either prior to hemorrhage or during the early phases of a prolonged hypotension. Neither situation adequately duplicates the usual clinical sequence of events. In this study the morphine was administered following restoration of normal blood pressures by blood transfusion after a prolonged hemorrhagic hypotension, as well as prior to and during early 'shock.' Based on blood pressure recordings and direct visual observation of omental peripheral blood vessels, usual doses of morphine (1-3 mg/kg intravenously) in unhemorrhaged dogs produce no significant or persistent vasomotor disturbances. When given early in hemorrhagic shock (1-2 hr after initial bleeding) morphine administration is followed by a transient fall in blood pressure, accompanied by temporary vasodilatation with slowing of peripheral blood flow. In spite of restoring normal blood pressure by transfusion, after prolonged hemorrhagic shock, morphine in these doses is followed in all instances by a sharp severe fall in blood pressure. This coincides with observed arteriolar dilatation, depression of arteriolar reaction to topically applied epinephrine, and a severe slowing of peripheral blood flow, with stagnation occurring in the venular components of the capillary bed. Although immediate further transfusions were given each animal, 3 of the 12 dogs died in profound peripheral vascular collapse within 15 minutes after receiving morphine.

Physiological effects of bacterial polysaccharides upon the cardio-vascular and neuro-muscular systems. CHESTER E. LEESE and K. VIRGINIA GREENE (by invitation), *Dept. of Physiology, School of Medicine, George Washington Univ., Washington, D. C.* Rabbits were given doses of polysaccharide from 2 to 25 gamma/kg. Most of the animals showed a strong pyrogenic response associated with the usual maladjustments. Many animals lost the use of their body-righting reflexes. Lethal doses produced convulsive seizures, urination, defecation, and vomiting preceding death. Cats were less affected by the dosage level effective in rabbits. Pyrogenic reaction was noted before decerebration. Decerebration was often fatal when the animal was under the influence of the drug. If the drug was withheld until the animal recovered from decerebration, it tolerated a higher dosage without pyrogenic response. The cat showed lowering of arterial blood pressure, and an increased venous pressure. Both electrical and chemical stimulation of the vascular reflexes indicated a loss in the controlling power of the autonomic nervous system, or a lesser ability of smooth muscle to accept the stimuli. The vagus, splanchnic, and cardiosympathetic nerves required an increased strength of stimulus under polysaccharide influence. Assays with adrenalin, acetylcholine, pitressin, and histamine pointed to a loss in nervous response, rather than a decrease in the ability of smooth muscle to accept stimulation. Turtles perfused with polysaccharide showed cardiac changes. First to appear was an occasional dropped beat, followed by the various stages of heart block, and finally, complete dissociation. Tremendous dilation of both auricles and ventricles was noted. There occurs a progressive loss in the effectiveness of adrenalin or acetylcholine when added to the perfusate. Stimulation of the vagus also becomes less effective.

Food packages as a supplement to the German nutrition. G. LEHMANN (introduced by D. B. DILL), *Max Planck Inst fuer Arbeitsphysiologie, Dortmund, Germany.* The German nutrition of a man doing no heavy work reaches 1850 cal per day. This includes 47 gm of protein, of which only 11 gm are of animal origin, and about 20 gm of fat. A food parcel raising the nutrition of a family of 4 for one week to a normal level therefore, should contain foodstuffs rich in protein and fat, for instance the following: 2.5 lb of corned beef, 1 lb of whole milk powder, 1 lb of cheese, 0.5 lb of powdered egg, and 1 lb of margarine. Instead of corned beef, dried and smoked meat could be used. In contrast to the view of some nutritionists, the housewife will not take soy beans or other legumes as a valuable substitute for meat. The German housewife is not fond of pre-cooked meals, but

prefers basic foodstuffs. The German nutrition is very rich in carbohydrates. Therefore, foodstuffs that are predominately carbohydrate should be avoided. Chocolate and cocoa are very welcomed and useful. Coffee and tea are important because of their stimulating effect. The addition of fruit juices, marmalade, sweets, spices, etc. is less a matter of nutrition than a matter of making people happier. We are wrong in looking upon the question of food parcels from the point of view of the scientist only. Apart from raising the German nutrition to a normal level, they have the ideal task of intensifying friendship and understanding between men and nations.

Central hyperglycemic action of 1-(3,4-dihydroxyphenyl) 2-isopropylaminoethanol (isuprel)
A LEIMDORFER, *Dept of Pharmacology, Loyola Univ, Chicago, Ill.* In dogs, under nembutal, the effects of intravenous (i v) and intracisternal (i c) injection of isuprel on blood-sugar, blood pressure, and electrocardiogram were studied. Nembutal itself produced no changes in blood sugar. After i v isuprel, 75 micrograms/kg, only a small, short lasting increase in blood-sugar occurred (onset about 15 minutes after injection, highest increase 40 mg per 100 ml). The preinjection level was reached 45 to 60 minutes after injection. In contrast, after i c injection of the same amount, a much greater and long lasting increase in blood-sugar was found (onset about 15 minutes after the injection), the blood-sugar continued to rise for entire period of the observation (3½ hours), at that time it had risen to 180 mg per 100 ml.

After i v isuprel, a marked fall in blood pressure, a marked tachycardia and an extremely deep depression of the S-T segments were recorded. These changes were much smaller after i c isuprel. There seemed to be no direct relation between changes in blood-sugar and those in blood pressure and heart rate. Of importance might be the fact that after i c isuprel the blood-sugar was continuously rising whereas the changes in blood pressure and electrocardiogram were approaching the pre-injection levels. This fact and the particular blood-sugar curve after i c isuprel in contrast to that following i v isuprel suggest that the action of i c injection of isuprel in raising blood-sugar implies action on structures (centers) bathed by cerebrospinal fluid. These observations about isuprel which has an action like sympathin I are in conformity with our previous findings.

A method for collecting end-expiratory air from the oral cavity GERSON LESSER, MORTON GALTSTON, and MARVELIN PRUSS (introduced by J MURRAY STEELE) *Research Service, Third New York Univ Medical Division, Goldwater Memorial Hospital, New York City.* In elderly or diseased patients it has often been impossible to collect alveolar air samples by the Haldane-Priestley

method due to their inability to cooperate in performance of the necessary respiratory maneuvers. To overcome this difficulty and to maintain an undisturbed respiratory pattern, a simple, inexpensive method for collecting end-expiratory samples is being studied. Air samples are drawn at the end of a usual expiration through a narrow, semi-rigid tube, threaded through a rubber mouth-piece with its proximal open end 4-5 cm within the oral cavity. The other end of the tube is connected to a small 3-way stopcock which is attached to the end of an evacuated mercury gas sample bottle. The stopcock permits washing out the tube with expired air just prior to collection of the end-expiratory sample. Preliminary evaluation of 40 observations in 4 young, healthy subjects suggests that for each individual there is a consistent relationship between the composition of end-expiratory air and alveolar air (Haldane-Priestley, end-expiratory). The $p\text{CO}_2$ was always lower (individual means of $\Delta p\text{CO}_2$ were 1.6 to 4.1 mm Hg), and $p\text{O}_2$ was always higher (individual means of $\Delta p\text{O}_2$ were 2.4 to 7.9 mm Hg) in end-expiratory than in alveolar air. In any given subject the greatest variation of a single observation from his mean was 3.5 mm Hg $p\text{CO}_2$ and 6.4 mm Hg $p\text{O}_2$ for end-expiratory air (26 observations), and 1.1 mm Hg $p\text{CO}_2$ and 2.7 mm $p\text{O}_2$ for alveolar air (14 observations).

Analysis of the electrocardiographic pattern produced by injection of epinephrine and nor-epinephrine in cats EUGENE LEPECHKIN and W RAAB (introduced by F J SICHEL) *Division of Exptl Medicine, Univ of Vermont College of Medicine, Burlington, Vt.* Two to 6 seconds after intravenous injection of 20-40 micrograms epinephrine or nor-epinephrine in 4 cc Ringer, a transient elevation of the T-wave in unipolar precordial or direct leads from both ventricles is observed. This elevation lasts 2-7 seconds, appears on injection of corresponding amounts of plain Ringer, and is due to cooling of the subendocardial ventricular muscle layers. The subsequent changes are due to the epinephrine or nor-epinephrine proper. Ten to 15 seconds after injection T becomes depressed over the right ventricle and elevated anew over the left ventricle (Phase I). During Phase II (until the 40th-50th second), T is inverted over the right ventricle, flattened (sometimes inverted) over the left ventricle. Phase III extends until the 120th-160th second and is characterized by marked elevation of T over both ventricles. In some cats T may again become temporarily inverted over the left ventricle between the 300th-400th seconds (Phase IV). These changes of the T-wave are independent of the changes of the heart rate. They are not due to blood pressure elevation (increased heart work), as they appear even if this elevation is abolished or reversed through previous administration of certain sympatholytic drugs (933F, SY28, DHE180,

Priscol) Other sympatholytic drugs (DHE 45, SY30) inhibit both the hypertensive reaction and the electrocardiographic changes. The latter cannot be due to direct action of epinephrine on subendocardial muscle layers of the right ventricle, as they appear in similar sequence and magnitude on injection of epinephrine into the left auricle.

Lecithinase activity of epiphyseal cartilage of the foetal pig MILTON D. LEVINE and RICHARD H. FOLLIS, JR. (introduced by JOHN EAGER HOWARD) *Depts. of Medicine and Pathology, Johns Hopkins Univ., Baltimore, Md.* Lecithinase of the type described by Macfarlane and King (*Biochem J.* 35: 884, 1941) has been demonstrated in the epiphyseal cartilage of foetal pigs. Homogenates of such cartilage, when incubated with lecithin suspension substrates in the presence of Ca^{++} , results in the liberation of neutral fat and free choline while the phosphorus of the substrate goes from the fat soluble to the water soluble state. The mechanism is thought to be as follows: Lecithinase splits lecithin into phosphocholine and neutral fat. Phosphocholine is a substrate for phosphatase, hence we have a hitherto undescribed source of phosphate ion. Lecithin and the appearance of neutral fat in cartilage will be discussed.

Nature of the action of insulin on the level of serum inorganic phosphate R. LEVINE, S. D. LOUBE (by invitation) and H. F. WEISBERG (by invitation) *Dept. of Metabolic and Endocrine Research, Medical Research Inst., Michael Reese Hospital, Chicago, Ill.* It is well known that the administration of insulin, glucose or adrenaline causes a fall in the level of inorganic phosphate of the serum. We have previously shown (*Am J Physiol* 134: 40, 1941) that no decrease in serum phosphate occurs after the administration of glucose or adrenaline to depancreatized dogs, indicating that the phosphate lowering effect of these substances is mediated by insulin. It was then concluded that the fall in serum phosphate is one of the direct actions of insulin. The rate of entry of fructose into tissues, as measured by the rate of disposal of intravenous fructose, is not diminished by the complete absence of insulin (pancreatectomy) (*Federation Proc.* 6: 21, 1947). We have, therefore, determined the effect of intravenous fructose on the serum phosphate level of untreated depancreatized dogs. In 9 of 10 animals there was a significant drop in inorganic phosphate of the serum following fructose administration. Under similar circumstances intravenous glucose does not affect the phosphate level. It is, therefore, concluded that the fall in serum phosphate is an expression of the rate at which a hexose enters the tissues. Insulin enhances this rate for glucose. Fructose, which enters tissues at a rapid rate even in the complete absence of insulin, causes a phosphate fall independent of the hormone. We can therefore no

longer consider the fall in serum phosphate as a direct action of insulin. The only presently proven primary action of insulin is the increase in the rate of entry of glucose into tissue, specifically skeletal muscle.

Three dimensional electrocardiography RAPHAEL B. LEVINE (by invitation) and OTTO H. SCHMITT *Depts. of Zoology and Physics, Univ. of Minnesota, Minneapolis, Minn.* Much of the difficulty in the interpretation of electrocardiograms lies in correlating records of the several different potential variations which occur simultaneously. If it were possible to combine all of the information contained in the numerous individual lead electrocardiograms into a single interpretable pattern, compactness would be obtained with no loss in generality. The 'vector cardiogram' of Wilson and Johnston is an approach in two dimensions to this ideal. Development of the 3-dimensional cathode ray oscilloscope has made possible the extension of electrocardiographic presentation to three dimensions. This instrument is capable of utilizing three electrical signals in such a way as to form a picture which to the eyes is a stereoscopically correct perspective pattern in space. An application of such an oscilloscope which immediately suggests itself is the presentation of a true 3-dimensional vector electro-cardiogram. This requires development of orthogonal leads or their electrical equivalent. In practice, it may prove entirely satisfactory to present data from conventional non-orthogonal leads in orthogonal form. As a further improvement, a computer currently under development will permit rotation and translation at will of any three-dimensional display to the angle and range most advantageous for viewing. These 3-dimensional displays can readily be photographed on standard film for stereoscopic viewing, and lend themselves well to the preparation of wire models.

Fat metabolism in Alaskan Eskimos VICTOR E. LEVINE and CHARLES G. WILBER *Arctic Research Lab., Office of Naval Research, Point Barrow, Alaska.* The blood sera of Point Barrow Eskimos (adults and children of both sexes) were taken for estimation of various lipid components. To date 50 analyses have been completed. Mean values are as follows:

LIPID COMPONENT	ALASKAN ESKIMOS	CANADIAN ESKIMOS (CORCORAN & RADINOWITCH)
	mg/100 cc	mg/100 cc
Total fatty acids	701.3	535
Total cholesterol	221.6	141
Free cholesterol	124.9	46
Ester cholesterol	96.7	95
Lipid phosphorus	14.2	—
Phospholipids	355.0	164

The following were calculated cholesterol/lipid phosphorus, 21.4, free cholesterol/total cholesterol, 0.56, cholesterol/fatty acid, 0.31. Our results compared with normal values for whites are high. The blood was taken late in August and early in September 1948, during which time walrus and seal were plentiful. The Eskimos were living close to their native diet. The values may, therefore, reflect a pronounced 'alimentary fat tide' due to the ingestion of fat rich meals. Our findings are not in accord with those of Corcoran and Rabinowitch (*Biochem J* 31:343, 1937) who found low lipid values in the bloods of 27 Canadian Eskimos taken at random. They did, however, report higher lipid values for natives on a meat diet compared to those for natives on a mixed diet. Under field conditions in the Arctic it is extremely difficult to collect blood in true fasting condition. Our results reflect a high fat intake, while those of the Canadian investigators may reflect a low fat intake or even a diet close to a starvation level.

Measurement of the intracellular cation in the dog. MARVIN F. LEVITT and MARIO GAUDINO (introduced by HOMER W. SMITH) *Dept of Physiology, New York Univ College of Medicine, New York City*. The measurement of the intracellular concentration of any cation in the living animal requires the determination of the volume of intracellular fluid and of the total intracellular cation. In our present experiments the intracellular fluid was calculated as the difference between total body water (D_2O space) and extracellular fluid (inulin space, *Proc Soc Exptl Biol & Med* 68:507, 1948). The total amount of body cation (Na or K) was measured by *in vivo* dilution of Na^{24} and K^{42} . A known amount of isotope was injected intravenously and the ratio of isotope to total cation in the plasma was followed until equilibrium was attained. Equilibrium requires 3 hours for Na^{24} (Kaltreider et al *J Exptl Med* 74:569, 1941), and, according to our present observations, 9 hours for K^{42} in the dog. Observations with carefully purified K^{42} indicate that this cation penetrates tissues so rapidly that 85% of equilibrium is reached within 3 hours. At equilibrium, the counts retained in the body (considering urinary loss) divided by the total counts per mEq of Na or K in the plasma, gives the total Na or K in the animal. In 2 dogs the average values were

ELECTROLYTE CONCENTRATION² (MEQ/L)

	Na	K	Total
Extracellular (plasma)	150	4	154
Intracellular	34	111	145

Inhibition of a proteolytic enzyme system of blood. JESSICA H. LEWIS (by invitation) and JOHN H. FERGUSON *Dept of Physiology, Univ of North Carolina, Chapel Hill, N C*. In certain human pathological conditions such as shock, anoxia, and severe liver disease, the phenomenon of liquefaction of a formed blood clot (fibrinolysis) has occasionally been observed. Normal serum contains no active lysin, the enzyme causing fibrinolysis, but does contain large amounts of the inactive precursor, prolysin, and a potent inhibitor, antilysin. The present report, dealing with certain reactions of antilysin, is part of a series of studies by which we are endeavoring to shed some light upon the mechanism of fibrinolysis in various human disorders and hemorrhagic shock in dogs. Standard curves are presented for the determination of potency of lysins partially purified from dog, human, and bovine sera, as measured by the lysis of a standard fibrin clot. The three curves are apparently identical. There is no detectable species specificity of each antilysin for its homologous lysin, as tested by the antilysin assay method here presented. Serum antilysins do differ in important particulars from certain purified proteolytic enzyme inhibitors isolated from soybean, navy bean, and pancreas. Contrary to the suggestion that proteolytic enzymes may be inhibited by the products of protein digestion, it is shown that serum lysin is not inhibited by the products of fibrinolysis. Attempts were made to study the nature of the enzyme-inhibitor complex. It could not be split by differential heat destruction of the enzyme nor by chloroform treatment which has long been known to remove inhibitor from such prolysin-antilysin mixtures as serum.

Studies on lipoprotein in dog serum. LENA A. LEWIS and IRVINE H. PAGE *Research Division and the Bunts Inst of the Cleveland Clinic Foundation, Cleveland, Ohio*. Four protein peaks can be demonstrated by ultracentrifugation of dog serum previously diluted and dialyzed against 1.0, 1.5 or 2.0 M $NaCl$. They are similar to those described by Pedersen for human serum. The peak with the slowest sedimentation rate is not demonstrated at salt concentrations of 0.6 M $NaCl$ or less, and is only poorly resolved with 0.75 M $NaCl$. Using 2.0 M $NaCl$ —2% dog serum protein solution, prolonged centrifugation in the preparative rotor was done. The top layer which had a 'milky appearance' was removed and studied by electrophoresis and ultracentrifugation. With phosphate buffer pH 7.8, ionic strength 0.16 M, two fractions were demonstrated by electrophoresis. One had a mobility similar to that of serum albumin (dog) and represented 57% of the total area. The slower moving component had a mobility similar to that of α_2 globulin (dog) and represented 43%. The albumin in the dog

serum was 47.9% and the α_2 globulin 6.2% of its total protein. The slow sedimenting lipoprotein of human serum has the electrophoretic mobility of β_1 -globulin. In the ultracentrifuge two peaks were demonstrated in the lipoprotein concentrate when salt concentrations of 1.0 and 1.5 M NaCl were used. The faster sedimenting component had a sedimentation rate similar to serum albumin and represented 62% of the total area. The slower sedimenting fraction represented 38%. A slow sedimenting lipoprotein with the electrophoretic mobility of α_2 -globulin can be demonstrated and concentrated from dog serum by ultracentrifugation when salt concentrations between 1.0 and 2.0 M NaCl are used.

Metabolism of lactate by the isolated dog gastrocnemius studied with α, β - and carboxyl-labeled lactate. NATHAN LIFSON, AKIRA OMACHI (by invitation), and SHIRLEY L. MICHEL (by invitation) *Dept. of Physiology, Univ. of Minnesota, Minneapolis, Minn.* The isolated gastrocnemius of a nembutalized dog was perfused at physiological pressures and temperatures by means of a closed, pump-oxygenator circuit, the animal's own heparinized blood serving as the perfusion fluid. The muscle was either at rest or was stimulated via the sciatic nerve with maximal single shocks at a frequency of 2/sec for 2-3 hours. Either α, β - or carboxyl-labeled racemic C^{13} -sodium lactate was administered to increase the blood level by approximately 1.4 mM/100 cc. In 3 experiments with stimulated muscle it could be calculated from

$$\frac{\text{total mM } CO_2}{3} \times \frac{\text{atom \% } C^{13} \text{ excess in respiratory } CO_2}{\text{atom \% } C^{13} \text{ excess in administered lactate}}$$

that 0.56-0.94 mM/100 gm of muscle/hr of administered lactate was converted to respiratory CO_2 . The addition of non-isotopic acetate or succinate to give blood levels of about 0.8 mM/100 cc reduced these values by at least 50%. In these latter experiments acetate, succinate, and lactate were recovered from blood and/or muscle and analyzed for C^{13} . Resting muscles, to which was administered glucose with or without insulin, also converted administered lactate to respiratory CO_2 but at slower rates than did stimulated muscles. In the stimulation experiments the glycogen content of the perfused muscle decreased in comparison with that of the corresponding unperfused muscle of the other side. In the experiments with resting muscles, the glycogen content increased when insulin was administered. However, in no instance was definite significant excess C^{13} found in the muscle glycogen. From the above data it is

concluded that 1) lactate is converted to CO_2 by both resting and active mammalian skeletal muscle, 2) the presence of other substrates (acetate and succinate) decreases the rate of conversion of administered lactate to CO_2 , and 3) unequivocal evidence for the conversion of lactate to glycogen in either contracting or resting muscle was not obtained.

Further studies on the single muscle fiber membrane potential. G. LING and R. W. GERARD *Dept. of Physiology, Univ. of Chicago, Chicago, Ill.* Studies on the membrane potential of frog sartorius fibers, singly impaled with a capillary electrode (Ling, Ling and Gerard), have been extended. The A potential fraction, between 54 and 78 mV, has been shown to parallel fairly well the actual CrP content under the influence of tetanization, methylene blue, cyanide, azide, hydroxylamine, iodoacetate alone or with lactate. The B potential, between 0 and 54 mV, is not directly dependent on metabolism but varies with K^+ , increased K^+ lowers the plateau between A and B potentials which appears when the A potential has been abolished by some of the above conditions. Continuous irrigation of the muscle surface is necessary to avoid accumulation of leaking K^+ . Thus, in unstirred K -free solutions, the potential rises irregularly, seldom above 90 mV, in flowing ones, it can rise to 108 mV. Also, the fall in N_2 is less in flowing than still solutions, and that in IIA starts later. In general, potential varies inversely as log of external K^+ concentration (slope = 44 mV per tenfold change) to -7.0 mV at 4 times isotonic K^+ . The slope of the K^+ -potential curve is seemingly altered by addition of Ba^{++} but not by Ca^{++} . Exposure to CO_2 , in contrast to nerve, lowers the normal value by 5 to 10 mV during 1 to 2 hours, return in air is complete in 1 hour. In 20% or 100% CO_2 , alike, the A potential is lost in half to one hour, returns in a few minutes. Absence of K^+ from the irrigation fluid does not alter the size of the CO_2 depression. Conclusions can be drawn from these data as to the nature of the membrane.

Effect of temperature on the membrane potential of the frog sartorius muscle. G. LING and J. W. WOODBURY (introduced by R. W. GERARD) *Dept. of Physiology, Univ. of Chicago, Chicago, Ill., and Dept. of Physiology, Univ. of Utah, Salt Lake City, Utah.* Variations of membrane potential with temperature were measured in the sartorius muscle of *Rana pipiens* by inserting a micro electrode directly into a single fiber. Four to eight potential measurements were made on each muscle at from 4 to 6 different temperatures between 3° and 41°C on a total of 28 muscles. The muscles were allowed to equilibrate at each temperature for at least 20

minutes Temperature was progressively increased in 14 experiments, decreased in ten, and first increased then decreased in four The averaged data from 8 muscles (temperature increasing) are tabulated below

T° C	5	15	20	23	30	37	39	40	40.5	41
V mv	71	80	83	85	87	85	83	80	71	63

For progressively increasing temperature from 5°C to 25°C the relation between log voltage and reciprocal of absolute temperature was approximately linear with a Q_{10} of 1.1 (activation energy 1700 calories) The mean Q_{10} for all muscles was 1.08 (1400 calories) Membrane potential reached a peak at approximately 30°C and fell precipitously and irreversibly above 35°C, in association with contracture The low temperature co-efficient may possibly represent the difference between energies of activation for penetration of the membrane by positive and negative ions produced by metabolic processes

A modified procedure for inulin determination in plasma and urine J MAXWELL LITTLE, JUNE HARRIS (by invitation) and LEWIS FRANKLIN (by invitation) *Dept of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N C* Oxidative destruction of glucose by heating in the presence of NaOH has been utilized to replace the usual destruction by yeasting (Selkurt *Methods in Medical Research* 1 197, The Year Book Publishers, Inc., Chicago, 1948) The effects of NaOH concentration and duration of heating in boiling water upon glucose and inulin were studied Incomplete glucose destruction (with 50-300 mg % solutions) or production of an absorbing intermediate compound was indicated by light transmissions of 93-99% when compared with a water blank set on 100% This residual absorption was almost completely rectified by the addition of 1.0 cc of 100 mg % glucose to the blank, which was set on 100% *Procedure* plasma, or diluted urine, containing 5-30 mg % inulin, is precipitated with CdSO_4 and NaOH after the addition of 1.0 cc of 100 mg % glucose solution Shake the mixture vigorously for 10 minutes, centrifuge, and filter through washed cotton To 4.0 cc of filtrate, in a large test tube, add 1.0 cc of 4.0 N NaOH, cover with marbles and immerse in boiling water for 10 minutes Cool, add 10 cc of diphenylamine reagent and immerse in boiling water for 30 minutes Cool the tubes and read in an Evelyn colorimeter using filter 620 Blank tubes contain plasma or urine without inulin Mean recoveries

and standard deviations with theoretical concentrations of 5, 10, 15, 20, and 30 mg % inulin for plasma were respectively 5.10 ± 0.293 , 9.93 ± 0.357 , 15.21 ± 0.664 , 20.25 ± 0.846 , and 30.53 ± 0.700 , for urine 4.98 ± 0.235 , 9.78 ± 0.272 , 14.94 ± 0.224 , 20.16 ± 0.479 , and 30.49 ± 0.523

Atrial hemodynamics in the presence of experimentally produced interatrial septal defects R C LITTLE (by invitation), J G HAWLEY (by invitation) and D F OPDYKE *Dept of Physiology, Western Reserve Univ Medical School, Cleveland, Ohio* Interatrial septal defects were produced in open chest dogs Right and left atrial pressures were recorded simultaneously The results of experimental alteration of atrial pressures before and after producing the defect are compared The left-to-right pressure difference between the atria which was observed previously in similar preparations without septal defects persists after producing the defect, although the differential appears to be decreased Rapid saline infusion elevates both left and right atrial pressures in the presence of an interatrial defect, but does not appear to increase left pressure as effectively as in the control However, right pressure does not exceed left, although the two pressures tend to equalize after a considerable volume of infusion

As in the control series, positive pressure inflation of the lungs (open-chest) results in an elevation of both right and left atrial pressure, but right increases more than left with a subsequent reduction of the pressure differential Persistence of a pressure differential is probably due to a difference between the volume-elasticity characteristics of the atria At equilibrium the volume flow into each atrium is equal Since the left atrium has the greater volume-elasticity coefficient, the pressure will be greater than that in the right atrium Experiments with a physical model indicate that this is a major factor in maintaining the pressure difference

Effects of breathing CO_2 on blood pressure and renal circulation in normotensive and hypertensive subjects W J LITTLE, J W AVERA and S W HOOBLER (introduced by M H SEEVERS) *Dept of Medicine, Univ of Michigan Hospital, Ann Arbor, Mich* The mean blood pressure increased 21% in 6 normotensive subjects and 16% in 5 hypertensive subjects during inhalation of a gas mixture containing 10% CO_2 and 90% O_2 This rise was theoretically due to increased vasomotor tone, but was probably in part the result of an increase in cardiac output, since it occurred after caudal anesthesia had been established to dorsal 3 The procedure was also associated usually with

renal vasoconstriction, while control studies utilizing hyperventilation of room air and rebreathing 100% oxygen had little effect on renal circulation. The following table summarizes observations with 6 normals and 5 hypertensives, showing the average (and range) percentage change during CO₂ inhalation.

	NORMOTENSIVES	HYPERTENSIVES
RPF	-13% (0 to -39%)	-23% (-9 to -66%)
RR	+46% (+7 to +110%)	+75% (+23 to +228%)
GFR	-2% (-31 to +16%)	-12% (-59 to +12%)
FF	+10% (0 to +17%)	+17% (+9 to +25%)

RPF is renal plasma flow (PAH), RR is renal resistance (Mean BP/RPF), GFR is glomerular filtration rate (mannitol), and FF is filtration fraction (GFR/RPF). The changes were usually abrupt in onset and offset, occurring only during the 10 min period of CO₂ breathing. The failure of a 'rebound' phenomenon to occur in the ensuing urine collection periods excluded the possibility that the changes were due to retention of urine in the renal pelvis. The evidence seems to indicate that the renal vasoconstriction observed in the human subject, during inhalation of 10% CO₂, is primarily the result of an increase in neurogenic vasoconstrictor activity.

Post-tetanic potentiation of presynaptic actions in the spinal cord. DAVID P. C. LLOYD *Labys of The Rockefeller Inst for Medical Research, New York City*. Monosynaptic reflexes of a given muscle are greatly increased for several minutes following brief tetanization of the nerve afferent for that muscle, the phenomenon resembling that studied in ganglia by Larrabee and Bronk, in frog by Bremer and Kleyntjens. Tetanizing afferent pathways, from other muscles or skin, that converge, directly or after internuncial relay, with the monosynaptic pathway of the given muscle may influence transmission therein for 15 seconds, but prolonged potentiation is not realized. Furthermore, penetration into motoneuron somata of antidromic volleys, depressed for several seconds following presynaptic tetani, is not further influenced. If, however, the nerves of two muscles, synergists or antagonists within a myotatic unit, are stimulated by single shocks so related in time that the volley in one nerve, through monosynaptic connection, facilitates or inhibits the monosynaptic reflex elicited by stimulation of the other nerve, then, following tetanization of the first-mentioned nerve, its conditioning action, facilitatory or inhibitory, upon the test reflex, is enhanced for

several minutes, the test reflex in isolation remaining unaffected. Now, since any activity evoked by means other than stimulation of the tetanized afferent fibers is not potentiated, and since the known synaptic actions of those afferent fibers all are, it follows that the process underlying post-tetanic potentiation must be sought in the rhythmically stimulated afferent fibers themselves. Recordings from the intramedullary afferent fibers of post-tetanicly increased impulses displaying subnormal velocity suggest that hyperpolarization (positive after-potential) by increasing in size the presynaptic impulses causes them to be more effective synaptic agents.

Effect of sham-feeding on gastric motor activity of the dog. STANLEY H. LORBER (by invitation), S. A. KOMAROV and HARRY SHAY *Samuel S. Fels Research Inst., Temple Univ. School of Medicine, Philadelphia, Penna.* The effect of sham-feeding on the gastric motor activity of a dog with an esophagotomy and gastrostomy has been studied by a balloon-kymograph technique. A balloon was introduced into the gastric antrum through the gastrostomy opening and, after a 30-60 minute period of observation, the dog was sham fed. Results observed were of two types and depended upon the basal motor activity. In the active stomach, sham-feeding abolished peristaltic waves and diminished or abolished tonus waves. The diastole of tonus activity was not altered appreciably. The above effects occurred promptly at the start of sham-feeding and preceded acid secretion by 5-7 minutes. The motor inhibition lasted for several hours. In the inactive stomach, sham-feeding resulted in the initiation of motor activity and in an increase in tonus. The waves induced were predominately of the tonus type but for a few minutes after sham-feeding peristaltic waves also occurred. The effects preceded or immediately followed the termination of sham-feeding. The results indicate that sham-feeding can induce or inhibit gastric motility depending upon the state of basal gastric activity.

Conversion of propionate carbon to liver glycogen in the intact rat, studied with C¹⁴, C¹³-labeled propionate. VICTOR LORBER, MARGARET COOK (by invitation) and JULIEN BORDEAUX (by invitation) *Dept. of Biochemistry, Western Reserve Univ. School of Medicine, Cleveland, Ohio*. Solutions of C¹⁴H₃C¹³H₂COO-Na and glucose were given to fasted rats by stomach tube. Resulting liver glycogen was isolated, hydrolyzed to glucose and the position of C¹⁴ and C¹³ in the glucose molecule determined (*J. Biol. Chem.* 159:475, 1945). Prior experiments (*Federation Proc.* 6:272, 1947),

Lorber, Lifson, Sakami, and Wood, unpublished) in which $\text{CH}_3\text{C}^{13}\text{H}_2\text{COO-Na}$ and $\text{C}^{13}\text{H}_5\text{CH}_2\text{COO-Na}$ were fed to different animals have indicated that isotope administered separately in the α and β carbons appears in both cases mainly in the 1,6 and 2,5 carbons of glucose from liver glycogen, and is equally distributed between these positions. In the present experiments in which it was possible to study the fate of isotope from both the α and β carbons of propionate simultaneously in the same animal, the above result has been confirmed. Following feeding of $\text{CH}_3\text{C}^{13}\text{HOHCOO-Na}$, the isotope is incompletely randomized between carbons 1,6 and 2,5 of the glucose from liver glycogen (Lorber, Lifson, Wood, and Sakami, *Proc Am Physiol Soc*, in press), positions 2,5 predominating. The results with propionate may therefore be interpreted to mean that for the conversion of propionate to pyruvate some pathway other than a direct conversion via α oxidation must exist.

Role of insulin in the metabolism of amino acids

WILLIAM D. LOTSPEICH, *Syracuse Univ College of Medicine, Syracuse, N Y*. It has been repeatedly demonstrated that the injection of insulin results in a fall in the plasma amino acid nitrogen. The best evidence to date indicates that amino acids are being synthesized into protein under the influence of insulin. In order to further clarify this problem the following study was undertaken. Normal adult female dogs, fasted for 12 hours, were injected intravenously with insulin (Hletin-Lilly) in doses of 2 U/kg body weight. The whole blood levels of the natural forms of the 10 essential amino acids were followed for 1 hour thereafter. All amino acids were determined on tungstic acid filtrates of whole blood by microbiological assay techniques using *Streptococcus fecalis-R*, *Leuconostoc mesenteroides* and *Lactobacillus arabinosus* as the test organisms. It was found that the blood levels of all amino acids fell at different rates. Arranged in the order of decreasing extent of fall the amino acids were leucine, lysine, isoleucine, valine, threonine, arginine, phenylalanine, histidine, methionine and tryptophan. It was further found that there existed a direct correlation between the quantity of each amino acid leaving the blood after insulin and the concentration of that same amino acid in dog skeletal muscle protein. This was taken as presumptive evidence that insulin plays a role in the synthesis of protein from circulating free amino acids.

Action of epinephrine on the pyloric sphincter as recorded by the pyloric inductograph. HORTENSE LOUCKES (by invitation), DANIEL A. BRODY (by invitation) and J. P. QUIGLEY, *Division of Physiology, Univ of Tennessee, Memphis, Tenn*. We have employed a modified form of the pyloric inducto-

graph of Brody and Quigley (*J Lab & Clin Med* 29:3 1944). By increasing the primary coil input from 60 to 1500 cycles per minute the 50 ohms resistance coils become more sensitive and much smaller coils have adequate sensitivity. The amplifier output has been rectified so that coil or sphincter movements are recorded as a line instead of a band. This facilitates interpretation of the records. Normal dogs, thoroughly trained to participate in the studies, were provided with two coils of 50 ohms resistance sutured at the pyloric sphincter diametrically opposite each other. The pyloric sphincter of the dog usually exhibited spontaneous rhythmic contractions having a 10-14 second cycle. This interval was not altered by the fasting or postprandial state but the contractions became more regular, of greater amplitude and spontaneous periods of inactivity were rare when the stomach contained food. The intravenous injection of epinephrine (0.5-4 gamma/kg) completely abolished postprandial motility for 30 to 85 seconds respectively and depressed motility for an additional 10 to 60 seconds respectively. During the recovery period the frequency of sphincter contractions returned to the pre-injection rate. Epinephrine increased the sphincter tone and this increase was of longer duration (30 to 240 seconds) than the motor inhibition. The latent period of epinephrine effect ranged from 15 to 25 seconds. A reduction in pulse rate paralleled the decreased sphincter motility. Doses less than 0.5 gamma/kg epinephrine appeared to be subthreshold for they produced questionable inhibition of sphincter motility, no effect on tonus and a slight decrease in pulse rate.

Absorption of sulfamylon. E. W. MCCLESNEY, M. E. AUERBACH (by invitation) and H. W. ECKERT (by invitation), *Sterling-Winthrop Research Inst, Rensselaer, N Y*. Studies on the absorption of Sulfamylon (homosulfanilamide, marfanil) have been retarded by the lack of a satisfactory method for its determination in blood. The method of Heideman and Rutledge (*J Pharmacol & Exptl Therap* 93:451, 1948) is open to several criticisms, principally on the ground that the metabolic product, p-carboxybenzenesulfonamide, also absorbs light in the region 265-270 m μ , and absorbs it more strongly than Sulfamylon. An alternate method for the determination will be presented, based on the Ehrlich and Herter reaction for α -amino groups (*Z physiol Chem* 41:329, 1904). The method involves precipitation of the whole blood proteins with trichloroacetic acid, adjustment of the filtrate to pH 9, and reaction with β -naphthoquinone sulfonate. The yellow derivative is extracted with methyl n-amyl ketone, and is de-

terminated in the photoelectric colorimeter using a 420 m μ filter. This method has been applied in studies of absorption of the drug. Dogs given 625 mg/kg by intravenous infusion showed blood levels ranging from 15 to 80 mg %, 30 minutes after the end of the infusion, the blood was essentially cleared of Sulfamylon in 5 hours. When the same dose was given orally, the peak blood levels ranged from 7 to 35 mg %, 2 to 3 hours after medication. In human subjects an oral dose of about 110 mg/kg gave peak blood levels of the order of 7 mg %, 1 to 2 hours after medication.

Inhibition of the knee jerk from tendon spindles of crureus G P MCCOUCH, I D DEERING (by invitation), W B STEWART and W W CHAMBERS (by invitation) *Depts of Physiology and Anatomy, Univ of Pennsylvania, Philadelphia, Penna.* In decerebrate cats, the muscles of the lower extremity were silenced by denervation or tenotomy except for quadriceps and usually semitendinosus. The limb was fixed by drills and a pelvic clamp. Muscle tension was recorded isometrically and action potentials, from a bare needle through rectus and crureus. The knee jerk was elicited by a mechanical tapper, which stretched the muscle by raising the lever shank. Conditioning volleys, usually below and never significantly above threshold for direct or neuromyal response, were delivered through bare needles of which one passed through the patellar ligament, the other transversely through vastocruureus a few millimeters proximal to the tendon of crureus. Inhibition curves may be preceded by facilitation at intervals between conditioning and test stimuli up to 2 msec. This is followed by a descending limb, relatively constant in shape and duration, which reaches maximal depth within 8 or 9 msec. Total duration of inhibition is variable, being greater with more proximal electrode positions, which involve more receptors, than with distal positions, which engage fewer end organs. It ranges from 20 to 100 msec. Two factors may be postulated to account for the shape of the curve. The first is facilitation from the inevitable involvement of muscle spindles by the conditioning stimulus. The second results from the multisynaptic character of the inhibitory arc and its consequent afterdischarge.

Vascular changes in the warmed ear during oximetry W J MCCRACKEN (introduced by J K W FERGUSON) *Dept of Pharmacology, Univ of Toronto, Toronto, Canada.* A photoelectric oximeter developed by Dr W Paul employs amplification and meters such that fluctuations of blood content of the ear ('ear thickness') are observable with each pulse beat and amount to 0.25-1.5 ma. The reading with the ear compressed and bloodless

is less than one ma, while fully warmed ears give readings of 9-15 ma, or about 8 ma more than unwarmed ears. Over and above fluctuations with the pulse, changes have been observed after a) compression of the ear (tissue anoxia for 3-5 min), b) hyperventilation, c) anoxia by holding the breath, and d) breathing oxygen. After compression of the ear the current increased by 0-3 ma in 19 subjects (av 1 ma) above the precompression reading, and returned to the precompression reading in 3-5 minutes. This is interpreted as reactive hyperemia which temporarily increased the blood content of the ear by an average of about 20%. Hyperventilation in 2 subjects produced vascular constriction, as shown by a decreased current of about 1 ma. Subsequent anoxia by breath-holding without increased intrathoracic pressure caused an increased current of about 4 ma above normal, indicating additional vasodilatation in the ear. Breathing O₂ (50, 75, and 99%) caused a decrease of current of 0-2 ma in 19 subjects (av 0.75 ma). The magnitude of this vasoconstriction did not seem to vary with O₂ % from 50-99%.

Experimental studies with psoriatic blood DAVID I MACHT *Dept of Pharmacology, Labys of Sinai Hospital, Baltimore, Md.* For 25 years, the author has been employing plant-physiological methods for studying pharmacological properties of blood. In this way by testing 1% solutions of sera in plant physiological saline on root growth of *Lupinus albus* seedlings, the presence of toxic substances was demonstrated in menstrual blood and in the blood of pernicious anemia, pemphigus, leprosy, and trachoma. Numerous experiments with psoriasis blood however on normal seedlings gave readings exactly the same as with normal blood sera. Three years ago, the author began experiments on the action of drugs and sera on seedlings which had been previously vernalized. He found that ethyl-carbamate was much more toxic for vernalized seedlings than for normal ones. Similarly it was discovered that psoriatic serum was toxic for vernalized seedlings but not for normal seedlings, all the other phytotoxic sera mentioned above did not show such a difference.

Vernalization (or 'yarovization') in these experiments was produced by chilling seedlings at 42°F in the dark (Miller, S C *Plant Physiology*, 1938). This finding in psoriasis is useful for diagnosis and also for the evaluation of therapeutic procedures. Thus exposure of psoriatic sera to x-rays *in vitro*, or to ultra-violet rays in quartz produce detoxification of psoriatic blood. These physical agents are employed with some success in the treatment of this disease. Extracts of *Smilax sarsaparilla* also detoxify psoriatic serum *in vitro*, and that drug has

also been employed clinically (*New England J Med* 227 128, 1942)

Physiological, pharmacological and biochemical studies on blood of animals in rage DAVID I MACHT and THOMAS HOFFMASTER (by invitation) *Dept of Pharmacology, Labys of Sinai Hospital, Baltimore, Md* Six different experimental studies were made on the blood of rabbits and cats before and after enraging them 1) clotting time of whole blood measured by the Lee White method, 2) prothrombin time estimated by Quick's method, 3) blood sugar determined by microchemical methods, 4) phytotoxic effects on root-growth of seedlings measured on *Lupinus albus* by Macht's method, 5) reducing power of blood sera for methylene blue studied in a substratum of crushed vegetable cells by Thunberg's method, 6) similar studies with serum on brain and muscle tissues, made by Thunberg's method

Coagulation time of whole blood in rage is markedly reduced, as much as 50% or more Prothrombin time is but slightly affected, the fluctuations being of little significance Blood sugar is markedly increased for 6 hours or more Phytotoxic properties of sera after rage are very striking and are exhibited by them even after refrigerating the blood specimens for 3 days Control experiments with intravenous injections of epinephrine also shorten coagulation time and produce hyperglycemia, but render the blood phytotoxic only when tested a few hours after drawing it, the phytotoxicity disappears, probably through oxidation, on standing several hours Significant differences also are shown between the reducing power of the sera after rage and after injections of epinephrine, respectively The results indicate that the blood changes in rage cannot be attributed entirely to adrenal effects, but involve other metabolic or endocrine disturbances

A new chemical method for the determination of histamine in biological materials FLOYD C MCINTIRE, MURIEL SPROULL, and FRANCES B WHITE (introduced by R K RICHARDS) *Dept of Biochemistry, Abbott Research Labys, North Chicago, Ill* The reaction between histamine and 2,4-dinitrofluorobenzene may now be used for the determination of histamine in biological materials The histamine to be determined is purified by the cotton acid succinate method (McIntire, Roth, and Shaw, *J Biol Chem* 170 537, 1947) The cotton acid succinate eluate is not neutralized, but is evaporated to dryness The dry residue is dissolved in a small amount of an alkaline buffer, an alcoholic solution of dinitrofluorobenzene is added and after 20 minutes at room temperature the solution is acidified Under the conditions employed, histamine reacts with dinitrofluorobenzene to yield

β -(4-imidazolyl-N-(2,4-dinitrophenyl)ethylamine This histamine derivative is separated from the excess reagent and from a number of dinitrophenyl-amino compounds by a micro counter-current extraction procedure Interference from histidine and diamino acids is eliminated by a second counter-current extraction under alkaline conditions The quantity of histamine derivative is determined by measuring optical density at 358 m μ in a Beckman spectrophotometer By employing microcells in the spectrophotometer it is possible to determine as little as 0.2 μ g of histamine with an accuracy of $\pm 5\%$ The histamine derivative is very stable and the results are highly reproducible In our application of this method to rabbit whole blood and plasma, the chemical method has given good agreement with bioassays The method is particularly suited for the handling of a large number of samples at one time

Changes in the amount of protamine sulfate required to re-establish *in vitro* clotting of heparinized blood in certain pathologic states CHARLES L MCKEEN, RICHARD M ELGHAMMER and PETER V MOULDER (introduced by J GARROTT ALLEN) *Dept of Surgery, Univ of Chicago, Chicago, Ill* The amount of protamine sulfate required to allow coagulation of standardly heparinized blood is constant in normal human beings and dogs In certain human pathologic conditions, and in artificially produced toxic states in dogs, this protamine sulfate requirement increases In humans, this phenomenon has been observed in leukemia, menorrhagia and in certain patients with coagulation disturbances, otherwise unclassified, it has also been seen following internal and external irradiation and aminopterin and nitrogen mustard therapy It has been produced in dogs by total body irradiation, aminopterin and nitrogen mustard In these instances of changed protamine requirements, clotting times, though usually mildly prolonged, are only occasionally normal and rarely incoagulable This changed protamine requirement is independent of the thrombocyte count Intravenous or intramuscular protamine sulfate and intravenous toluidine blue return the *in vitro* protamine sulfate requirement to, or toward normal

Concurrent estimates of blood volume in rabbits by bleeding and dye methods PAUL L McLAIN, C H WILLIAM RUHE (by invitation) and T K T KRUSE *Dept of Physiology and Pharmacology, School of Medicine, Univ of Pittsburgh, Pittsburgh, Pa* In 20 rabbits, the blood volume by Guthrie's modified Welcker method averaged 126.2 ± 3.21 ml or $4.28 \pm 0.078\%$ of the body weight The animals were bled and simultaneously infused

with saline so as to reduce the circulating hemoglobin as much as possible before failure of the heart. Infusion was continued, with manual pumping of the heart, until the hemoglobin concentration of the effluent was well below 1% of the original. Finally, the limbs were massaged, all large vessels slashed, and the animal drained. All precautions were taken to insure that no significant amount of hemoglobin was left in the animal. In 15 of these animals, plasma volume was estimated concurrently by the dye T-1824. The line established by 3 points on the dye disappearance curve was extrapolated to zero time both rectilinearly and logarithmically. Blood volume was estimated from plasma volume and arterial hematocrit. Ordinary plotting yielded blood volumes $7.23 \pm 0.29\%$ of the body weight, logarithmic plotting $5.67 \pm 0.18\%$. Estimates by the 2 dye methods differed significantly from each other and from those obtained by hemorrhage, and were much less consistent when related to body weight. The average plasma volume obtained by rectilinear plotting exceeded the average whole blood volume estimated by the bleeding technique. The discrepancies between the results of hemoglobin and dye procedures seem too large for explanation by reasonable differences between arterial and 'body' hematocrit, and suggest serious loss of dye from the circulating plasma shortly after injection.

Assay of FSH, ICSH, and luteotrophin in the hypophysectomized rat: clinical applications
 WILLIAM O. MADDOCK (by invitation), EDWIN C. JUNGCK (by invitation), DEMETRIOS A. RIGAS (by invitation), and CARL G. HELLER. *Depts. of Physiology and Medicine, Univ. of Oregon Med. School, Portland, Oregon*. Current methods for assessing gonadotrophins in clinical materials usually indicate whether titers are greater or less than normal, but rarely indicate which of the 3 gonadotrophins are present or predominate. Purified FSH alone does not cause ovarian secretion. FSH plus ICSH stimulates ovarian secretion of estrogen, and the addition of luteotrophin stimulates progesterone secretion. This serves as a basis for the qualitative assay of a mixture of gonadotrophins. Thus, if an unknown extract causes estrogen elaboration from the ovary, FSH and ICSH must be present, if, in addition, progesterone secretion is produced, luteotrophin must also be present in the extract. Estrogen and progesterone secretion can be determined by the effects of these hormones on the immature rat uterus. Alpha-estradiol, administered to castrate or intact immature rats, produced thin-walled, translucent uteri, uniformly ballooned with fluid. Doses of alpha-estradiol 1000 times an effective dose still produced thin-walled, trans-

lucent, fluid-filled uteri. Simultaneous administration of estradiol and progesterone produced thick-walled, opaque uteri, devoid of fluid. Administration of extracts containing predominantly FSH and ICSH to hypophysectomized rats with intact ovaries produced fluid-containing uteri. The same extracts plus luteotrophin produced stimulated uteri devoid of fluid. Administration of extracts of the urine of castrate and normal men have produced uteri devoid of fluid, similar to those produced by estrogen plus progesterone treatment, or by administration of extracts containing FSH, ICSH and luteotrophin. It is concluded that all 3 gonadotrophins are excreted by normal and castrated men.

Possible relationship between cinchophen ulceration of the stomach or duodenum and the choleretic response to cinchophen
 D. F. MAGEE (by invitation), K. S. KIM (by invitation) and A. C. IVER. *Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago, Ill.* Cinchophen in dogs is both ulcerogenic and choleretic, whereas in rabbits neither action is observed. The object of this investigation was to determine if this relationship existed in other species. Two sets of experiments were made using cats, rabbits, guinea pigs and rats. 1) *Acute*. Under 'nembutal' anesthesia the common bile duct was cannulated, the gall bladder tied off and the half hourly bile output collected and measured. The differences in volume between the basal flows and those after 100 mg/kg of intravenously administered cinchophen dissolved in N/10 sodium hydroxide were determined. As a test of reactivity intravenous 'decholin' was given. The basal bile flow in cats was 0.5 ml/kg/hr. In guinea pigs 10 ml/kg/hr. Increases after cinchophen of 50% and 60% respectively were observed. The basal flow in rabbits was 4 ml/kg/hr. In rats 2 ml/kg/hr. No choleresis was observed after cinchophen, but it occurred after 'decholin'. 2) *Feeding experiments*. Capsules containing 100 and 200 mg/kg were administered daily. The rats were fed the same per kg dose dissolved in N/10 sodium hydroxide by stomach tube. Equal quantities without cinchophen were given to the control rats. Consistent ulceration was produced only in the cat. Some rabbits and guinea pigs developed gastritis and duodenitis but no ulcers were histologically demonstrable. All the animals, except the rats, fed the sodium salt, died in from one to eight weeks of cinchophen feeding. There is apparently no relation between cinchophen induced choleresis and cinchophen ulcer.

Effect of blood composition on the metabolic activity of the perfused brain in the living cat
 J. MAGNES, R. M. TAYLOR and A. GEIGER (Intro-

duced by H GRUNDFEST) *Dept of Psychiatry, College of Physicians and Surgeons, Columbia Univ, New York City* In continuation of experiments on the metabolism of the perfused cat's brain, attempts were made to facilitate the identification of essential brain metabolites by simplifying the composition of the perfusion blood Perfusion experiments were made with 1) fresh, defibrinated, heparinized beef blood passed through an isolated cat liver for removal of vasoconstrictor substances, 2) the same blood but dialyzed against 0.9% NaCl solution in the cold for 6 days, and reconstituted with electrolytes and glucose, 3) exhaustively washed beef blood corpuscles suspended in a Ringer's solution containing 7% bovine serum albumin All 3 preparations maintain brain functions Preparation (3) shows no vasoconstrictor properties Cerebral oxygen consumption and blood flow were highest with (3) and lowest with (1) with equally good functional activity of the brain The rate of O_2 consumption of the brain is related to the functional activity of the brain as long as reflexes can be elicited, but the actual rate with (1) is between 4-6 ml O_2 consumed by 100 gm brain per minute, while with (3) it is between 7-11 ml Physiologically inactive brains may also show a relatively high O_2 consumption with (3) It is suggested that by washing the blood corpuscles and by substituting a Ringer-albumin solution for plasma an inhibitor of brain metabolism is eliminated

Venous pressure as a factor limiting tolerance of acceleration in the prone position P J MAHER, JR (by invitation), O GAUER (by invitation), E E MARTIN (by invitation), J P HENRY *Aero Medical Lab, Air Materiel Command, Wright-Patterson Air Force Base, Dayton, Ohio* Practical considerations of flight control has stimulated the modification of the full prone position in order to permit better forward vision The torso is raised until the head and chest make an angle of 25° with the horizontal Standard light tests of visual symptoms and electrocardiograph recordings were carried out with 7 unrelaxed subjects in the range of 10-12 g Visual symptoms were absent or mild The electrocardiographic changes were of the same type and degree as those found during positive acceleration of approximately three g in the upright seated posture However, 5 (5) subjects complained of intense pain in the legs or forearms during high accelerations Petechiae were observed in 3 subjects, extending up the arms to the biceps region Venous pressures were measured directly in 2 (2) subjects at 10 g using a vein in the forearm and recording pressures by means of a trimount inductance gauge Pressures of 175 mm Hg were recorded in both cases Since the vertical heart-log distance was 50% greater than the heart-arm dis-

tance of approximately 19 cm, even larger pressures probably occur in the legs These results suggest that pain in the extremities may determine the upper limit of g-tolerance in the prone position

Repeated explosive decompressions DAVID I MAHONEY (introduced by J W WILSON) *Aero Medical Laboratory, Air Materiel Command, Wright-Patterson Air Force Base, Dayton, Ohio* As a result of increasing interest in the explosive decompression of pressurized aircraft, the question has arisen concerning the possibility of producing irreversible damage in individuals exposed to explosive decompressions of moderate intensity repeatedly carried out within a period of several hours In order to test this possibility, a series of experiments was conducted using dogs as subjects Thirteen test animals were used during the study and were subjected to no surgical procedure The animals singly or in pairs, were placed in a small cabin which was located within a low-pressure chamber The small chamber was held at ground level until the large chamber was evacuated to the altitude required to give the desired pressure differential (6.55 psi) A membrane of several layers of brown wrapping paper covering a $10\frac{1}{2}$ in diameter orifice in the small chamber was broken at the desired time of decompression The chamber was then immediately brought to ground level to preclude any effects of hypoxia The time for all explosions varied between 0.120 to 0.126 seconds The total time elapsing during a series of 7 explosive decompressions on each dog ranged from 45 to 75 minutes There was no evidence of ill effects from the explosive decompressions in any of the dogs either immediately after the decompressions or during a subsequent observation period of 2 weeks The lack of evidence of irreversible damage in this series plus the results of Hitchcock *et al* (*J Av Med* 17, 6, 1946) lead to the conclusion that repeated explosive decompressions of moderate intensity are not a significant factor that may lead to mortality in dogs

Behavior of actomyosin threads SAMUEL MALLOV (by invitation) and JANE SANDS ROBB *Dept of Pharmacology, Syracuse Univ College of Medicine, Syracuse, N Y* Actomyosin from skeletal muscle of the rabbit, dog and guinea pig has been extracted and also purified by methods described by Szent Gyorgyi Mincing favors greater output in shorter time but renders further separation more difficult Purified actomyosin is stable for several weeks Threads can be drawn but in contrast to unpurified threads, these respond to ATP only if calcium and/or magnesium ions are added and even then the degree of shortening is less Images of these contracting threads have been projected to a screen and moving pictures taken

Movement, generally beginning at the ends, is preceded by increase in density. As shortening proceeds, spiraling occurs. It is our impression that threads drawn into about 0.015% magnesium chloride develop tighter spirals (than is the case when drawn in potassium chloride) and that shortening is followed by partial relaxation. Behavior of threads drawn into 1:2,000,000 solution of crystalline cardiac glycoside is standard. If the glycoside is mixed with actomyosin and allowed to stand for an hour or two before threads are drawn, considerable relaxation precedes contraction, spiraling is pronounced and maximum shortening appears. We have not been able to demonstrate activation by barium which was attempted because of the report of Deutsch and Lundin that it is barium and not ATP which is responsible for automatic cardiac activity. Using standard methods, less actomyosin can be extracted from a given amount of heart muscle, it is less viscous, forms threads less well, and these in spite of being thinner are less reactive than threads composed of skeletal actomyosin.

Intestinal Perfusion in a patient with chronic uremia N. S. R. MALUF *Surgical Research Unit, Brooke General Hospital, Fort Sam Houston, Texas*. Intestinal perfusion was tested in a severely uremic patient by the triple-bore tube. Thirty-one liters of 2 to 2.27% anhydrous sodium sulfate were dripped in 24 hours for intestinal and, then, gastric irrigation. The small quantity of liquid which regurgitated into the stomach while the intestine was being perfused was sucked through a gastric tube admitted through the other nostril, this completely prevented vomiting. Inevitable leakage through the anus was through a rubber-tube (1.6 cm O.D., 1.1 cm I.D.) inserted into the rectum and attached to the buttocks by adhesive tape, this almost totally prevented soiling. Perfusion averaged 22 cc/min, which was much slower than previously used in non-uremic patients (Maluf, 1948, *Federation Proc.* 7:77, 1948, *J. Urology* 60:307, 1948). Because of the high plasma-urea and slow perfusion, the urea clearance was only 1.8 cc/min. Nevertheless, 50 gm of urea were extracted in 24 hours and plasma-urea fell from 357 to 246 mg/100 cc in spite of intestinal perfusion occupying only 10.5 hours, the remaining 13 hours being exclusively gastric irrigation. Intestinal irrigation was at least 8 times more effective in eliminating urea than gastric. Edema, ascites and weight dropped markedly in spite of 6.5 liters liquid iv. Elevated arterial pressure fell and the patient was mentally improved. 662 mg of Ca, 2.3 gm of K and 48.3 gm of Cl were lost in the perfusate. Plasma Cl (as NaCl) fell from 574.6 to

523.9 mg/100 cc. Besides dextrose, water and vitamins, 15 gm NaCl and 4 gm calcium gluconate were given intravenously.

Inactivation of thromboplastin by serum F. D. MANN and MARGARET HURN (Introduced by T. B. MAGATH) *Division of Clinical Labys, Mayo Clinic, Rochester, Minn.* Most of the thromboplastic activity of extract of rabbit brain disappeared on incubation with an equal volume of human serum for 1 hour at 37°C. By dilution and acidification of the mixture, thromboplastin was precipitated and recovered in active form. Decalcification of the serum with oxalate largely prevented the inactivation of thromboplastin. Over the pH range 7 to 8, alkali decidedly favored and acid inhibited the inactivation of thromboplastin by serum. A rather similar pH effect was observed for conversion of prothrombin to thrombin in the two-stage system.

Effect of dibenamine on ventricular fibrillation following sudden coronary occlusion G. W. MANNING, R. C. A. F. *Institute of Aviation Medicine, Toronto, and the Dept of Physiology, Univ of Western Ontario, Ontario, Canada*. In 75% of normal conscious dogs sudden occlusion of the left circumflex coronary artery results in ventricular tachycardia followed by ventricular fibrillation and death. It has been shown that cardiac sympathetic denervation and the sympatholytic drug, dihydroergotamine, significantly inhibits the development of this fatal ectopic rhythm. In view of the results reported by Nickerson *et al.*, with dibenamine, the effect of this sympathetic blocking agent on the immediate mortality due to ventricular fibrillation was studied. Under anesthesia a loose ligature was placed around the left circumflex coronary artery. Dibenamine (20 mg/kg) was given intravenously in 250 cc of normal saline, over a period of $\frac{1}{2}$ to $\frac{3}{4}$ of an hour. ECG and blood pressures, in some cases, were recorded before and after dibenamine and before, during and after ligation. Sudden ligation in the conscious dog was carried out the following day by traction on the ends of the ligature which had been brought out through the chest wall (at a tangent to the heart). In the ECG the most constant changes noted were an increase in the heart rate, and increase in amplitude of the T and P waves and a reduction of the amplitude of the R waves occurred in some experiments following the injection of dibenamine. Following ligation of the left circumflex the usual sequence of events was noted in the ECG. There was a progressive elevation of the S-T segments, frequent extrasystoles and bouts of ventricular tachycardia followed by fatal ventricular fibrillation in 55% of the animals within the 24-hour period following ligation—(all occurred within the first 12 minutes

except in one animal which died in 10-12 hours following ligation) In the control series the same changes were observed and 75% of the animals died in ventricular fibrillation within the 24-hour period Although an insignificant reduction in mortality occurred, the frequency of extrasystoles and ventricular tachycardia appeared to be no different from that seen in untreated control animals It would appear from these experiments that dibenamine is not an effective agent in preventing the fatal ectopic cardiac rhythm which occurs following sudden coronary occlusion in the conscious dog

Characteristics of the thigh muscles with a double action J E MARKEE and MAUDE WILLIAMS (by invitation) *Dept of Anatomy, Duke Univ School of Medicine, Durham, N C and Division of Physiology, Womens College of Univ North Carolina, Greensboro, N C* Previously, evidence was presented that muscles which pass over both the knee and hip function as double muscles, one part shortens to move the hip, another part shortens to move the thigh This different participation of the separately innervated areas of the biceps femoris, semitendinosus, semimembranosus, rectus femoris, sartorius and gracilis occur during 1) direct stimulation of the nerve branches, 2) reflex flexion and crossed extension elicited in decerebrate preparations, and 3) flexion and extension induced by cortical stimulation In the present investigation, evidence is presented indicating that these thigh muscles which act as double muscles possess the functional characteristics of simple flexor and extensor muscles That is, one part of the biceps femoris flexes the knee and resembles a simple flexor muscle in that it fatigues rapidly and develops less tension per unit of weight In addition the development of effective tension occurs through a longer proportion of the range of shortening of the fibers On the other hand, the part of the biceps femoris which extends the hip resembles muscles which are simple extensors in that this part fatigues less rapidly and develops more tension per unit of weight In addition the development of tension is more dependent on the length of the fiber in the extensor portion of the muscle The flexor and extensor portions of the semitendinosus and gracilis respectively possess these flexor and extensor characteristics

Specific resistance of regenerating Dugesia in different media and the electrical work of polarity control GORDON MARSH and JEAN DIMMITT (by invitation) *Zoological Labys, State Univ of Iowa, Iowa City, Ia* Pieces of *Dugesia tigrina*, regenerated 3 days in solutions of different specific resistance, were transferred individually to a chamber 0.149 mm deep, which confined them at virtually uniform thickness The voltage difference

producing constant current with and without the piece, divided by that current, gives the difference in resistance, under the two conditions, of a chamber segment of length equal to the piece During current flow the orientation, geometric shape and critical dimensions of the piece were determined, enabling calculation of the 'specific resistances' (ohm-cm at 24°C)

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Midpieces showed the highest and posterior pieces the lowest resistance, with significance doubtful Resistance was independent of current strength, which was usually that capable of reversing polarity The previously demonstrated constancy of potential gradient producing a given morphogenetic effect in media of varying resistance, gains significance from the fact that the internal current varies The electrical energy expended in a 1 mm³ piece regenerating 4 days in the current varies from 0.06 to 0.25 cal, depending upon specific resistance and potential gradient (regressive bipolarity 1.78 volts/cm, reversal 2.17 volts/cm) Assuming 1.05 for piece density and 5 cal/cc caloric equivalent for oxygen, these energies would represent the QO₂'s above if provided by the regenerating material All lie within the limits of published data

Purification and possible histochemical localization of renin JOHN MARSHALL (by invitation) and G E WAKERLIN *Depts of Physiology and Pathology, Univ of Illinois College of Medicine, Chicago, Ill* Purified hog renin was prepared by an extension of the ethanol fractionation method previously described (*Federation Proc* 7 78, 1948), with a resulting yield of 22,000 DU (Goldblatt) at a purity of 125 DU/mg N This preparation gave a polysaccharide reaction to the Hotchkiss technique (*Arch Biochem* 16 131, 1948) Accordingly, sections of 22 dog kidneys of widely varying renin content were stained by the Hotchkiss method Renin content varied from 0 to 22 DU/gm The results showed a good correlation between the renin content and granules containing glycoprotein in the juxtaglomerular apparatus The solubility of the glycoprotein granules in the juxtaglomerular apparatus of tissues prepared by freezing and drying was compared with that of dog renin Using the same procedures employed for the fractional separation of purified renin, it was found that the solubilities of both substances mentioned above corresponded Both were insoluble at 0°C, ionic strength 0.005, pH 5.10, at 20% ethanol con-

centrations or higher, and were soluble at lower ethanol concentrations

Presence of multiple cortical foci in the primary sensory projection systems of the cat WADE H MARSHALL *National Institutes of Health, Bethesda Md* In connection with work on the recovery cycle of the somatic sensory system of the cat, this author located 3 cortical regions at which responses occurred following tactile stimulation of the contralateral forefoot. These regions were specified in a joint paper on cortical reactions (Marshall *et al*, *J Neurophysiol* 4 1, 1941) Adrian (*J Physiol* 100 159, 1941) has questioned the presence of 3 such regions. Woolsey, in his published work on mapping the somatic sensory cortex of the cat, has discussed only 2 of these regions. One, the sensory region of Campbell, he has logically labeled *Somatic I*, the other, the anterior ectosylvian region, he has labeled *Somatic II*. The question of the third he does not discuss. The point has been reexamined and the author's original findings have been confirmed. There is a third area extending laterally from the region of the junction of ansate and lateral sulci across the posterior part of the *Somatic I* face area toward the anterior ectosylvian *Somatic II*. Multiple primary projection foci are not peculiar to the somatic sensory system. The visual system in the cat contains 2 demonstrable areas in addition to the striate and contiguous boundary region. One of these is in the posterior suprasylvian gyrus. The other region is in the middle ectosylvian gyrus extending medially across the suprasylvian sulcus onto the dorsal surface of the suprasylvian gyrus.

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Occurrence of potassium U/P ratios lower than 1.0 F MATEER (by invitation), L GREENMAN (by invitation), J H PETERS (by invitation), R C GOW (by invitation), and T S DANOWSKI *Dept of Research Medicine, Univ of Pittsburgh School of Medicine, Pittsburgh, Penna* It has been demonstrated that during periods of reduced or absent intake of food and water the urinary excretion of sodium decreases to zero while potassium continues to be present in the urine (*J Clin Invest* 23

807, 1944) This diminished excretion of sodium has been interpreted as a salt-conserving response preventing sodium depletion. The continued urinary loss of potassium is often associated with transfers of potassium out of cells, in part with the negative balance of protein and in part with dehydration (*J Clin Invest* 23 93, 1944). These transfers may not occur during renal suppression (*J Clin Invest* 27 74, 1948). If urine is elaborated, excretion of potassium continues, thereby permitting movement of cellular potassium without production of toxic extracellular levels. This urinary loss of potassium frequently continues even in the presence of hypokalemia and is one of the important routes of loss resulting in potassium depletion (*J Clin Invest* 27 557, 1948). This would suggest that the kidney is incapable of conserving potassium when body stores of this cation are depleted. Our studies of subjects with hypokalemia indicate, however, that the urinary loss of potassium may sometimes be markedly reduced, resulting in potassium U/P ratios lower than 1.0. In the 6 instances in which this was observed, 3 patients recovering from diabetic acidosis and 3 infants with protracted vomiting, the decrease in urine potassium concentrations below those in serum did not necessarily appear coincidental with hypokalemia but rather became evident after several days of persistent deficits of potassium.

Measurement of renal venous pressure in normal man and in patients with congestive heart failure M. HARRISON MAXWELL, ERNEST S. BREED and IRVING L. SCHWARTZ (introduced by HOMER W. SMITH) *Dept of Physiology, New York Univ College of Medicine, New York City*. Direct measurements of renal venous pressure were recorded in 7 normal male subjects and in 5 male patients in severe chronic congestive heart failure by means of a no. 10 Courmand radio-opaque catheter passed under fluoroscopy from an antecubital vein into the right renal vein. A saline manometer was used with repeated elevation above equilibrium. The normal subjects were all supine, the cardiacs supine or semi-recumbent. An arbitrary zero point of 10 cm above the table top was assumed in all cases. Lateral X-rays taken on 6 subjects showed the tip of the catheter *in situ* varied from 5.3 to 15.2 cm above the table top, with an average of 9.9. Since the true zero point coincided so closely with the assumed one, no corrections were necessary. The renal venous pressure in normal subjects ranged from 13.0 to 19.8 cm of saline, with an average of 15.4; in failure the pressure ranged from 16.5 to 28.0 cm, with an average of 24.3. In 6 normal patients and 3 cardiac patients, right atrial pressures were recorded during withdrawal of the catheter, the zero point being taken as 5 cm below the angle of Louis. The normal subjects had pressures varying from 0 to 5.3 cm of saline, with an

average of 2.5. The cardiac subjects had pressures varying from 11.0 to 15.0 cm, with an average of 13.0.

Determination of blood flow through the finger at high rates of flow JERE MEAD and ROBERT C. SCHOENFELD (introduced by H. S. BELDING) *The Quartermaster Corps, Climatic Research Laboratory, Lawrence, Mass*. When venous occlusion is accomplished in the finger without occlusion artifact, the slope of the tracing at the instant following occlusion depicts a volume rate of change which is the algebraic sum of two components: 1) the volume rate of change varying through each cardiac cycle to produce the finger pulse, 2) the volume rate of change which relates directly to the obstruction of outflow from the finger. If the instantaneous pulse slope derived from corresponding points on preceding pulse waves is subtracted algebraically from the initial occlusion slope, the volume rate of change of the finger independent of the pulse volume rate of change is obtained. This represents the instantaneous rate of blood flow through the finger.

Utilizing an electric solenoid air valve 'triggered' through a variable time delay circuit by the QRS potential of the ECG, venous occlusions were produced at specific points through the cardiac cycle, and variations in blood flow during the finger pulse cycle were studied. In vasodilated individuals the rate of flow in the terminal phalanx of the finger increased during the rising phase of the pulse to maximum values as high as 200 cc/100 cc of tissue/minute, and decreased during the diminishing phase of the pulse to roughly $\frac{1}{4}$ of the maximum flow. Average flows over the pulse cycle as high as 160 cc/100 cc of tissue/minute were computed. The markedly pulsatile character of blood flow through the terminal phalanx of the finger suggests that a considerable part of the finger's blood flow may be through the arteriovenous shunts described in this region.

Effect of carbohydrate on starvation metabolism in the presence of a high caloric deficit G. W. MELLINGER (introduced by J. W. HEIM) *Aero Medical Laboratory, Air Materiel Command, Wright-Patterson Air Force Base, Dayton, Ohio*. During a study of airborne survival ratios, metabolic observations were made to determine the effect of feeding 100 gm of carbohydrate daily on the body protein loss, ketosis, water balance and physical efficiency of men performing strenuous physical exertion in a cold environment. Two subjects resided continuously in an all-weather chamber at -29°C for 6 days. During this period they expended 4000 to 4500 calories while ingesting only 100 gm of carbohydrate (400 cal) daily. Clothing consisted of an experimental arctic clothing assembly having a clo value of approximately four. Sleeping bags were used at night. As a control, the subjects performed a similar

amount of work for 6 days while fasting. The serum carbon dioxide, organic acids, urinary ammonia and titratable acidity showed that there was a moderate degree of ketosis during the fasting experiments, but that all but slight changes were prevented by carbohydrate in amounts of 100 gm daily. Body protein loss was spared to the extent of 40-50% of the fasting loss when the carbohydrate was given. The water balance was favorably affected by 100 gm of carbohydrate as was shown by the marked reduction in the minimum urine water and total urine solutes, and by the moderate reduction in the minimum water intake. While maximum efficiency was not maintained during the experiments, the subjects were in sufficiently good condition at the end of the 6 days to perform tasks necessary for survival.

Further studies on complete parenteral alimentation. H. C. MENG (introduced by HOWARD J. CURTIS) *Dept. of Physiology, Vanderbilt Univ. Medical School, Nashville, Tenn.* A fat emulsion has been prepared for intravenous feeding (described elsewhere), and the present report describes the nutritional value and toxicity of this emulsion in dogs and rabbits. In the control period, 5 adult dogs were fed a complete basal diet by mouth. Immediately following this they were fed entirely by vein for 4 weeks and then killed for pathological examination. Four were given the same diet as during the control period, of which 34% of the calories were from fat, while the 5th dog received the same carbohydrate and protein diet but without the fat. The dogs on the complete diet were normal and healthy in all respects, and all laboratory findings were negative. The dog on the fat-free diet lost 14% of his weight and was very weak and apathetic. The injection of relatively large amounts of the emulsion in rabbits produced no appreciable pyrogenic effects. Further, the daily injection in rabbits of large quantities of both the emulsion and the emulsifying agents for as long as 4 weeks produced no deleterious effects. As tested in rabbits, it has been found that the emulsion is not antigenic. It is concluded that this emulsion is non-toxic and that the body is capable of utilizing fat in this form.

Physiologic effects of bilateral simultaneous removal of Brodmann cytoarchitectural areas in the human. FRED A. METTLER *Dept. of Neurology, College of Physicians and Surgeons, Columbia Univ., New York City.* In the 'combined-operations' study, known as the Columbia-Greystone Project, the physiology of 23 psychotic individuals from whom localized areas of frontal cortex were simultaneously, bilaterally removed, was studied pre- and postoperatively. Data were collected relating to formed elements of the blood, blood chemistry, blood pressure, body weight, gastrointestinal motility, electroencephalography, vision,

audition, somatosensation, reflex state, motor function and higher cerebral functions. The studies were begun 40 days prior to operation and have been continued, when pertinent, for over 1½ years. In certain physiologic fields (basal metabolism, somatic activity) no data could be obtained.

Bilateral removal of Brodmann areas of granular frontal cortex or of various combinations of these produced no definite, enduring, physiologic changes in any of the fields noted above except, in some cases, electroencephalography. Temporary effects were short-lived and generally not of such a nature as to demonstrate a causal effect between the nature of the ablation and the physiologic disturbance. Aphasia does not follow removal of area 44. The effects of removal of agranular cortex were also relatively unproductive. As previously reported for the simian (by Hines *et al.* and by the present author) cortex of area 6 may be removed without evoking evidence of spasticity. Neither does such a procedure necessarily produce forced grasp in the human.

III Effect of low barometric pressure on the brains of five vertebrates. BERNARD METZ (introduced by CHARLES G. WILBER) *Biological Lab., Fordham Univ., New York City.* Five vertebrates (the goldfish, *Carassius auratus*, the frog, *Rana pipiens*, the painted turtle, *Chrysemys picta*, the pigeon, *Columba livia*, and the Wistar rat, *Rattus norvegicus*) were subjected to simulated altitudes of 18,000, 33,000, 48,000, 64,000 and 90,000 feet, respectively. The survival rates, behavior and effects upon the vascular tissue and nervous elements of the brain were studied. The methods used and the survival rates were previously reported by Metz and Wilber (*Anat. Rec.* 99: 676, 1947; 101: 738, 1948; in the latter paper, survival rates for rats at 48,000 feet should be amended to read 66.2 seconds, not 66.2 minutes). Concerning the effects upon the brain, the most striking pathological changes are the intracranial hemorrhages (table 1). Visible damage to the nervous elements was noted only in a few cases. This nerve cell damage, as in the case of the hemorrhages, was widely dispersed in the optic lobes (in the fish, frog and turtle), cerebrum, cerebellum and medulla oblongata.

Table 1. Numbers of cases showing pathological changes

A. TIAL	NO. OF EXPERI- MENTAL A. TIALS	HEMOR- RHAGES	NERVE CELL DAMAGE
Goldfish	50	36	5
Frog	50	23	3
Turtle	50	25	9
Pigeon	50	21	4
Rat	50	27	5

The fact that there was only a small number of cases showing nerve cell damage does not imply that the nervous elements of the brains of the other experimental animals escaped unharmed. There exists the possibility that the changes which may have occurred are not morphological in nature, but rather are biochemical phenomena at a sub-microscopic level (Page *Chemistry of the Brain*, 1937).

Renal hemodynamics during short and long periods of salt poor albumin administration
ALEXANDER J. MICHIE (by invitation), NICHOLAS S. GIMBEL (by invitation) and CECILIA RIEGEL Harrison Dept of Surgical Research, Schools of Medicine, Univ of Pennsylvania, Philadelphia, Penna. The daily intravenous administration of salt-poor albumin to normal subjects caused a progressive expansion in plasma volume, an enlargement in extracellular fluid volume, an increasing venous pressure, a dilation of the roentgen heart shadow and an engorgement of the liver. The distended plasma volume produced a renal hyperemia and relaxation of efferent arteriolar tone. In renal hyperemia absence of a maximal filtration rate might be indicative of some systemic circulatory derangement. One week after termination of the albumin therapy and in the presence of a continued renal hyperemia the filtration rate increased as plasma volume and venous pressure dropped. Five months later the same subject was given 75.0 gm of salt-poor albumin intravenously in 20 minutes. Renal plasma flow was raised 700 cc/min, and filtration rate showed a maximal increase of 40.0 cc/min above the control values. The filtration fraction was decreased to a low of 0.117. The protracted renal hyperemia apparently led to an increase in T_{mPAH} . As the albumin was slowly metabolized during the 2- to 3-week experimental period, these subjects approached a protein-free diet, which in the experimental animal tends to diminish filtration rate, renal plasma flow and tubular mass. In the acute experiment 75.0 gm of albumin caused no significant change in T_{mPAH} or T_{mG} . The unchanged T_{mG} shows that no glomerular arteriovenous shunts were opened by this hyperemia and that the diminution in the PAH extraction ratio is probably due to increased albumin binding.

Influence of adrenaline on the cardiodepressor effect of acetylcholine and the vagus nerve
S. MIDDLETON and J. TALESNIK (introduced by HAROLD C. WIGGERS) *Physiological Institute, Univ of Chile, Santiago, Chile*. In isolated guinea pig and cat hearts adrenaline perfusion with concentrations of 3–20 $\mu\text{g/l}$ produced a marked increase of submaximal cardiodepressor (negative chrono and inotropic) effects of acetylcholine (0.05–0.5 μg). This augmenting effect was less marked with concentrations of adrenaline of 30 $\mu\text{g/l}$ or more, while concentrations of 1 $\mu\text{g/l}$ or less

were ineffective. The effects of adrenaline were not influenced when nicotine in concentrations of 1–2 $\mu\text{g/l}$ was added to the perfusion fluid. This would indicate that adrenaline acts by modifying the sensitivity of the cardiac effectors (peripheral effect), an influence on the intracardiac ganglia should be discarded. Adrenaline did not influence the cardiodepressor action of KCl.

In 28 experiments performed on isolated cat hearts, perfusion with adrenaline in concentrations of 0.05–0.1 $\mu\text{g/l}$ produced a very marked increase of submaximal cardiodepressor effects induced by electrical excitation of the vagus nerves. Smaller concentrations of adrenaline were ineffective. Concentrations of 1 $\mu\text{g/l}$ or more produced a decrease of the vagal effects. In view of the above-described results with nicotine the effect of high concentrations of adrenaline are probably due to a blocking action on the intracardiac ganglia. Eight experiments were performed on frogs, in which perfusion of the isolated heart (Straub's heart with Krayner cannula) with adrenaline (0.5–1 $\mu\text{g/l}$) produced a marked increase of the cardiodepressor effect of the vagus.

Influence of the cardiac sympathetic nerves on the cardiodepressor action of the vagus nerve
S. MIDDLETON and J. TALESNIK (introduced by HAROLD C. WIGGERS) *Physiological Institute, Univ of Chile, Santiago, Chile*. Twenty-five experiments were performed on isolated cat hearts, which were removed with the vagi and part of the surrounding mediastinal structures (including the stellate ganglia and adjacent thoracic sympathetic chains) attached. The preparations were made in such a way that, when desired, the stellate ganglion could also be perfused. In various experiments the vagi or the preganglionic or postganglionic sympathetic nerves were electrically stimulated. It was observed that stimulation of the cardiac pre- or postganglionic sympathetics in 6 experiments induced increase of submaximal cardiodepressor effect of the vagus. In 8 experiments, however, the sympathetic produced depression of the vagal effects and in 11 experiments the latter were not significantly modified. Nine experiments were conducted on intact cats. Much more regular results were obtained in this series, compared with the isolated heart. In all the animals, stimulation of the sympathetic cardiac nerves which did not by themselves produce changes in cardiac rate (sub-threshold), induced, however, marked increases of submaximal bradycardic effects of the vagus. Similar results were obtained with threshold stimulation of the sympathetic. It seems interesting to point out that in the intact cat the cardiac response to stimulation of the sympathetic nerves is very minimal and rare. Usually only negligible effects were observed even with supramaximal stimulation. In the isolated heart, on the contrary,

the effects of the sympathetic nerves on cardiac activity are very manifest

Effect of pH on the three waves of the protein polarogram *G J Millar* (introduced by L B JAKUES) *Dept of Physiology, Univ of Saskatchewan, Saskatoon, Sask, Canada* Protein added to an ammonia buffer containing cobalt yields a polarogram exhibiting 3 waves (Jurka, *Collection Czech Chem Commun* 11 216, 1939, Millar, *Proc Can Physiol Soc* 1947) When the pH of the medium is altered, the magnitudes of the first two waves are either unaffected or are decreased as the pH falls, while that of the third ('prenatrium') wave is enhanced The following modification of Brdicka's reactions is proposed to account for the behaviour of the second wave $-SH + e \rightarrow -S^- + H$, $-S^- + H_3O^+ \rightleftharpoons -SH + H_2O$ The observed pH effect seems to depend on the concentration of cobalt- NH_3 complex The cobaltous complex dissociates easily as the pH is lowered In the presence of the stable cobaltic complex, the pH of the medium may be lowered to 3.5 with little effect on the height of the first two waves Hence, these waves are only indirectly dependent on pH

Experiments with acetylated proteins and some organic compounds suggest that the third wave depends on the free amino groups of the protein and possibly on histidine residues The following reactions are proposed $-NH_2 + e \rightarrow -NH_2^- + H$, $-NH_2^- + H_3O^+ \rightleftharpoons -NH_2 + H_2O$ When the pH is lowered a greater proportion of free amino groups are in the zwitterion form and the third wave is enhanced to a maximal value Its height is probably related to the protein dissociation curve

Effect of bacitracin on renal function JOHN H MILLER (by invitation), ROGER K McDONALD (by invitation) and NATHAN W SHOCK *Section on Cardiovascular Diseases and Gerontology, National Heart Institute, National Institutes of Health, Bethesda, Md, and the Baltimore City Hospitals, Baltimore, Md* The parenteral administration of bacitracin is productive of proteinuria in patients treated for various infections In the present study, serial renal function tests were performed on 12 subjects, free of infection, who received intramuscular injections of 1500 U of bacitracin per kilogram body weight on two successive days Proteinuria appeared in all individuals at 48-60 hours after the first injection, was maximal during the first 24-48 hours after its appearance (range of maximal 24-hour proteinuria was 0.7-9.7 gm), and persisted for an average of 10 days Microscopic examination of the urine revealed numerous tubular epithelial cells and an absence of red cells at the time of maximal proteinuria Tm_{PAH} was decreased at the time of maximal proteinuria from 15-85% (mean 39%) Tm_G was decreased from 20-76% (mean 52%) The maximal tubular excretory capacity for PAH and the maximal tubu-

lar reabsorptive capacity for glucose returned gradually over a period of 3 to 8 weeks to 88% or more of the baseline value in 10 to 12 subjects In the other 2 subjects, Tm_{PAH} was still depressed 25% at 8 weeks Inulin clearance was decreased from 2-75% (mean 32%), and the PAH clearance was depressed from 0-76% (mean 24%) during maximal proteinuria Both PAH and inulin clearances tended to return to baseline levels more rapidly than did the Tm values Unless the nephrotoxic principle contained in present preparations of bacitracin is an impurity which can be eliminated, these findings preclude the general use of this antibiotic by parenteral administration

Effects of growth hormone on the blood sugar and insulin sensitivity of the rat ANNE E MILMAN (by invitation) and JANE A RUSSELL *Dept of Physiological Chemistry, Yale Univ, New Haven, Conn* Observations were made concerning the effects of a highly purified preparation of growth hormone (prepared by Wilhelm, Fishman, and Russell) on the blood sugar and on the insulin sensitivity of fasted normal and diabetic rats When doses of 3 to 5 mg/100 gm were injected intraperitoneally into mature, normal or adrenalectomized male animals, a significant and prolonged depression of the blood sugar followed In alloxanized or partially depancreatized rats, on the other hand, these same preparations of growth hormone produced a marked rise in blood sugar This occurred in animals which were only mildly diabetic as well as those with more severe symptoms Normal animals pretreated with growth hormone 4 to 6 hours before were less sensitive to insulin Similarly, in diabetic animals, the duration of the blood sugar lowering effect of subcutaneously administered protamine-zinc-insulin was curtailed by injections of growth hormone (given 2-3 hours after the insulin) Growth hormone did not appear to alter the glucose tolerance of normal animals under our experimental conditions Bioassay of some of the growth hormone preparations showed traces of adrenotrophic hormone to be present, these amounts of ACTH were found to be to be insufficient to reproduce the effects observed in diabetic animals These results suggest that in the normal rat, the administration of growth hormone may provoke secretion of extra insulin In the animal lacking sufficient islet tissue, hyperglycemic or contra-insulin effects of the growth hormone are seen instead

Influence of intestinal motility on intestinal blood flow M SIDKY MOHAMED (by invitation) and JOHN W BEAN *Dept of Physiology, Univ of Michigan, Ann Arbor, Mich* The artery and vein to a loop of intestine of dogs anesthetized with pentothal were cannulated and the isolated loop set up in a perfusion apparatus Oxygenated heparinized dog blood was supplied to the tissue

under constant or pulsatile pressure. The venous outflow of blood was continuously recorded and the motility of the loop recorded from the lumen by tambour. On initiating the perfusion of quiescent gut the venous outflow was continuous but with the return of rhythmical contractions it became intermittent, increasing sharply during the contraction phase, decreasing or even momentarily stopping with relaxation. Since flow speeded up during the contraction phase the intermittence cannot be due simply to a cutting off of the arterial supply by occlusion of the vessels during the contraction, thus indicating the gut contraction causes an active propulsion of the blood from the tissue. Interruption of rhythmic contractions by periods of relative quiescence, at which time the arterial pressure should be most effective in increasing the flow, resulted in a decreased flow. The flow was also markedly augmented during the contraction phase of the longer tonic waves upon which the rhythmic contractions are superimposed but excessively strong tonic contractions, after initially increasing the flow, stopped the flow completely during period of high tonus presumably by passive vascular occlusion. On experimental occlusion of the venous outflow, venous pressure increased progressively and intermittently, coincident with successive rhythmical contraction phases. The experiments demonstrate the importance of intestinal motility in blood flow through the gut. Similar effects were with pulsatile and constant perfusion pressure.

Studies on vasotropic principles in blood (VEM and VDM) and renal hemodynamics in chronic heart failure. R. MOKOTOFF, D. J. W. ESCHER, I. S. EDELMAN, J. GROSSMAN and L. LEITER (all by invitation) and R. E. WESTON, B. W. ZWEIFACH and E. SHORR. *Medical Division, Montefiore Hospital, and the Dept. of Medicine, Cornell Univ. Medical College, the New York Hospital, New York City.* Since the increased renal arteriolar constriction in chronic congestive heart failure is probably not neurogenic, the possible role of VEM and VDM, newly described vasotropic principles, was investigated. Blood samples from the renal and hepatic veins, and the femoral artery or normal controls and of patients in chronic failure, were assayed for VEM and VDM by the rat meso-appendix test, in which the potentiation or inhibition of the arteriolar responsiveness to epinephrine is measured. Simultaneous renal hemodynamic and renal and hepatic oxygen extraction studies were performed. In all cases of congestive failure were both an increased filtration fraction and an increased oxygen extraction ratio were demonstrated, there were significant amounts of vasoexcitor principle (VEM) in the renal vein blood. In contrast, with one exception, almost no VEM was detected in the controls. The cardiacs had markedly reduced glomerular filtration rates and renal

blood flows with renal oxygen extraction increased to 14-29% as compared to 5-9% in the controls. Pronounced vasodepressor activity (VDM) was found in all hepatic vein samples of congestive failure patients. Only one control showed mild VDM. Although femoral arterial blood containing a mixture of VEM and VDM showed a slight predominance of the vasoexcitor principle in the majority of cardiacs, several did show a peripheral blood VDM predominance. This is important in view of the recent demonstration by Baez, Mazur and Shorr, that VDM has a profound antidiuretic effect. The relation of these principles to impaired renal hemodynamics in congestive failure will be discussed.

Method for the determination of heparin in blood. F. C. MONKHOUSE (by invitation), MARY STEWART (by invitation) and L. B. JAKES. *Dept. of Physiology, Univ. of Saskatchewan, Saskatoon, Sask., Canada.* A method for the determination of heparin in blood has been developed. It is applicable to the estimation of raised quantities of heparin in blood, as after intravenous injection, or anaphylactic shock. Blood is received into citrate and the heparin precipitated from the plasma with 7% octylamine hydrochloride. The precipitate is hydrolysed with 0.1N NaOH and the octylamine removed with ether. The heparin is then precipitated with 5% brucine phosphate at pH 6.0, and the brucine-heparin is washed with alcohol and ether and dried. The heparin present is assayed for its metachromatic activity with Azure-A using the method of Jakes, Mitford and Ricker (*Rev. Can. Biol.* 6: 740, 1947) or for antithrombin activity. Using the metachromatic method of assay, recoveries of 80-90% were obtained for heparin added to blood in concentrations of 0.2 to 3.6 mg/100 ml, while complete recovery of heparin was obtained from plasma. Normal human blood without added heparin gave a value equivalent to 0.009 mg % of heparin by this method. Owing to the extremely small amounts of heparin thus found in normal blood, certain modifications of the method are indicated in applying it to the estimation of normal blood heparin.

Further studies on the stimulating effects of light on sexual activity of the female opossum during the nonbreeding season. CHARLES F. MORGAN. *Dept. of Physiology and Biophysics, Georgetown Univ. School of Medicine, Washington, D. C.* Opossums were subjected to radiant energy from incandescent lamps, some with and some without monochromatic filters. The filters used were 'black' infra-red ($\lambda 8500$), red ($\lambda 6400$) and green ($\lambda 5460$). The radiant energy was determined by means of a chromel-constantan thermopile connected to a sensitive Rubicon galvanometer. Increase in the size and weight of the reproductive tract appeared directly proportional to the amount

of radiant energy received through lamps with and without filters. Thus radiant energy changes show greater effect than changes in wave lengths.

One group of opossums was exposed to a total amount of unfiltered radiant energy given 10 hours per day and compared with another group of opossums given the same total amount of radiant energy extending 20 hours per day with both groups receiving the treatment the same number of days. The sizes and weights of the reproductive tracts in both groups were similar. The degree of stimulation was the same as above when the animals received an equal amount of radiant energy from flashing lights given over a 20-hour daily period. Direct observations of the ovaries at laparotomy revealed ovulations in some animals receiving the stimulating effects of infra-red radiations. Ovulations have not been obtained in animals injected with varying amounts of gonadotropic hormones.

Sudden increases of radiant energy to a greater constant daily amount resulted in stimulation as great as that obtained in experiments previously reported (Morgan, Chas. F. *Federation Proc.* 6:359, 1947) in which incandescent light was increased by small increments each day such as occurs in nature.

Effect of spinal cord transection on the ventilatory response to exercise. DONALD P. MORGAN (by invitation) and FRED S. GRODINS, *Dept. of Physiology, Northwestern Univ. Medical School, Chicago, Ill.* The ventilatory response to electrically induced exercise of the hind limbs in barbitalized dogs was studied before and after section of the spinal cord at T₁₀. Respiratory rate, minute volume and oxygen consumption were determined at rest and during a steady state of exercise. Percutaneous electrical stimulation with 60-cycle AC modulated at 100/min. was used to produce oxygen consumptions up to seven times the resting value. A linear relationship was found between ventilation and O₂ consumption in 56 tests on 11 intact dogs. The regression equation was $Y = 0.56 + 0.031X$, where Y is ventilation in l/min., BPTS, and X is oxygen consumption in cc/min., STPD. The correlation coefficient was 0.97. A linear relationship was also found in 50 tests on 11 dogs after cord section. The regression equation was $Y = 2.31 + 0.027X$ and r was 0.78. Covariance analysis showed no significant difference between the regression coefficients, but a significantly higher adjusted mean ventilation in the sectioned animals. The latter was most apparent in the considerably higher resting ventilation after section. Respiratory patterns were different in the two groups, the tidal volume being less and the rate correspondingly greater at a given total ventilation in the sectioned animals.

Activity of single units in the cerebellar cortex. G. MORUZZI (by invitation), JOHN M. BROOKHART

and R. S. SNIDER, *From the Depts. of Anatomy and Physiology, Northwestern Univ. Medical School, Chicago, Ill.* Spike potentials have been recorded from single units having somas located in the cerebellar cortex. Glass micropipette electrodes having tip diameters of 5 to 20 microns have been used for unipolar recordings in unanesthetized decerebrate cats. The recorded spikes have the following characteristics: 1) duration of approximately 1 msec., 2) amplitudes reaching 250 microvolts, about 3 times the amplitude of the largest spontaneous cerebellar waves, 3) unbroken contour with rising phase shorter than falling phase. The unitary origin is indicated by 1) constancy of amplitude and contour, 2) possibility of identifying multiple units in the same record and 3) parallel modulation of amplitude of two units by slight cerebellar movements. Modification of spike frequency by local application of strychnine gives evidence of cortical localization of soma.

Spike activity falls into three classes: 1) injury, 2) normal spontaneous, and 3) convulsive. Injury discharges are characterized by normal spike contour, by frequencies higher than 100/sec. and by durations usually less than one minute. Normal spontaneous spikes have been recorded at frequencies from 25 to 150/sec. for many minutes; they usually occur in outbursts of variable duration. Occasionally, spike discharges have been evoked or inhibited by sensory stimulation. Convulsive outbursts of spikes have been produced by local strychninization of the cerebellar cortex. After an initial increase in frequency, resulting from strychnine, characteristic outbursts of convulsive activity occur, starting at frequencies above 200/sec., increasing to terminal frequencies up to 500/sec., and terminating abruptly.

Influence of bulbo-reticular stimulation upon electrical activity of cerebral cortex. G. MORUZZI (by invitation) and H. W. MAGOUN, *Dept. of Anatomy, Northwestern Univ. Medical School, Chicago, Ill.* The effect of stimulating the suppressor bulbar reticular formation upon cortical electrical activity has been investigated in cats under chloralose anesthesia. The high voltage, 5/sec. waves, characteristically pronounced in the sensori-motor area with this anesthesia, were abolished during such bulbar stimulation, while lower voltage, faster, background activity continued. This effect was most complete in the ipsilateral sensori-motor cortex, but was observed also in the visual area and contralaterally. It was prevented by section of the midbrain tegmentum, but not by section of the tectum or both bases pedunculi. It remained after complete paralysis with curare, and was not reproduced by antidromic stimulation of the bulbar pyramid. This effect was obtained with stimuli to the reticular formation of 300/sec. and 2-3 V. with individual shocks of 1

heparinized blood, usually have a moderately increased clotting time, and only rarely is it incoagulable. The blood is unusually dark and in most instances gelation precedes coagulation. Normal color, 'normal clot', normal or nearly normal clotting time and a decrease to or toward normal of the amount of titrating protamine are produced by administration of toluidine blue or protamine sulfate. Strikingly dissimilar, however, are the protamine titration changes relative to the clotting time. Only 0.75 mg/kg body weight of intravenous heparin is required to make the blood of a normal dog incoagulable (15 minutes after injection). The protamine requirement is increased when 1.0 mg/kg of body weight or more is given, and 3-5 mg must be injected to produce the changes similar to those seen in the patients and dogs.

Thalamic representation of tactile sensibility in the Macaque monkey. VERNON MOUNTCASTLE and ELWOOD HENNEMAN (introduced by PHILIP BARD). *Dept. of Physiology, Johns Hopkins Univ., School of Medicine, Baltimore, Md.* The thalamic terminations of afferent systems conveying cutaneous tactile sensation have been mapped by an application of the evoked potential technique. The responsive area is contained within the nuclei ventralis posterolateralis and posteromedialis. No responses were recorded from lateral, medial or anterior nuclear groups. The contralateral body surface is represented in nucleus ventralis posterolateralis in dermatome sequence, the caudal segments being most lateral, followed by lumbar, thoracic, and cervical segments in successively more medial positions. Those portions of the body most heavily innervated by sensory neurons, the apices of the limbs, receive greatest volume representation in the thalamus. The enlargement of the thalamic zone for the lower cervical segments (hand) has separated the zone for the upper cervical segments (head and neck) from that for the trigeminal nerve.

The face and mouth are represented in nucleus ventralis posteromedialis. The pattern suggests a para-oral, segmental representation of the trigeminal fields, with lips and mouth placed medially. The intra-oral structures are represented bilaterally. In the most medial position is a small area for ipsilateral face. Responses to tactile stimulation of ipsilateral body and face have been recorded from points in and near the external medullary lamina, but a zone of thalamic transfer of impulses destined for the second somatic area of the cerebral cortex has not been located definitely.

Studies on the renal secretion of potassium in the dog. GILBERT H. MUDGE (by invitation), JAMES G. FOULKS (by invitation), ADELBERT AMES, III (by invitation), and ALFRED GILMAN. *Dept. of Pharmacology, College of Physicians and Surgeons, Columbia Univ., New York City.* Potassium (K) clearances (CK) greater than simultaneous creati-

nine or thiosulfate clearances (CF) are considered evidence that a part of urinary K results from renal tubular secretion. CK/CF ratios over unity are most readily obtained by the intravenous infusion of K salts, a procedure which yields average ratios of approximately 1.3, occasionally rising higher. The intravenous infusion of hypertonic solutions of urea and sodium chloride also promotes the excretion of K. During water diuresis CK/CF remains low. A variety of drugs known to affect renal function has been studied for their effects on K excretion. Mercurial diuretics were the only agents to possess a significant action. These sharply diminished the excretion of K during K administration and during states of hypertonicity, and slightly elevated CK/CF during water diuresis. The effects of mercury were rapidly reversed by BAL. Although it is not possible from the data thus far obtained to determine the exact contribution of the secretory process to the excretion of K, the available results may be explained by the hypothesis that tubular secretion is a response to hypertonicity or to elevated cellular K following K infusion and that the action of Hg^{++} is to depress this process. If this hypothesis is correct it follows that a major portion of excreted K results from secretion.

Polarographically determined protein index. Nature of the cobalt-catalyzed reaction of cysteine. OTTO H. MÜLLER. *Dept. of Physiology, Syracuse Univ. College of Medicine, Syracuse, N. Y.* In continuation of the studies of the 'protein index' (Müller and Davis, *Arch. Biochem.* 15: 30, 1947) it became necessary to know more about the nature of the electrode reaction which produces the catalytic wave of sulfhydryl groups in the presence of a buffered cobalt solution. Consequently cysteine was polarographed over a large range of concentrations in solutions containing 0.1 N NH_4OH , 0.1 N NH_4Cl and several different concentrations of $CoCl_2$. The catalytic wave was observed at cysteine concentrations as low as 2×10^{-7} M. Its height is proportional to the surface area of the mercury drop but it is not a linear function of the Co^{++} concentration. It plotted against the logarithm of the cysteine concentration, it yields an S-shaped curve, typical of catalytic reactions. At higher cysteine concentrations a new wave was discovered which just precedes the above catalytic wave, it usually overlaps with it at lower concentrations. The height of this new wave is proportional to the concentration of Co^{++} ions and to the sixth root of the drop time, which suggests a type of diffusion current, its relationship to the cysteine concentration is more complex. From this and other evidence it is concluded that the first mentioned wave is produced by the catalytic action of the cobalt amalgam which is formed at the dropping mercury electrode, whereas the new wave is produced by the action of a

number of factors upon which these differences depend, *in vitro* as well as *in vivo*. Even working with the virtually pure enzyme solution, the reaction rate depends at least on three variables—enzyme, inhibitor and substrate. Variations of the concentration of each of these three components affect markedly the inhibitory effect. Considerable differences are observed between reversible and irreversible inhibitors, but even compounds belonging to the same group may show entirely different behavior. A few examples will be discussed. Still more factors determine the effect *in vivo*. Of special importance is the structural barrier surrounding the axons. Lipid insoluble compounds act only upon the synapse. But between lipid soluble inhibitors exist interesting differences. Some physiological implications of these observations will be discussed.

The spread of excitation and the order of depolarization in the human heart. L. H. NAHUM and H. M. CHERNOFF (by invitation). *Laby of Physiology, Yale Univ. School of Medicine, New Haven, Conn.* To determine the nature of VR, VL and VF in man it is necessary to know the 'proximal' and 'distal' zones of each lead. This information was obtained by projecting solid cones from the position of each electrode upon the heart, the area subtended being considered the proximal zone. The position of the heart was taken from X-ray views obtained at various angles. A geographic map was then constructed, showing the overlapping of the proximal and distal zones. By analyzing the positions of the beams from moment to moment in the simultaneously recorded QRS complex of each lead, the order of arrival of the excitation process at the various segments of the heart was determined.

The region first to receive the excitation process was lower anterior septum. Excitation then spread rapidly to involve anterior and posterior right ventricle, then to left apex and left posterior ventricle. Excitation terminally involved a region of the right anterior ventricle (conus area). This order of spread corresponds to that already described in the dog and agrees with observations made upon the exposed human heart. The order of repolarization was determined from analyzing the position of the ST-segment and direction of the T-wave in unipolar extremity leads and in simultaneously recorded CV1 to CV6. It was found that the apical and diaphragmatic region of the heart is the site of fastest repolarization.

Nitrogenous excretion in *Colpidium campylum*. ROY and M. NARDONE (introduced by CHARLES G. WILBER). *Biological Laby, Fordham Univ., New York City*. Wilber and Scamen (*Biol. Bull.*, 93: 214, 1947) found that *Colpidium campylum* can synthesize fat from protein. The following investigation was undertaken in an attempt to ascertain the nature of nitrogenous excretion in *Colpidium*

campylum when grown on a protein medium (proteose-peptone). Quantitative analyses for ammonia, in pure, sterile cultures of colpidia, were made using the permutit method (*J. Biol. Chem.* 29: 329, 1917). No ammonia was found 24 hours after inoculation. A straight line increase in ammonia with time was obtained from the 2nd day after inoculation to the 14th day after inoculation. The mean population reached a maximum of approximately 68,000 organisms per ml of culture on the 9th day after inoculation. The population then decreased gradually to approximately 41,000 colpidia per ml on the 14th day after inoculation. The results shown below are mean values of 5 analyses each day.

NO. DAYS AFTER INOCULATION	POP. /ML	AMMONIA N
		γ /ml
1	5,500	0.00
2	17,025	0.71
3	18,450	1.43
4	46,620	4.28
5	52,500	6.09
6	61,200	7.85
7	63,855	9.10
8	62,550	8.20
9	67,965	10.00
10	50,110	13.85
11	53,100	13.20
12	43,240	16.40
13	40,350	18.05
14	40,890	17.90

Using a qualitative spot test (Feigl), no uric acid was detected throughout the experiment. Urea, however, was produced during the logarithmic phase of growth.

Effects of eserine and pilocarpine on the parasympathetically denervated iris of the cat. ENID A. NEIDLE (introduced by WALTER S. ROOT). *Dept. of Physiology, College of Physicians and Surgeons, Columbia Univ., New York City*. According to Anderson (1905) the iris of the cat which, immediately after removal of the ciliary ganglion, responds to eserine soon ceases to do so and remains unresponsive. There is then a gradual return of the eserine response in the denervated sphincter. This has been taken as evidence for nerve regeneration. In 5 cats in which one ciliary ganglion had been removed and which showed a return of responsiveness to eserine, the oculomotor nerve was sectioned intracranially. Ten days later, the response of the sphincter was tested by instilling 0.4 mg of eserine salicylate into the conjunctival sac. In every instance there was complete or partial loss of response. This supports the belief of Anderson (1905) that preganglionic cholinergic fibers can replace postganglionic cholinergic fibers. Instillation of 0.04 mg of pilocarpine nitrate in the conjunctival

sac produces marked constriction of the ganglionectomized iris but has no effect on the normal iris. The sensitivity of the denervated iris to pilocarpine diminishes slightly but significantly with time after operation. Section of the oculomotor nerve, some time after removal of the ciliary ganglion, is followed by a complete or partial restoration of sensitivity to topically applied pilocarpine. It is suggested that the decrease in sensitivity to pilocarpine may be related to the occurrence of regeneration to the denervated sphincter of the iris.

Effect of hydration on the survival of fasting rats. DOROTHY NELSON (introduced by A. C. Ivr) *Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago, Ill.* Rats deprived of food dehydrate themselves even in the presence of adequate water. There is considerable evidence in support of the hypothesis that rats ingest any available material that is of benefit to them. In sharp disagreement, a growing literature indicates that in many situations rats fail to make advantageous selection of foods. Is the voluntary dehydration of the fasting rat protective or detrimental? To clarify this point we have compared the survival of several groups of mature females. With no food or water the rats lived about 11 days. The injection of 7.5 cc of 0.6% NaCl solution every 12 hours prolonged life about 3 days, a significant increase. Similar injection of 0.9% NaCl solution had no effect on survival, though less weight was lost than in the control groups. It appears from this, that the rat's refusal to drink in the absence of food, does not represent an attempt to maintain an optimal water balance, but probably results from a break down of the mechanisms for fluid adjustment.

Effect of elemental iodine and iodocasein in hypothyroidism induced by methyl thiouracil. WARREN O. NELSON and EILEEN B. HINES (by invitation) *Dept. of Anatomy, State Univ. of Iowa, Iowa City, Iowa.* Young male and female rats weighing 40-50 gm. were placed on a stock diet containing 0.2% methyl thiouracil. After 30 days the animals in each experiment were divided into 3 groups. One continued on the methyl thiouracil diet, a second received the same diet with 0.033% iodocasein, while the third received the same diet, but in addition received 4.0 or 6.0 mg. elemental iodine daily by subcutaneous injection. The second phase of the experiment was continued for 30 or 60 days. Control animals received the regular stock diet throughout the experimental period. For males in the longer experiments the averages for final body weight and total length were respectively: normal controls, 375 gm., 41 cm.; methyl thiouracil only, 95 gm., 28.5 cm.; methyl thiouracil and iodine, 175 gm., 33 cm.; methyl thiouracil and iodocasein, 252 gm., 38 cm. The animals which received methyl thiouracil only showed markedly reduced testicular function, both gametogenic and endocrine. The adrenals, thymus, spleen, liver, heart, submaxillary

glands and kidneys all showed marked reduction in weight as compared to the controls. However, all of these except the thymus corresponded to the reduction in body weight. Iodine induced definite improvement in testicular function and in organ weights, but was less efficacious than iodocasein. Females of the several groups showed the same trends for body weight, body length, ovarian function and organ weights. Estrous cycles were markedly improved by both iodine and iodocasein. Determinations of plasma protein-bound iodine revealed high levels for animals which received iodine or iodocasein.

Transmission spectra of the human ear (pinna and lobe) at arterial occlusive pressures. J. F. NEVILLE, JR., J. O. ELAM, K. SUGIOKA, and A. ROOS (introduced by H. L. WHITE) *Laby of Applied Thoracic Physiology (Surgery) and Dept. of Physiology, Washington Univ. School of Medicine, St. Louis, Mo.* Factors related to the accuracy of the oximeter have been investigated by spectrophotometry of the intact human ear. Instrumentation consisted of a Littrow type monochromator (resolution of 80 Å), S-4 and S-1 response phototubes, AC amplifier, and continuous ink recorder. The monochromatic beam was directed via quartz to the ear of the recumbent subject. The recording spectrophotometer automatically scanned the spectrum between 4000 and 11000 Å. Ear tissue was compressed with translucent rubber diaphragm by pneumatic inflation to 200 mm. Hg. Spectra were calculated as percentage absolute transmission ($\%T = \frac{I_t}{I_0} \times 100$, where I_0 = phototube response).

Data obtained in 10 male and 2 female normal white subjects indicated: 1) the bloodless pinna and lobe transmissions were zero at wavelengths shorter than 5100 Å, 2) the maximal transmissions of the bloodless pinnae were uniformly at 8800 Å, 3) the maximal transmissions of the bloodless lobes covered a broad spectral band from 6500 to 10000 Å, 4) the absolute transmissions of the bloodless pinnae averaged 15% between 6000 and 10000 Å while comparable values for the lobes averaged 26%, 5) the spectra of the bloodless pinnae indicated marked light scattering between 8800 and 5100 Å (decreasing transmission with decreasing wavelength) while this effect was much less evident in the lobe spectra, and 6) the absorption band of water between 8500 and 9800 Å was evident particularly in the pinna spectra. The lobe of the ear appears to offer optical advantages superior to the pinna as the site for oximetry.

Effect of diets containing certain tissues on liver regeneration in the rat. E. NEWMAN (by invitation) and A. C. Ivr *Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago, Ill.* Previous studies in this laboratory had shown that liver regeneration in partially hepatectomized rats was more rapid in rats fed whole cooked liver or

dried whole liver than in those fed casein or beef heart. The growth promoting factor was shown not to be in crude liver extracts. In order further to analyze this effect diets containing certain organs other than liver were fed to partially hepatectomized rats. The organs studied included spleen, kidney and duodenum. None of these organs were equal to liver in promoting liver regeneration. Studies were also made using nucleic acid or uracil supplements to a standard synthetic diet. Nucleic acid (derived from yeast) was as effective as whole liver in promoting liver regeneration. Uracil had no effect.

A 'vertical tube' method for the study of pressure-flow relations in vascular beds. J. NICHOL (by invitation), F. GIRLING (by invitation) and A. C. BURTON, *Dept. of Biophysics, Univ. of Western Ontario, London, Canada*. In the perfused preparation, as the isolated rabbit's ear, a vertical small-bore glass tube is connected (as a 'T') to the arterial cannula through which perfusion is made, while in the 'intact' preparation, as the hind-limb it is connected to a T-cannula inserted into the artery. In the latter case, the fluid in the vertical tube is separated from the flowing blood by a slack rubber membrane. The liquid in the tube stands at a height giving the pressure in the artery at that point. The artery, or tube from the perfusion bottle, is then clamped on the high pressure side of the T. The circulation in the preparation then proceeds, maintained by 'bleeding' from the vertical tube, at a steadily diminishing driving pressure. The height of the vertical column is plotted against time, giving an exponential-like curve. Tangents drawn to the curve at specific pressures give the flow through the vascular bed at any pressure. It can be shown that the length of the sub-tangent is a direct measure of the resistance to flow. Complete pressure-flow relations are thus obtained. These agree with those obtained by altering the pressure head and measuring flow by a micro-flow meter, but the vertical tube method is much more convenient and very accurate at low pressures and flows, where theoretical interest is greatest.

Time course of failure of the visual pathway in rabbits during anoxia. WERNER K. NOELL and HERMAN I. CHINN (introduced by HARRY F. ADLER), *USAF School of Aviation Medicine, Randolph Field, Texas*. In order to determine the factors responsible for the sequence of anoxic failure, potentials resulting from illumination of the eye and from electrical stimulation of the optic nerve were recorded from different parts of the visual pathway. Amplitude and timing of these responses were measured during nitrogen breathing. The curves for the decline in responsiveness thus obtained reveal, as one factor, the effectiveness of a decreased excitability on differently organized neuronal systems. Complete irresponsiveness at the surface of the striate area to either

photic or electrical stimuli was reached after 88 seconds of nitrogen breathing (blood was oxygen free within 30 to 45 seconds). The immediate post-synaptic potentials from the geniculate disappeared after 113 seconds. Potentials of the optic tract to strong photic stimuli became undetectable after 256 seconds and to maximal electrical stimuli after 293 seconds. One portion of the b-wave of the electroretinogram decreased simultaneously with the optic tract potentials, 25 minutes were required until all positive components of the electroretinogram had disappeared. A small negative electroretinogram persisted for many hours. All figures represent mean values. Intravenous administration of iodoacetate quickly abolished retinal excitability during air breathing. Neither the anoxic decline of cortical responsiveness nor the failure of the optic nerve to direct stimulation was hastened. Lethal doses of fluoride did not specifically affect retinal excitability immediately after injection. However, during subsequent anoxia all positivity of the electroretinogram disappeared more rapidly, while the survival times of cortex and optic nerve were not decreased.

Incorporation of glycine in the tissues of acutely starved rats. T. R. NOONAN, K. I. ALTMAN, G. W. CASARETT, and K. SALOMON (introduced by WILLIAM F. BALE), *Dept. of Radiation Biology, School of Medicine and Dentistry, Univ. of Rochester, Rochester, N. Y.* The effects of acute starvation upon the incorporation in tissues of glycine, labelled in its alpha-carbon atom with C_{14} , have been studied in the rat. One microcurie of labelled glycine (specific activity 1.83 $\mu\text{C}/\text{mg}$) was injected intravenously into normal rats and into rats which had been starved for 5 to 7 days, 24 hours after injection, the animals were killed and tissue samples secured for analysis of C_{14} content. Samples from the liver, gastrointestinal tract, kidneys, testes, and brain of animals starved for 5 or 7 days showed a markedly higher radioactivity per gram than corresponding samples from control rats. On a unit weight basis, the muscles of starved animals showed lower C_{14} concentration than control animals, this effect being more marked after 7 days of starvation. When calculated on the basis of percent of injected C_{14} activity found per total organ, the starved animals showed a pattern of decreased incorporation in muscular tissue with an unchanged or even increased incorporation in visceral tissues.

Time-dose study of biochemical responses of rats to x-radiation. N. NORTH (by invitation) and L. F. NIMS, *Brookhaven National Lab., Upton, L. I., N. Y.* A time-dose study of biochemical responses of the adrenal-pituitary system in fasted rats following whole body x-radiation indicates a pattern of changes comparable to that following other stresses. Male albino rats were subjected to radiation doses of 500 to 2000 r at a rate of 50 r per minute and sacrificed at periods of time ranging

from 3 to 48 hours following exposure. Analyses of tissue constituents show an immediate and progressive increase in liver glycogen and no change in testis glycogen. There is a simultaneous decrease in the adrenal and liver cholesterol but little change in the testis cholesterol and a late increase in the plasma cholesterol. The adrenals also show an initial decrease in ascorbic acid content with a subsequent return to normal, while little variation from the unirradiated controls is seen in pituitary, liver or testis ascorbic acid. There are no evident effects of radiation on total body, adrenal or pituitary weights in fasted animals.

Acclimatization to histotoxic anoxia DAVID W. NORTHUP and ROSS BELL, JR. (by invitation) *Dept of Physiology, School of Medicine, West Virginia Univ, Morgantown, W Va*. In order to determine whether acclimatization to histotoxic anoxia occurs in a manner similar to that seen during anoxic anoxia, the red cell count, reticulocyte count, and hemoglobin concentration of the blood of three rabbits were followed during the administration of potassium cyanide. Determinations were made daily during an 11-day control period and then KCN was administered subcutaneously in doses ranging from 1.0 to 3.1 mg/kg/day, the dose being increased as the animals developed tolerance (Sufficient cyanide was given to produce distress). The average red cell count of the 3 rabbits rose from 4.97 million during the control period to 5.37 million during the period of cyanide administration, significant at the 0.1% level, the hemoglobin from 10.95 gm to 11.92 gm significant at the 0.1% level, the reticulocyte count from 1.0% to 2.3%, significant at the 0.1% level. The results indicate that the rabbit responds to histotoxic anoxia by increased activity of the bone marrow.

Inside the cell Part I Enzymes in intracellular chemistry (Motion picture) ALEX B. NOVIKOFF (introduced by F. J. SICHEL) *Dept of Pathology, Univ of Vermont College of Medicine, Burlington, Vt*. Produced by the United States Army Medical Illustration Service, under the supervision of the Surgeon General, this film is intended for teaching professional medical personnel of the Services and civilian medical and university students. It suggests the importance to medical practice of a knowledge of enzymes and cell metabolism. After briefly sketching the historical development of our knowledge of enzymes, the film deals in detail with the known steps of glucose metabolism, including glycolysis, the tricarboxylic acid cycle, hydrogen transport via the cytochrome system, and the phosphate or energy cycle. It suggests points of contact of the metabolism of carbohydrates and other foodstuffs, and it indicates the relation of vitamins and minerals to enzyme structure. Most of the film is in color animation. There are also live action demonstrations of several sections, photographed in the laboratories of the individuals mentioned.

J. B. Sumner shows the essential steps in the crystallization of urease. Otto Meyerhof demonstrates the need of DPN and ATP in glycolysis. Severo Ochoa shows the enzymatic decarboxylation of oxalosuccinic acid and the oxidation of isocitric acid. Carl F. Cori and Gerty Cori demonstrate the *in vitro* synthesis of glycogen and starch. Carl F. Cori and M. E. Krahli show the effect of insulin and adrenal hormone on the uptake of glucose by diaphragm muscle. Van R. Potter demonstrates the low succinic dehydrogenase activity of tumor homogenates as compared with those of normal organs.

Response to intravenous injection of small quantities of insulin JOHN A. NUETZEL (by invitation) and NORMAN S. OLSEN *Dept of Internal Medicine and the Oscar Johnson Inst, Washington Univ School of Medicine and Barnes Hospital, St Louis, Mo*. A modified intravenous insulin tolerance test, using $\frac{1}{10}$ unit of insulin/kg body weight, was performed in a series of normal, hypertensive, and other patients. Blood sugar levels were determined at 0, 15, 30 and 60 minutes after intravenous injection, by the method of Nelson. The data were analyzed on the basis of the percentage fall from the fasting level. Certain clinical groups were resistant to the action of intravenously administered insulin in that they showed significantly less fall in 15 and 30 minutes than did the normal group. These groups consisted of patients with malignant hypertension, with hypertension and severe complications (such as stroke and heart failure), with advanced neoplastic diseases, liver diseases, collagen diseases, and psychosomatic disorders. Resistance was also found, as expected, in active infections and certain endocrine disorders. Hypertensive patients having an endocrine or neurogenic basis and not in a malignant phase were not resistant.

Blood pressure after nephrectomy ERIC OGDEN, ELEANORE TRIPP (by invitation) and GEORGE CONSTANT (by invitation) *Dept of Physiology, Univ of Texas Medical Branch, Galveston, Texas*. Grollman reported occasional elevations of blood pressure in rats and dogs after unilateral nephrectomy. In the present group of 5 well-trained dogs series of measurements were made before and 5 months after removal of the left kidney. The table indicates the average values for the individual dogs.

DOG	BEFORE NEPHRECTOMY		AFTER NEPHRECTOMY	
	Syst./Diast.	P rate	Syst./Diast.	P rate
1	165/80	115	194/105	100
2	168/83	90	188/107	101
3	133/60	103	166/80	86
4	189/59	95	167/95	95
5	165/81	150	171/97	91

Blood pressure was computed from records made without anesthesia by puncture of the femoral artery with the needle connected to a glass spoon manometer with optical recording, the pulse counts were computed from the same records. It is believed that the lowered pulse rates after nephrectomy are indications that the dogs were better trained. The elevation of both systolic and diastolic pressures in each animal considered separately was statistically examined and found unlikely to be due to chance. That such an occurrence would appear in 5 consecutive animals is overwhelmingly likely to be due to something other than chance even though the changes are small and only two of the dogs showed blood pressure levels which might be considered mildly hypertensive.

Treatment and prophylaxis of experimental hypertension with amines. E. A. OHLER (by invitation), G. E. WAKERLIN and G. A. ALLES (by invitation) *Dept of Physiology, Univ of Illinois College of Medicine, Chicago, Ill.* Paredrine HBr has been previously reported (*Federation Proc* 7 87, 1948) to be effective therapeutically in experimental renal hypertension. Since our last report 3 neurogenic (debuffered) hypertensive dogs have been treated with 4 mg/kg of Paredrine orally for periods of 3 to 6 months. Two neurogenic dogs showed excellent reductions in blood pressure, one, a good response. Of 5 dogs treated prophylactically with 4 mg/kg of Paredrine orally daily for periods of 3 to 6 months, all showed a rise in blood pressure following bilateral renal artery constriction. Two animals exhibited rises of 55 mm Hg, one dog developed malignant hypertension and died, and the remaining 2 showed rises of 20 mm Hg or more. The following compounds, closely related chemically to Paredrine, have failed thus far to exhibit any antihypertensive activity: 2-amino-1-cyclohexylpropane, levoamphetamine, ephedrine, hydroxy-tyramine, hydroxy-paredrine, and dl-1-parahydroxyphenyl-3-aminobutane. Tyramine in a dosage of 2 mg/kg IM daily produced a modest fall in the blood pressure of 1 renal hypertensive animal. Of 3 renal hypertensive dogs treated with Paredrinol (dl-1-parahydroxyphenyl-2-methyl-aminopropane) in a 4 mg/kg dose orally only one showed a reduction in blood pressure. Tuamine (2-aminoheptane) at a dosage level of 4 mg/kg IM has proved ineffective in 2 hypertensive animals.

Diuretic response of normal and edematous dogs to intravenous administration of succinate and fumarate sodium salts. ELIZABETH O'LEARY (by invitation) and SAMUEL A. CORSON *Dept of Physiology, School of Medicine, Howard Univ, Washington, D. C.* Hypertonic solutions (1.4M) of disodium succinate and fumarate salts were administered intravenously to trained unanesthetized dogs by means of a constant infusion pump

at the rate of 2.5 ml/min, injecting a total dose of 10 ml/kg of body weight. Such an infusion increased the rate of urine flow from an average control value of 0.4 ml/min to an average value of 4.2 ml/min for a period of about 300 minutes. The net quantity of fluid lost by the animals as a result of the administration of these salts (after subtracting the quantity of fluid injected and the quantity of urine that would normally have been excreted) was on the average 67 ml/kg body weight. Administration of these salts to dogs with experimental hypoproteinemic edema (produced by a combination of massive plasmaphereses and a low protein diet) led to a net average increase in urine output of 76 ml/kg body weight. We must therefore conclude that the diuretic responses of dogs with hypoproteinemic edema to the intravenous injection of succinate and fumarate salts are of the same order of magnitude as (or possibly greater than) those of normal animals.

Graphic measurement of arterial pressure in the intact rat. F. OLMSTEAD and O. GLASSER (introduced by A. C. CORCORAN) *Research Division of the Cleveland Clinic Foundation and the Bunts Institute, Cleveland, Ohio.* The method previously described (*Federation Proc* 7 88, 1948) for measurement of systolic pressure from the foot pulse of rats had been modified for greater accuracy and convenience. A light weight pickup has been developed which consists of 2 miniature coils separated by a small layer of sponge rubber. This device is readily attached to the front or hind foot of the rat and substitutes for the strain gauge previously employed. A frequency discrimination circuit is used to transfer the pulse into electrical change. For greater compactness and accuracy in recording cuff pressure, a Selsyn generator and motor are used. The generator is driven by a large metal bellows. The motor is mounted in the Brush ink oscillograph and drives a recording pen. Seventy-five simultaneous optical recordings were made of carotid pressure by a Hamilton manometer and of foot systolic pressure as indicated from the foot pulse. The mean deviation of indicated pressure in the hind foot from that in the carotid artery was -5.4 mm Hg. Variation around this mean was ± 3.5 mm Hg. These measurements were made over a range of systolic pressure from 52 to 170 mm Hg.

Effect of intrapulmonic and intrathoracic pressure variations on left atrial pressure. D. F. OPDYKE and G. A. BRECHER (by invitation) *Dept of Physiology, Western Reserve Univ Medical School, Cleveland, Ohio.* Mechanical factors of extra-cardiac origin which exert pressure on the heart chambers become of great importance in determining pressure-flow gradients in the presence of intra-cardiac shunts. If these extraneous forces do not affect all cavities equally it is possible that their effect may be directly opposite to that

desirable for physiologic compensation. Experiments have been undertaken to assess the effects of two of these factors, intrapulmonic and intrathoracic pressure, on left atrial pressure, since the pressure variations in this heart chamber have been so little studied.

Positive pressure inflation of the lungs in an open chest dog results in an increase of both right and left atrial pressure with a decrease in aortic pressure. This effect is intensified by maintenance of the lungs in the inspiratory position for a few seconds. Right pressure increases considerably more than left. Ventral traction on the pericardium, lung roots, aorta, and other thoracic structures does not reproduce this effect. Experiments dealing with the effect of intrathoracic pressure changes and with the effects of positive pressure breathing on left atrial pressure in the closed chest dog are described and the results presented.

Diuretic effect of adrenal cortical hormones in mildly hydrated and dehydrated animals. C. M. OSBORN and W. J. EVERSOLE (introduced by ROBERT GAUNT) *Dept. of Zoology, Syracuse Univ., Syracuse, N. Y.* Experiments were made to determine the conditions under which the diuretic action of adrenal cortical hormones could be demonstrated in intact rats. In previous experiments with water given *ad lib.*, desoxycorticosterone acetate (DCA) caused a diabetes insipidus-like state (Selye and Bassett), this condition was intensified by addition of salt to the diet (Corey and Britton, Ragan *et al.* Richter). When large multiple doses of water were force-fed, both DCA and cortical extract enhanced markedly the diuretic rate and prevented water intoxication (Gaunt). The possibility exists that such effects are manifested only in the presence of high water loads induced either indirectly by salt retention or directly by water administration. Forty-eight fasted male rats were given a single hydrating dose of water by stomach tube (4 ml/100 cm² of body surface, average dose = 12 ml). One group received 5 mg DCA one-half hour before water was given, 90 minutes later they had excreted 11% more urine than controls, a result similar to that following chronic DCA treatment (Selye and Bassett). With same water dosage, another group received 4 ml of Upjohn's adrenal cortical extract (alcohol removed), force-fed as a portion of the water dose. These animals excreted 31% more urine than saline-treated controls. Forty-eight rats were deprived of food and water for 20 hours. Beginning at the 13th hour, part of the animals were given DCA at intervals until dosage totaled 4 mg and others received a total dosage of 4 ml of cortical extract. With such dehydration and acute treatment, DCA had no effect on urine volume, but the cortical extract doubled urine

output. It appears, therefore, that the diuretic action of DCA may be somewhat dependent on water load, while cortical extract enhances diuresis under all observed conditions of water balance.

Application of Gray's theory of respiratory control to the hyperpnea produced by 'passive' exercise. ARTHUR B. OTIS *Dept. of Physiology and Vital Economics, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.* Movements of the limbs produced by external forces cause an increase in the minute volume of ventilation as, has been observed by several previous investigators. Such movements, however, are not entirely passive but produce an increased oxygen consumption. Continuous measurements of the alveolar gas tensions make possible the estimation of the ventilatory stimulus due to the limb movements *per se* by application of Gray's multiple factor theory. Such calculations show that the stimulus from such movements is enough to produce a ventilation increment 100% to 150% of the resting ventilation.

Anemia producing properties of hexahomoserine and the antagonizing effect of lysine. EDOUARD PAGÉ and R. GINGRAS (by invitation) *Depts. of Nutrition and Biochemistry, Medical School, Laval Univ., Quebec, Canada.* Hexahomoserine (α -amino- ϵ -hydroxycaproic acid) produces anemia and inhibits growth in the rat. It also causes a considerable rise in blood plasma amino nitrogen. Simultaneous lysine supplementation prevents the anemia, improves partially the growth rate but has no effect on the plasma amino nitrogen. These observations were made on rats receiving either a complete basal ration or one containing no lysine. Amino-adipic acid, a biological derivative of lysine, antagonizes neither hexahomoserine, nor lysine. Liver concentrates and folic acid are ineffective in the prevention of the anemia caused by hexahomoserine.

Mechanisms of the vascular action of tetraethylammonium chloride. IRVINE H. PAGE, RALPH PRINCE (by invitation) and JOHN J. REINHARD, JR. (by invitation) *Research Division and Bunts Institute of the Cleveland Clinic Foundation, Cleveland, Ohio.* Acheson, Moe, Lyons and Hoobler introduced tetraethylammonium chloride as a means of blocking autonomic ganglionic transmission to measure autonomic activity. Although we were unable to show a significant difference in response to TEA after renal hypertension developed, individual responses were so variable that blockade of ganglionic transmission probably was not the only action of TEA. Ninety-four normal dogs showed widely variant responses. Occasional 'normal' animals showed a rise in blood pressure rather than the more usual short rise followed by a fall. On repeating the doses, the responses became progressively less depressor and,

finally, pressor Destruction of the spinal cord from C₆ caudad caused invariably a pressor response to every injection, and inactivation of the carotid sinus mechanism either did not change the response or somewhat augmented it In 2 patients with severe central nervous system lesions pressor responses were observed with the initial dose Bilateral nephrectomy done 2 days before testing usually accentuated the pressor component while bilateral adrenalectomy very moderately reduced it Hepatectomy greatly increased the depressor action TEA caused little or no rise in pressure after hepatectomy, hepatectomy-nephrectomy-adrenalectomy, or in animals with spinal cord destroyed, carotid sinus mechanism inactivated, kidneys and liver removed The pressor response was abolished by prior administration of Dibenamine and Priscol sufficient to reduce or abolish the action of arterenol and reverse adrenalin The response to TEA appears to be composed of 2 components one dependent on blockade of autonomic ganglionic transmission, the other, dependent probably on liberation of an arterenol-like substance

Observations on pulmonary lymph flow and edema ROBERT PAINE (by invitation), FRANK A HOWARD (by invitation), HARVEY R BUTCHER (by invitation) and JOHN R SMITH *Cardiovascular Division, Dept of Medicine, Washington Univ School of Medicine, St Louis, Mo* Lymph flow from the lungs of dogs has been studied Using Drinker's method, the pulmonary lymphatics were isolated, and intact, or open thorax preparations under positive pressure respiration, were made The lung lymph was measured by cannulation or by catching the fluid on weighed pledgets Obstruction of the pulmonary veins producing moderate elevation of venous tension and pulmonary congestion showed no effect on the rate of lymph flow However, maximal pulmonary venous congestion results in increased lung lymph (Drinker) The reduction of plasma proteins to 2.5 gm % or less by plasmaphoresis, and by the administration of Locke's solution, caused a marked increase in lung lymph flow Furthermore, when pulmonary lymphatic drainage was curtailed by obstruction of the superior vena cava, a rise in lung lymphatic pressure occurred as noted by direct manometry of the lymphatic system However pulmonary edema was not observed following any of these procedures When lymphatic outflow was obstructed, the critical diminution of plasma proteins produced gross pulmonary edema The effects of increased hydrostatic pressure and decreased osmotic pressure indicate the operation of Starling's hypothesis in the lung The curtailment of lymph outflow due to peripheral venous hypertension from heart failure, in conjunction with pulmonary congestion or

hypoproteinemia, may be important in the precipitation of pulmonary edema

Variability and reliability of cardiac index estimations with the high-frequency, undamped ballistocardiograph ROBERT M PAINE (by invitation) and NATHAN W SHOCK *Section on Cardiovascular Diseases and Gerontology, National Heart Inst, Bethesda, Maryland, and the Baltimore City Hospitals, Baltimore, Maryland* The ballistocardiograph used was horizontal, undamped, and had a natural frequency of 12.6 cycles/sec when loaded with 150 lbs The calibration was such that a force of 280 gm applied to the bed caused a displacement of 1 centimeter on the photographic paper record The largest and smallest complexes in each of 2 representative respiratory cycles of each tracing were selected independently by 2 observers and the average areas (mm-sec) under the I-waves and J-waves were inserted into Tanner's formula stroke volume in cc per minute = $\sqrt{21 + J\sqrt{C}}$ C represented the duration of a cardiac cycle in seconds Cardiac index (L/min/M²) was estimated in the basal state, after a standard meal, at hourly intervals throughout a day of ordinary activity, and at 5-minute intervals, through a 40-minute period while resting supine Ten young male hospital inpatients without cardiovascular disease and 10 young male laboratory personnel served as subjects The entire routine was repeated on the inpatients the following day A significant elevation of mean cardiac index (12%) occurred within $\frac{1}{2}$ hour after eating which lasted 3 $\frac{1}{2}$ hours There was no difference between inpatients and laboratory personnel, in either the basal state or following ordinary activity, and there was no change through forty minutes of rest, similar results were obtained on the second day The reliability of the cardiac index was high and variation in any case determination depended largely on the complexes chosen No significant advantage was obtained by increasing the number of complexes measured or the number of observers

Adrenal cortical hormone levels in blood following 'alarming stimuli' K E PASCHKIS, A CANTAROW and D BOYLE *Jefferson Medical College, Philadelphia, Penna* The reaction of the adrenal cortex to 'alarming stimuli' was studied by assay of adrenal cortical hormone in peripheral arterial blood of dogs Hormone levels were determined in plasma by the mouse liver glycogen method of Venning It had been shown previously (*Fed Proc* 7:90, 1948) that levels in the arterial blood rise following the injection of ACTH or of adrenalin A rise of cortical hormone in arterial plasma occurred within the first hour after subcutaneous injection of formalin A surgical procedure, exposure of the adrenal gland under anesthesia, resulted in higher levels of cortical hormone in the adrenal vein blood than had been previously obtained in the unanes-

thetized, trained dog (London cannula) Insulin injection was followed by an increase in cortical hormone, suggesting the participation of adrenal cortical hormone in counteracting insulin-induced hypoglycemia Attempts to determine whether the effects of these stimuli were mediated by epinephrine (according to the theory of Long) led to an investigation of the action of Debenamine in this connection Injection of Dibenamine itself was found to be an 'alarming stimulus', leading to an increase in blood levels of cortical hormone The effect of sympathetic blockade by Dibenamine upon the response to formalin and insulin was studied

Influence of a conditioning injection of estrogen on the hematologic and organ weight response to X-irradiation HARVEY M PATT, MARGUERITE N SWIFT (by invitation), ROBERT L STRAUBE (by invitation), ELLA B TYREE (by invitation) and DOUGLAS E SMITH *Biology Division, Argonne National Lab, Chicago, Ill* Estradiol benzoate (0.166 mg I/M) in a single injection 5 to 15 days before exposure greatly enhances resistance of mice to total body X-irradiation Progesterone and testosterone are ineffectual To elucidate this phenomena we have studied the effect of estradiol on organ weights, blood count, and microscopic anatomy in control and irradiated mice Estrogen increases adrenal weight by 35-40% 4 days after injection and accelerates thymic involution but has no appreciable effect on weight of spleen, inguinal nodes or kidney Leukopenia with maximal depression around 10-14 days after injection has also been observed Decreases are noted in lymphocytes and heterophils Little change is seen in erythrocytes Significantly, the maximal estrogenic protective effect is observed when mice are irradiated during their leukopenic period Estrogen injection at the time of irradiation or 25 to 35 days before exposure does not protect Radiation induced involution of spleen, thymus and inguinal nodes is not altered by estradiol However, the increase in adrenal weight is somewhat greater in these animals Leucocytes are decreased equally in both estrogen and control (sesame oil injected) irradiated animals, although maximal depression and recovery occur somewhat earlier in the former Erythrocyte count of irradiated controls is decreased by 45% at 11 and 14 days after exposure Estrogen treated animals manifest a smaller decrease in red count (25%) 7 days after irradiation and normal levels at 11 and 14 days The possibility that the protective action of estrogens is mediated by the adrenals is being investigated

Oxygen consumption and water balance in the X-irradiated frog HARVEY M PATT, MARGUERITE N SWIFT (by invitation) and ELLA B TYREE (by invitation) *Biology Division, Argonne National Lab, Chicago, Ill* As part of a study of the radiation response of frogs and of factors contribut-

ing to their radiosensitivity, we have investigated 1) oxygen consumption after X-irradiation, and 2) rate of water uptake in dehydrated irradiated frogs Oxygen consumed by individual unfed Autumn frogs, sedated with nembutal, was measured volumetrically at atmospheric pressure and 23°C Results of a pilot study in which determinations were made during a control period of several days and at frequent intervals after X-irradiation with 3000 R (completely lethal, mean survival time 28 days), suggest a slight early decrease in oxygen consumption with maximal depression (34%) 15 days after exposure This is followed by return to control levels (0.086 ± 0.002 cc/gm/hr) around 25 days after irradiation Although there is evidence that ionizing radiation accelerates catabolism in mammals, especially terminally, we failed to observe any increase in oxygen consumption in these frogs Body weight of unfed irradiated frogs also did not change appreciably Frogs kept in dry individual containers at 23°C for 3 days lose 35-40% of their water After irradiation with 3000 R and immediate return to water, such partially dehydrated irradiated frogs regain water at the same rate as non-irradiated dehydrated controls In both groups, recovery is essentially complete by 4 hours There is no change in water content of non-dehydrated irradiated frogs over this period Experiments are in progress to evaluate the influence of water content at the time of exposure on radiosensitivity in the frog

Effect of urine extracts from hypophysectomized dogs on gastric secretion T L PATTERSON, J KAULBERSZ, D J SANDWEISS and H C SALTZSTEIN (by invitation) *Depts of Physiology and Surgery, Wayne Univ College of Medicine and Harper Hospital, Detroit, Mich* In continuation of our earlier studies related to the influence on gastric secretion of urine extracts from dogs deprived of their pituitary gland, new series of experiments were made with extracts prepared from dogs hypophysectomized by transtemporal methods The effect of the extracts were tested on several dogs either with gastric fistula or with Heidenhain pouch by the double-histamine technique In 21 experiments (*series A*) the extract was administered during the second period of the double-histamine test, and in 25 experiments (*series B*) during the first period Extracts from the urine of hypophysectomized dogs did not exhibit a prompt inhibitory effect which is characteristic for urogastrone prepared from normal urine In relatively small doses (1 mg) hypophysectomized extracts produced as an immediate effect an increase in secretory response to histamine which is statistically significant in both animal series No significant delayed effect on secretion could be detected In larger doses (3 mg or more) there was no significant immediate effect on secretory response to

histamine, although in some dogs varied degrees of stimulation or inhibition were observed. However, statistically significant evidence of a strong delayed inhibition lasting more than 9 hours is revealed. Therefore, it appears that urine extracts prepared from hypophysectomized dogs contain both stimulatory and inhibitory agents. The stimulatory effect is prompt and more noticeable in small doses. The inhibitory effect is more evident in larger doses of the extract. In contrast to that of typical urogastrone, inhibition develops slowly and lasts longer than 9 hours.

Manifestations of potassium intoxication in the dog heart-lung preparation FRANCIS E. PAYNE (by invitation), LESLIE L. BENNETT, SANDOR G. BURSTEIN (by invitation), and JAMES HOPPER, JR. (by invitation). *Divisions of Physiology and Medicine, Univ. of California, Berkeley and San Francisco, Calif.* Heart-lung preparations were arranged for the continuous recording of right auricular pressure, aortic pressure, ventricular volume, and left ventricular output minus the coronary flow. Unipolar electrocardiographic tracings were taken directly from the anterior surface of the left ventricle using a wick electrode. The plasma potassium concentration was elevated by the addition of isotonic KCl and the plasma level was determined by the use of a flame photometer. Eight heart-lung preparations were studied. The venous return and peripheral resistance were not altered during an experiment. It was found that the major mechanical phenomena of potassium intoxication did not appear until a plasma level of approximately 7 mEq/l was reached. While approaching this level of potassium concentration only minimal increases in ventricular volume and minimal decreases in cardiac rate and output were observed. During this period of increasing potassium concentration there were progressive electrocardiographic changes which consisted of decreased amplitude of P waves and QRS complexes, depression of the S-T segments, prolongation of the P-R intervals, and increased amplitude of the T waves. When potassium levels of approximately 7 mEq/l were reached the following marked changes of abrupt onset occurred: increase in right auricular pressure, ventricular volume, and aortic pulse pressure (reflecting the increased stroke output due to the slow rate), and decrease in cardiac output and heart rate (the result of auricular standstill). The most striking associated electrocardiographic changes were the disappearance of the P waves, further displacement of the S-T segments and still higher T waves.

Hepato-renal factors in circulatory homeostasis

XXV. Low protein diets and renal VEM formation MARY ANN PAYNE (by invitation) and EPHRAIM SHORF. *Dept. of Medicine, Cornell Univ. Medical College and The New York Hospital, New York City.* Previous studies from this laboratory demon-

strated the importance of the renal vasoexcitor, VEM, for compensatory peripheral vascular reactions to hemorrhagic and traumatic shock, in its absence, these compensatory reactions failed to occur. Under certain conditions the renal capacity to form VEM was abolished, e.g., anoxia (2 hours for rat, 4 for dog kidney), adrenal insufficiency and nutritional cirrhosis (rats, on 10% protein diets for 4 months). The present study deals with the acute effects, in rats, of fasting and a lower protein diet (5% casein) on the renal capacity to form VEM, as measured by anaerobic incubation of kidney slices *in vitro*. Adequate vitamin supplements were supplied throughout. Fasting rats sustained a significant reduction in the capacity to form VEM by the 4th, and a total loss by the 6th day of the fast. Those receiving the 5% casein diet showed appreciable reductions in VEM formation by the 8th, and complete abolition from the 12th through the 39th day, when the experiment was terminated. Desoxycorticosterone acetate, which restores VEM formation to kidneys of adrenalectomized rats, failed to prevent this defect, when given from day 1 to 14, or to correct it, when given from day 14 to 39 of the low protein regime. This VEM defect was not associated with significant reductions in renal oxygen consumption. It is suggested that, in addition to the adrenal cortical hormones and an aerobic environment, an adequate protein intake is essential for the integrity of the renal mechanism for forming VEM.

Studies on the fat-mobilizing hormone of the anterior pituitary gland RICHARD W. PAYNE (introduced by E. B. ASTWOOD). *Joseph H. Pratt Diagnostic Hospital, and Dept. of Medicine, Tufts Medical School, Boston, Mass.* The fat-mobilizing effect of various anterior pituitary preparations was assayed by fat deposition in the livers of fasted mice by the method of Campbell (*Endocrinology* 23: 692, 1938). Increasing doses induced a progressive increase in the liver fat from control values of 4.5 to 6.0% to maximal values of 12.0 to 14.0%. Higher doses caused a submaximal response. Some activity was found to be present in all anterior pituitary preparations tested (whole beef anterior pituitary and pork and sheep pituitary powders, Armour and Parke-Davis thyrotrophic hormone, Armour's adrenocorticotrophic hormone, Armour's growth hormone, and Searle's anterior pituitary gonadotrophic hormone). Posterior pituitary preparations showed no fat-mobilizing properties. Purified adrenocorticotrophic and thyrotrophic hormones were the most active on a weight basis. Purified growth hormone and gonadotrophic hormones showed little activity as compared to other purified preparations. One week after adrenalectomy, the test animals exhibited no fat-mobilization in response to any anterior pituitary preparation. Thyroidectomy or depression of thy-

roid function with propylthiouracil did not inhibit the effect. Single injections in oil of progesterone (10 mg), testosterone (25 mg), desoxycorticosterone acetate (5 mg), and stilbestrol (5 mg) had no effect. One cc of aqueous adrenal cortical extract produced no significant fat-mobilizing effect.

Comparison of a volumetric respirometer with the Warburg method in measurement of brain respiration

C. N. PEISS and R. WENNESLAND (introduced by J. FIELD) *O. N. R. Arctic Research Lab., Point Barrow, Alaska, and Dept. of Physiology, Stanford Univ., Stanford, Calif.* Six aliquants (each about 25 mg wet weight) of rat cerebral cortex slices were prepared from the brains of each of 8 adult albino rats. The moist cold box technique was used (Field, *Methods in Medical Research*, 1948). Oxygen uptake was measured on 3 aliquants by the Warburg method and on the other 3 by means of volumetric respirometers (see abstract by Wennesland). Thus 48 Warburg runs were made and 47 volumetric (one sample lost). The liquid phase was Krebs Ringer's phosphate containing 0.01M glucose and 0.01M bicarbonate. Gas phase oxygen. Temperature $37.5 \pm 0.01^\circ\text{C}$. Initial pH 7.4. Final pH 7.4 to 7.5. The mean Q_{O_2} s were 14.12 and 14.08 for the Warburg and volumetric methods respectively. The corresponding standard errors were ± 0.169 and ± 0.171 . Statistical analysis showed that the two series did not differ significantly either in respect of the means (Student's 't' test) or of the variability (Fisher's 'F' test).

Blood gas content of dogs during immersion hypothermia

K. E. PENROD and FELIX R. ROSENHAIN (by invitation) *Dept. of Physiology, Boston Univ. School of Medicine, Boston, Mass.* Simultaneously drawn samples of arterial blood from the carotid artery and mixed venous blood from the right ventricle were handled anaerobically and analyzed for O_2 and CO_2 content. Samples were drawn before immersion in an iced bath and at rectal temperatures of 30° , 25° and 20°C while breathing air, and before immersion, at 35° , 30° and 25°C in a second series breathing 100% O_2 . All dogs were lightly anesthetized with sodium pentothal. Hematocrit determinations were made on each blood sample. In 2 of 9 dogs breathing air a mounting arterial CO_2 , evidence of approaching respiratory inadequacy, appeared above 25°C rectal temperature. In 4 it was between 25° and 20° and in the other 3 below 20°C . Beginning circulatory inadequacy was not yet evident at 20°C rectal temperature in 7 of 9 dogs. In one it occurred between 25° and 20° and in one failure was progressive during the entire period of immersion. In 5 of 8 dogs surviving to below 20°C the venous O_2 content at 20° was equal to or greater than pre-immersion levels. Hematocrits showed a 20% increase during hypothermia which is partially accounted for by pentothal depression of the control value. When breath-

ing 100% O_2 throughout the hypothermic period 4 of 9 dogs showed some respiratory failure above 25°C . In nearly every case respiration was depressed throughout, as reflected in a high arterial CO_2 content. Circulatory impairment was evident in only 1 dog at 25°C . The venous O_2 content was higher at 25°C in 7 of the 9 dogs than it was before immersion.

Cooling as a stimulus to certain smooth muscles

JOHN F. PERKINS, JR. and CHARLES H. NICHOLAS (introduced by R. W. GERARD) *Dept. of Physiology, Univ. of Chicago, Chicago, Ill.* Cooling alone causes contraction of the retractor penis muscle of the dog (De Zilwa, *J. Physiol.* 27: 200, 1901), of the cat's nictitating membrane (Perkins and Li, *Fed. Proc.* 7: 1948) and apparently also of the smooth muscle of the arteriovenous anastomoses of the skin (Perkins et al., *Am. J. Physiol.* in press). Further studies were made on the dog's retractor penis, which was placed in Tyrode's solution in a jacketed bath. Temperature was measured by a thermistor in contact with the muscle. Provision was made to cool the muscle gradually, or rapidly, from 36°C down to as low as 0°C . Cooling alone acts as a stimulus, producing as quick a contraction as that caused by epinephrine. Rapid cooling is a more effective stimulus than gradual. When degrees of rapid cooling (abscissae) were plotted against heights of contraction, an S-shaped curve resulted, with a maximum at 24° of rapid cooling (cooling from 36 to 12°C). Lowering the pH to near 7 causes a gradual, marked contraction, on which the effect of cold can be superimposed. The separate effects of epinephrine and of cooling are additive when tested together. After Dibenamine the muscle lengthens with epinephrine but still shortens with cold. With both together, the effects tend to cancel (algebraic addition). Thus, cold apparently acts on a different excitatory system than epinephrine, or else it acts directly on the contractile system. Against a direct action is the failure of rabbit intestine, uterus, aorta, and iris to contract when cooled.

Effect of estrogen and relaxin on the connective tissue of the pubic symphysis

E. PERL (by invitation) and H. R. CATCHPOLE *Dept. of Pathology, College of Medicine, Univ. of Illinois, Chicago, Ill.* Female guinea pigs castrated for 1 month were treated daily for 4-5 days with $1.0\text{ }\mu\text{g}$ estradiol, then given an injection of pregnant rabbit serum containing relaxin. Following relaxation (6-8 hours) each of one group of animals received intravenously 4.0 ml 1.25% Evans blue (intravital) dye, and was killed 10 minutes later. The symphysis was frozen in liquid air and frozen-dried *in vacuo*. The dried block was embedded in paraffin and sectioned at $120\text{ }\mu$. Uninjected controls and animals receiving estrogen only were similarly studied. In another group, symphyses of control, estrogen treated and

estrogen-relaxin treated animals were frozen-dried, embedded as above, sectioned at 5-10 μ and stained for glycoproteins by the McManus-Hotchkiss periodic acid-leucofuchsin reagent. Connective tissue between the symphyseal cartilages is unstained by Evans blue in control, and virtually unstained in estrogen treated animals, in the anterior and posterior notches bordering the symphysis, estrogen treated animals show slight staining. Following relaxin the connective tissue of the joint proper stains more or less blue in all cases and that of the notches stains very markedly. The glycoprotein material of the symphysis, which stains with periodic acid-leucofuchsin reagent, was found to become more soluble in aqueous solvents following relaxin. The interpretation is made that relaxin induces breakdown (depolymerization) of the glycoproteins of the symphyseal region, which provides the physical basis for relaxation, these components become more water soluble and are able to bind Evans blue.

Mixing time of Brilliant Vital Red injected into dogs. RAYMOND W. PICKERING (by invitation) and PHILIP DOW *Dept of Physiology, Univ of Georgia School of Medicine, Augusta, Ga.* The mixing time of intravascular dyes injected into normal animals has long been controversial. Its prolongation in circulatory collapse has been recognized but not (so far as we have found) quantitatively measured. An opportunity to obtain estimates of both was presented in the course of efforts to follow fluid shifts during serial hemorrhages in dogs (currently reported by Remington). Following a rapid jugular injection of Brilliant Vital Red, samples at frequent intervals were drawn simultaneously from a femoral artery and the corresponding vein. Under control conditions, arterial dye concentrations declined and venous concentrations rose, the two becoming acceptably identical after four minutes. After an estimated 35% hemorrhage (7 steps, 1 hour) the results were highly variable. In one type the concentration curves were like controls in form but greatly prolonged, their slow approximation permitting an estimate of dilution volume only after 20 to 30 minutes. In another type both arterial and venous concentrations continued to rise for 30 minutes, suggesting sequestration elsewhere and slow mixing of a large fraction of the injected dye. When supplementary samples from the non-injected jugular were added to the series they coincided with the arterial curve and would thus have given false assurance of mixing had they been the only ones available for comparison. The results emphasize the hazardous aspect of estimating completeness of mixing from a series of samples from any single vessel. They supplement and extend the previous observations of the Greger-en group and of Phillips.

Potent pressor action of an extract of a phaeo-

chromocytoma after adrenolytic doses of Dibenamine. D. M. PITCAIRN (by invitation) and W. B. YOUNG *Dept of Physiology, Univ of Oregon Medical School, Portland, Ore.* A 13-year-old boy with severe and relatively sustained arterial hypertension was given Dibenamine, and the blood pressure decreased rapidly to the normal range with the patient remaining in the supine position. Following surgical removal of the left adrenal gland, which contained a phaeochromocytoma, his hypertension was relieved. An aqueous extract of the phaeochromocytoma was prepared 2 hours after removal, and effects of the extract upon the blood pressure of dogs under pentobarbital sodium were determined before and after Dibenamine. The extract had a very potent pressor action which was not blocked by full adrenolytic doses of Dibenamine. The pressor substance was present in slightly diminished amount in the refrigerated phaeochromocytoma 24 hours later, and a relatively small amount was detected after 5 days. Extracts of the thin layer of cortex, which could not be cleanly separated from the phaeochromocytoma, had much less pressor potency than extracts from the central portion of the tumor. Aqueous extracts of fresh normal dog and beef adrenal medulla, like epinephrine, had pressor action before Dibenamine and depressor action after Dibenamine. Aqueous extracts of dog and beef adrenal cortex produced either a slight fall in blood pressure or no response following Dibenamine. Persistence of the major part of the pressor action of the extract of the phaeochromocytoma after Dibenamine indicates the presence in the tumor of a considerable amount of a pressor substance other than epinephrine.

Filtration rate and sodium and water excretion following mercurial diuretics. ROBERT F. PITTS and JOHN J. DUGGAN (by invitation) *Dept of Physiology, Syracuse Univ College of Medicine, Syracuse, N. Y.* The rate of elimination of sodium and water by the normal human subject following the intravenous administration of 2 cc of mercurhydrin is determined in part by the glomerular filtration rate. Two subjects hydrated with 500 cc of water per os prior to the start of the experiment showed evidence of mild to severe peripheral circulatory failure from 1.5 to 2.5 hrs following the mercurial. Glomerular filtration rate was depressed during the period of circulatory collapse, as was the rate of excretion of sodium and water. When the subjects were hydrated by the infusion of saline at 10 to 15 cc per min for 2 hr prior to the start and throughout the course of the experiment, glomerular filtration was maintained at a normal or elevated rate for the subsequent 5 hr period of observation. The quantities of salt and water eliminated in the latter experiments far exceeded those of the preceding ones. Salt and water balances and hematocrit changes suggest that diuresis in the

water hydrated subjects rapidly reduced circulating blood volume and led to circulatory collapse with its attendant fall in filtration rate. A greater fluid reserve in the saline hydrated subjects, prevented these changes. It is inferred that a decrease in filtration rate is attended by reduced delivery of proximal tubular fluid into the distal segment, the sodium and water absorptive capacity of which is depressed by mercurial diuretics. Accordingly little sodium and water may be excreted even though distal absorption is severely curtailed.

Macrophage promoting factor (MPF) in mammalian blood serum. C. M. POMERAT and M. F. ORR (by invitation) *Tissue Culture Lab, Medical Branch, Univ. of Texas, Galveston, Texas.* Fragments of spleens from 18-day chick embryos approximately 1 mm square show a circular zone of migrating cells about 5 mm in diameter within 24 hours when cultivated at 37°C in a clot formed of cockrel plasma and chick embryo extract. The peripheral elements of such cultures consist of myelocytes with acidophilic granules. While the inclusion of 25% mammalian serum in the composition of the medium gave typical cultures, it was found that the blood of some rabbits induced the appearance of large numbers of highly phagocytic cells which engulfed all outwandering myelocytes within 24 hours. This macrophage promoting factor (MPF) has been found to be thermolabile, precipitated by 1/3 saturated ammonium sulfate and to be present in an acetone insoluble fraction. It does not appear to be related to the Forssman antigen or to anti-organ sera with high or low complement fixation or hemolytic titres. It was not found possible to reproduce the phenomenon of macrophage induction under the conditions described with the use of various concentrations of histamine, 17-hydroxy-11-dehydrocorticosterone, choline or various quaternary ammonium compounds. MPF does not appear to be identical with leucotoxin, the leucocyte-promoting factor, necrosin, pyrexin or the leucopenic factor which have been found by Menkin in experimental inflammatory exudates.

Estimate of the kinetic and potential energy of the work of the human heart. O. PREG (by invitation), L. N. KATZ, R. ROSENMAN (by invitation) and L. W. SENNETT (by invitation) *Cardiovascular Dept., Medical Research Inst., Michael Reese Hospital, Chicago, Ill.* The recent techniques of angiocardiology and right heart catheterization offer data from which an approximate calculation of the work of the human heart may be determined from the formula $W = \left(PV + \frac{Mv^2}{2g} \right)_L + \left(PV + \frac{Mv^2}{2g} \right)_R$.

The significance of the various symbols are those currently accepted. V and M are calculated from the Fick formula, v is calculated from the stroke output, cross sectional area of the aorta, just above the sinuses of Valsalva, and of the pulmonary ar-

tery, and the ejection time. The last is obtained from the pressure pulse within the main pulmonary artery. Data on a typical case are as follows: aortic diameter, 3.10 cm, pulmonary artery diameter, 3.23 cm, stroke output, 104 cc, ejection period, 0.26 sec, mean aortic pressure (estimated), 135 mm Hg, mean pulmonary arterial pressure, 14 mm Hg. Work in gm cm/sec = $18,250 + 160 + 1900 + 130 = 20,440$. Kinetic energy equals 6.8% of the total work of the right ventricle, and 0.9% of the left ventricle. The kinetic energy equals 1.4% of the total work of both ventricles.

Studies on the accuracy of pressures recorded through a catheter. W. H. PRITCHARD (by invitation), R. W. ECKSTEIN, R. ECKEL (by invitation), C. L. PARSONS (by invitation) and T. E. LOWE (by invitation) *Western Reserve Univ., Dept. of Medicine, Cleveland, Ohio.* The wide use of the catheter for pressure recording demands a critical comparison of its recorded curves with those recorded by more adequate means. For these studies, pressures were recorded through catheters connected to a high frequency Gregg optical manometer (with and without various lengths of intervening lead tube). These were compared to pressures simultaneously recorded through large needles connected by short lengths of lead to a second Gregg optical manometer. Pressures were measured in a diaphragm type of pump, and the right ventricle of the anesthetized open chest dog. With the pump perfect pressure agreement occurred at slow pump speeds. Higher speeds and the addition of lead tube produced artefacts with marked overshooting and unreadable curves. Time lags were small, from 0.008 to 0.015 sec. In the dog the catheter also produced overshooting with secondary oscillations making true pressure estimations impossible. This inaccuracy was accentuated by the addition of 1 or 2 meter lengths of lead tube. The curves were markedly improved when a certain degree of clotting occurred in the catheter tip. Therefore, damping was done by partially closing a stopcock placed between the catheter and manometer. At damping coefficients of about 7 accurate readable curves were obtained. Readable curves were also obtained from the human right ventricle after ideal damping with a stopcock.

Heat loss and heat production of cats at different environmental temperatures. LAWRENCE R. PROUTY (introduced by EUGENE F. DU BOIS) *Dept. of Physiology, Cornell Univ. Medical College, New York City.* Shaved, unanesthetized cats were studied during 77 one-half hour periods in environments ranging from 19° to 39°C. have shown that the thermoregulatory mechanisms of a cat differ greatly from man and that the cat is a poor laboratory subject for the study of human temperature regulation. Simultaneous determinations of respiratory metabolism, heat loss, rectal and average skin

temperatures and thermal conductance were made in the gradient calorimeter previously described (Prouty, Barrett and Hardy *Fed Proc* 7 96, 1948) Relative humidity was kept low enough not to interfere with the efficiency of the evaporative process As environmental temperatures were decreased below thermal neutrality, heat loss became progressively greater than heat production In 20 experiments between 20° and 24°C heat loss was 16% greater Vaporization values of 9 to 15 gm/m²/hr were approximately the same in this range as in the zone of thermal neutrality Man can alter peripheral thermal conductance 5-6 fold but the cat shows a relatively flat conductance curve until it licks itself In the cold, the cat relies upon the protection of its fur and the ability to decrease its surface area or increase its heat production Without fur, rectal temperature drops to 35°C then falls precipitously and death would result if the animal could not find a warmer climate As environmental temperature is increased above thermal neutrality, heat production becomes increasingly greater than heat loss At 38.5°C, it is 75% greater At 39°C, respiration approximates 200/min after 2 hours There is a rapid rise in rectal temperature to 43.9 and skin temperature to 43.5°C The animal does not sweat appreciably but coats accessible parts with saliva Nausea, vomiting and convulsions occur and the animal must be removed quickly

Effect of protein in the diet upon certain aspects of renal function THEODORE N. PULLMAN (by invitation), ALF S. ALVING, and MILTON LANDOWNE *Dept of Medicine, Univ of Chicago, Chicago, Ill* Ten normal young adults were placed on each of 3 diets, containing different amounts of protein, for successive 2-week periods The low, intermediary, and high protein regimens contained respectively 0.3-0.4, 1.0-1.1, and 2.3-3.0 gm of protein/kg of body weight/day Simultaneous inulin and diodrast clearances were determined after 2 weeks on each diet Eight subjects showed higher glomerular filtration rates on the high than on the medium protein diet, the total average increase being 21.9% This difference was highly significant statistically Six subjects showed lower glomerular filtration rates on the low protein than on the control diet, but the average difference could not be proved significant Seven individuals showed a higher renal plasma flow on the high than on the medium protein diet, the total average increase being 20.0% This difference was also statistically of high significance Eight subjects had lower renal plasma flows on the low than on the control diet, but the average difference could not be proved significant No significant change in filtration fraction occurred Preliminary studies on 6 subjects indicate that urea clearances are lower on the low protein diets and that such changes cannot be accounted for solely by diminution in the glomerular filtration rate In all groups,

the tubular reabsorption rate of urea seemed related linearly to the filtration rate of urea, the slope being higher when dietary protein was low than when medium or high This relation is currently under further investigation

Effects of unequal blood flow and ventilation upon the alveolar-arterial gradient HERMANN RAHN *Dept of Physiology and Vital Economics, Univ of Rochester School of Medicine and Dentistry, Rochester, N Y* It has previously been shown that, given a mixed venous blood gas content and inspired air tension, all the possible simultaneous combinations of pO₂ and pCO₂ existing in the lung or parts thereof can be determined If the metabolic R Q is known then in addition the mean alveolar air composition can be defined Any particular alveolar gas tension is determined by a definite ratio of alveolar ventilation (V) to pulmonary blood flow (F) When both of these quantities are measured in the same units then

$$\frac{V}{F} = \frac{864 \times (A-V) \text{ O}_2 \text{ difference} \times R Q}{\text{alv pCO}_2}$$

Thus a mean alveolar gas composition is determined by a definite mean V/F ratio But to calculate the mean arterial pressures one needs to know not only the alveolar pO₂ and CO₂ produced by the mean V/F but also by the variance of V/F This variance is determined by the degree of unequal pulmonary perfusion and alveolar ventilation While the gradient for CO₂ is negligible, the O₂ gradient depends upon the magnitude of the deviations assumed

Relations between blood flow, skin temperature, evaporation rates and sweating in various skin regions WALTER C. RANDALL and ALRICK B. HERTZMAN *Dept of Physiology, St Louis Univ School of Medicine* Simultaneous measurements of cutaneous blood flow, evaporation rates, and surface temperatures following mecholyl iontophoresis suggested certain important correlations between these functions in maintaining thermal flows at the skin surface In these experiments involving high sweating rates, the skin may be partitioned into three zones 1) areas such as the dorsal surfaces of the hand and foot where sweating rates provide for an evaporative heat loss in excess of the heat delivered by the blood, 2) surfaces of the face where sweating rates are low with relatively high blood flows, 3) areas of the trunk where both maximal blood flows and sweating rates are intermediate between these extremes The largest total surface area falls within the latter group Calculations of total possible evaporative heat losses if all the sweat were vaporized from each of these areas suggest a marked initial thermal deficit on the part of the cutaneous blood flow Part of this deficit may be corrected by direct conduction from deeper tissues Such thermal deficits have been observed immediately following stimulation of the sweat glands and blood vessels by mecholyl, but the deficit rapidly de-

creases to a point where appropriate caloric balances may be established

IV An electronic, multi-range, multi-channel, direct-writing pressure recorder MAURICE RAPAPORT and STANLEY J SARNOFF (introduced by WILLIAM H FORBES) *Dept of Physiology, Harvard School of Public Health, Boston, Mass* An apparatus has been developed which combines the characteristics thought to be desirable in a pressure recording apparatus 1) Linearity over all pressure ranges 2) Small fluid shift with change in pressure (0.02 mm ³ for 100 mm Hg) Change of sensitivity without changing the natural frequency of the system thus enabling full-scale deflection for 400 mm Hg, or for 1 mm Hg pressure change (with appropriate intermediate sensitivities) by simple adjustment of the amplification circuit 3) Recording by means of a direct-writing galvanometer 4) Rectilinear rather than curvilinear coordinates 5) Multi-channel recording 6) Portability of the single channel apparatus 7) Multiple chart speeds ranging from 0.25 mm to 50 mm per second with intermediate speeds 8) Sterilizability of all components in contact with the fluid system 9) Additional recording channels for synchronization with the electrocardiogram, pneumotachogram, pneumogram, and phonocardiogram, electrokymogram, etc The pressure pick-up is a condenser microphone which is an element of an alternating current bridge, operating at radio frequencies When zero pressure is applied to the pressure pick-up, the bridge is balanced and the output from it is zero When pressure is applied, the bridge is proportionately unbalanced, and a voltage output therefore occurs across the bridge This voltage is amplified and rectified and fed into a direct writing or photographic galvanometer

Multiple avitaminosis-like syndrome produced by adenine SIGWIN B RASKA *Dept of Pharmacology, Univ of Mississippi School of Medicine, University, Miss* Orally administered adenine in daily doses of 30 to 50 mg/kg given for a period of about 3 to 5 weeks produces the syndrome of multiple avitaminosis in dogs receiving complete normal diets both before and during the experimental period (Raska, *Science* 105 1947, *J Biol Chem* 165 743, 1946) Gross and microscopic examination of the tissues of these animals disclosed the following scattered areas of atrophy of the epithelium of the inner surface of the lip with dense cellular infiltration in the underlying tissue, general atrophy of the buccal mucous membrane with erosion in the depth of the buccal folds and moderate inflammatory leucocytic infiltration in the subepithelial tissues, varying grades of necrosis of the tongue, from moderately severe to marked, characterized by extensive pyknosis and even by obliteration of cross-striations and fibrils as the consequence of coagulation necrosis of the muscle fibers,

extensive necrosis of the gastric mucosa extending as deeply as the muscularis and accompanied by considerable hemorrhage in the necrotic tissue, deposition of brownish crystalline material in the distal convoluted tubules and collecting tubules of the kidney and monocytic infiltration in the interstitial tissue, deposition of calcareous material in some tubules, granulomatous lesions scattered throughout the renal cortex, moderate congestion of the pancreas, considerable congestion of the adrenal medulla and the liver In these animals, blood NPN, blood urea and blood uric acid were markedly elevated Oliguria and abnormal urinary findings (e g, large numbers of WBC, hyaline casts and large amounts of amorphous sediment) were present In the early stages, arterial blood pressure was moderately but definitely increased The motor activity of the animals indicated pathologic changes in the central nervous system Vitamins of the B complex were able to counteract partially the effects of adenine only in the early stages of the syndrome

Age and kidney renin in rabbits G CARL RAU (introduced by M M ELLIS) *Dept of Physiology and Pharmacology, Univ of Missouri Medical School, Columbia, Mo* Grossman and Williams (1938) reported that kidneys of young rats contained more renin than those of older rats, using extracts which were not hypertensinase-free and may have contained other pressor substances, for Helmer and Shipley (1947) suggest that kidney contains another pressor principle distinct from but extractable with renin It seemed desirable to determine whether the amount of renin alone in the kidney varies with age, using extracts prepared by newer methods and known to be free from other pressor substances and hypertensinase With modifications the preparation of renin, hypertensinogen and hypertensin, and the assay procedures were according to Dexter, Haynes and Bridges (1945) Sixty rabbits of three age groups, 20-24, 180 and 365 days old, were used, i e, two groups of immature and one of adult rabbits Each group of 20 was divided into two lots of 10 and the renin extracted from the pooled kidney masses of each lot On the basis of gram of kidney, considering the 365 day group as 1, the renin yield for the 180-day group was 5 and for the 20 to 24-day group, 2 When the proportional kidney weight was used, i e kidney weight divided by body weight, the renin yield per gram of kidney, considering the 365-day group 1, was 5 for both the 180 and the 20 to 24-day group The renin content of kidneys of immature rabbits as found was definitely greater than that of adult rabbits

Effect of position upon the respiratory rate of anesthetized dogs E A REED and J C SCOTT *Dept of Physiology, Hahnemann Medical College, Philadelphia, Penna* Respiration was recorded by

a tambour and endotracheal tube in dogs anesthetized with nembutal. Oxygen was supplied by a second endotracheal catheter. Compared with the lateral, the supine position usually increases the rate while the prone markedly decreases it. The rate is greatly increased in the supine position by tilting head downward 27° . Tilting head upward 27° decreases the rate. The head upward and the prone positions may completely inhibit respiration for several minutes. A return toward the rate of the previous position occurs during the first few minutes. When the change of position is one producing acceleration, the increased rate during the first minute may be due partly to extraneous stimulation from handling the animal. However, when the change of position produces a decreased rate, the same apparent adaptation proceeds in the reverse direction. Bilateral cervical vagotomy greatly reduces or abolishes rate changes due to position. In many cases, the effect of the prone and the head downward positions is reversed. In most instances, there is an acceleration lasting for approximately one minute after change to any position. Rate changes in the intact animal are probably due mainly to the Herring-Breuer reflex. The cranio-ventral slope of the diaphragm would permit compression of lung tissue by gravity shift of abdominal viscera when the animal is in the supine position and distension when in the prone position. Elevation or depression of the head end of the animal also distends or compresses lung tissue by shift of abdominal viscera.

Reduction of the plasma potassium concentration by vivodialysis and its restoration in non-visceral regions. ROGER M. REINECKE and FRANCIS L. STUTZMAN (by invitation). *Dept of Physiology, Univ of Minnesota, Minneapolis, Minn.* The arterial plasma potassium level of the dog was substantially reduced by vivodialysis. Concurrently there was a definite arterio-venous increase in the plasma potassium concentration of blood flowing through the hind limb. This arterio-venous increase was also found for blood flowing through a muscle that had been isolated from the body except for its blood and nerve supply.

Hemodynamic changes in response to hemorrhage with and without Dibenamine. JOHN W. REMINGTON. *Dept of Physiology, Univ of Georgia School of Medicine, Augusta, Ga.* Circulatory changes accompanying serial hemorrhages have been followed in 20 control dogs, and in 16 animals given Dibenamine. On the average, the first of the 6 or 7 bleedings (5 cc/kg each, at 10-min intervals), was followed by a 7% fall in mean blood pressure (optical manometer) and a 26% reduction in flow (calculated from pressure pulse contour), indicating 18% increase in resistance. Mean pressure was then held almost constant through the 4th hemorrhage, while flow declined slowly to -39%,

and resistance increased to +30%. Cardio-acceleration started usually after the 2nd hemorrhage, reaching its maximum after the 5th. In the late hemorrhages, when pulse rate was nearly maximal, mean pressure declined rapidly, with a reduction in resistance to pre-hemorrhage levels. The changes in the above factors were parallel in the two groups, which did not differ significantly. During the cardio-acceleration, the fraction of total blood volume held in the heart and lungs (measured by the dye injection technique) was reduced. X-ray heart volume and electrocardiographic changes in the two series are being studied. There was little if any dilution of the plasma (falling drop) until after the 4th hemorrhage, when dilution began at a rapid rate. The causal basis of the time of onset and the degree of dilution are not clear. Central venous pressure declined during the early hemorrhage, but later changes were variable.

Analysis of out-of-doors thermal stress due to radiation with the pan-radiometer. CHARLES H. RICHARDS (by invitation) and JAMES D. HARDY. *New York Hospital and the Dept of Physiology, Cornell Univ Medical College, New York City.* The fact that the solar spectrum and the emission spectrum of the low temperature radiation of the environment do not overlap appreciably makes it possible to measure separately these sources of radiant energy with the pan-radiometer. This instrument now consists of 3 hollow silver spheres, each containing a heating coil and thermocouple. The outer surface of one sphere is highly polished, a second is dull black, and the third is matte white reflecting the sun's radiation. When this instrument is exposed out of doors in sunlight, the black sphere becomes warmest, the polished sphere next and the white sphere least. Heat is put into the white and polished spheres bringing them to the temperature of the black sphere. From a measurement of this heat and the black sphere temperature the following can be calculated: the direct radiant heat of sun, the albedo of the environment, and the equivalent radiant temperature of the environment. These data, combined properly with the air temperature and velocity, will give the operative temperature which is a measure of the thermal stress of the environment.

Viscosity of muscle protoplasm. PETER RIESER (introduced by L. V. HEILBRUNN). *Zoological Lab, Univ of Pennsylvania, Philadelphia, Penna.* Small oil drops were microinjected into muscle fibers from the adductor magnus of the frog, and the rise of the drops through the protoplasm was observed in a horizontal microscope. The protoplasmic viscosity was determined from Stokes' law. An average value of 29 centipoises was obtained. Some higher values were interpreted as representing cases where a slight degree of injury had occurred. Visible clots always caused stoppage of the

movement of the drops Oil drops were able to move only in one direction through the fibers No movement across the fibers was ever observed Moreover, no movement of the drops could be noted in moribund fibers in which longitudinal fibrillar elements had become visible These facts, as well as the ovoid shape of the drops, suggest the existence of some longitudinal structural arrangement within the fibers Microinjection of long stretches of fibers with oil or air always showed that a peripheral region directly within the sarcolemma could never be displaced by these substances This outer region had a thickness of approximately 10μ By microinjecting aqueous solutions it was possible to push the protoplasm ahead through the fibers In every instance where such a flow occurred, there was a peripheral region of approximately the same thickness as noted above, and this peripheral region did not flow The outer region thus appears to be similar to the gel-like cortex of other types of cells

Analysis of ventilation-perfusion relationships in the lungs R L RILEY and A CURNAND *Dept of Medicine, Columbia Univ College of Physicians and Surgeons, New York City* The composition of alveolar air and of the blood leaving the alveolar capillaries in any part of the lung depends upon the ventilation-perfusion ratio, and conversely, analysis of the ventilation-perfusion ratio requires that the composition of the alveolar air be known Since the alveolar air differs in different parts of the lungs, especially in diseased states, calculations are facilitated by the use of an 'ideal' value, defined as the one and only value for the composition of alveolar air which satisfies both the blood R Q and gas R Q equations, assuming perfect equilibrium between the blood and gas leaving each alveolus This is the only value for alveolar air which could exist homogeneously throughout all functioning alveoli and still be compatible with the quantitative aspects of gas exchange which actually exist in a given subject The concept of 'ideal' alveolar air makes possible the calculation of a value for 'dead space' which includes a contribution from alveoli which are well ventilated but poorly perfused (high V_a/P ratio) It also makes possible the calculation of a value for 'venous admixture' which includes a contribution from alveoli which are well perfused but poorly ventilated (low V_a/P ratio) These relationships have been studied quantitatively in normal individuals and in patients with chronic pulmonary disease

Measurements of heart output with the electrokymograph GORDON C RING, M J OPPENHEIMER and MIRIAM BALABAN (by invitation) *Temple Univ Medical School, Philadelphia, Penna* Measurement of the stroke volume of the heart by means of the roentgenkymograph (*Am J Physiol* 126:741, 1939) has never been widely used probably because the heart borders are so poorly defined The

use of x-rays with the electrokymograph (EKY) appears to offer a more satisfactory method because the movements of the heart borders can be amplified and the records very accurately measured To test whether such records are suitable for calculating heart output, we have compared the results using our method with those obtained from ballistocardiograms (*J Clin Invest* 26:1, 1947) simultaneously recorded It was found that the measurement of heart output by means of the E K Y gives results comparable with those obtained by the ballistocardiograph Only 5 of 30 records on normal medical students and nurses showed differences between the two methods of more than 20% Since the cardiac output measured by ballistic methods checks the Fick no closer than this, our procedure may be as good as or better than the ballistocardiograph It also provides other valuable information With the subject lying on a ballistocardiograph table, the head of the electrokymograph is so placed that its fluorescent screen which is 3 cm in diameter is judged to be over the middle of the ventricular x-ray shadow Changes in intensity of the fluorescent light are signaled by a current from a 931 A tube which activates a string galvanometer These records are calibrated by passing a piece of pressed wood into and out of the x-ray beam The formula used in the calculations is $\text{Stroke} = 10.55 (T^2 \text{ diast} - T^2 \text{ syst})$ where T is the dorso-ventral thickness in centimeters

Electrograms of contracting actomyosin threads JANE SANDS ROBB and SAMUEL MALLOV *Dept of Pharmacology, Syracuse Univ College of Medicine, Syracuse, N Y* Electrodes penetrate a drop of solution into which threads of actomyosin are drawn Connections by way of Offner amplifiers allow records to be made either with an ink writer, a research electrocardiograph or a Dumont oscilloscope During control procedures many possible sources of extraneous currents were investigated, eliminated whenever possible, and at least are recognized when unavoidably present Under controlled conditions potentials appearing when ATP is added to salt solution die away quickly leaving a steady base line If ATP is made to disintegrate (heat or acid) potentials last far longer No potential waves are recorded from a quiescent actomyosin thread When a thread is activated potentials do appear which continue until full contraction is reached, after which time the base line is again steady If another thread is drawn into, the same drop contraction and further potential differences occur The intervening steady base line favors a theory associating these potential changes with thread activity The form of the recorded waves varies with the setting of the instrument Multiple channels allow simultaneous recording with varied settings If high frequencies are favored bursts of fine peaks appear If the setting is as for electro-

cardiography, the characteristic record consists of a series of slow monophasic waves each of which seems to precede a local contraction. Amplitude varies with the nearness and orientation of the thread to electrodes but less obviously to thread diameter.

Effect of some xanthine derivatives on gastric secretion C R ROBERTSON (by invitation) and A C ILL *Dept of Clinical Science, Univ of Illinois College of Medicine, Chicago, Ill*. Some xanthine derivatives have been reported to show an anti-histamine effect on smooth muscle in the cat. This led us to investigate their effects on gastric secretion. In dogs with pouches of the entire stomach 0.0125 mg histamine dihydrochloride was given subcutaneously every 10 minutes. When the secretory rate became relatively constant (2 to 3 hours) the drug to be tested was injected intravenously and the histamine injections were continued. Five tenths gm theophylline was found to potentiate the histamine-stimulated acid secretion (6 tests). The difference was statistically significant. When the theophylline was given alone (without histamine), 3 tests, it did not stimulate gastric secretion. Krasnow in our laboratory has shown that theophylline given alone (intragastrically) in man, will stimulate gastric secretion. Xanthine (0.2 gm—six tests) and theobromine (0.5 gm—7 tests) increased the average secretory response to histamine but the differences were not statistically significant. Caffeine has been shown to stimulate gastric acid secretion in cat and man but not in the dog. It also potentiates histamine-induced gastric secretion in cat and man. We have investigated the effect of caffeine on histamine-induced gastric secretion in the dog and found that 0.5 g (8 tests) potentiated the secretion (statistically significant). These results show that certain xanthine derivatives which, when given alone, fail to stimulate gastric secretion in the dog are able to augment the response to histamine.

Effect of epinephrine on the water and electrolyte composition of cardiac muscle WM A B ROBERTSON and PIERCE PEYER (introduced by F J SICHEL) *Division of Exptl Medicine and Dept of Biochemistry Univ of Vermont, College of Medicine, Burlington Vt*. The hearts of anesthetized cats were removed at intervals up to 11 minutes after intravenous injection of 5 to 500 μ g of epinephrine per kilo, and were analyzed to determine whether changes in concentration of water and electrolytes in cellular and extracellular phases had occurred. In all cases an increase in extracellular water as derived from chloride concentration according to the theory of Boyle and Conway (*J Physiol* 100:1 1941) was found. Direct confirmation of this extracellular increase was obtained by measuring changes in sucrose space. Increases in cell water were also found.

EPINEPHRINE	TIME	(H ₂ O) _E	(H ₂ O) _{SUCROSE}	(H ₂ O) _C
μ g/kg	min	m/kg of dry muscle		
0		1340		3130
5	4	1590		3020
5	11	1390		3170
50	2	1860		3410
50	4	1800		3300
50	11	1690		3260
500	6	2040		3810
0 (sucrose)		1100	1010	3170
100 (sucrose)	4	1610	1400	3220

An increased sodium and decreased potassium concentration in the cell water was observed only after injection of 500 μ g of epinephrine per kilo.

Factors correlated with resistance to decompression sickness TRUE W ROBINSON *Yellow Springs, Ohio*. In studies made on the resistance to decompression sickness of 18 men in a series of 12 altitude chamber flights, a variety of physiological data was obtained which has now been analyzed for factors which might correlate with this resistance. This data included pre-selection tests for the human centrifuge, accelerational or G-tolerance measurements, basal metabolism determinations, and studies on physiological responses to rest and different kinds of exercise both on the ground and at altitude. In all of the foregoing procedures, information on heart rate, blood pressure, oxygen consumption, and respiratory rate was obtained, as well as personal data regarding weight, height, surface area, anthropometric type, use of alcohol and tobacco, sleeping habits etc. Studies of these various factors indicate that most of these elements show little or no correlation with decompression sickness tolerance (as determined by average time at altitude for 8 or 12 flights). Low correlation is shown by the resting diastolic pressure and the systolic, diastolic and pulse pressures, pulse rate and respiratory rate obtained one minute after a step up exercise used as pretreatment before a chamber flight. Personal habits and characteristics also do not show a good correlation. There is a fair correlation with the resting pulse rate and a good correlation with resting systolic blood pressure and respiratory rate.

Blood pressure changes produced by local thermal stimulation of the brain S ROBBAND, L TAYLOR (by invitation) and D FRANKSON (by invitation) *Cardiovascular Dept., Medical Research Institute Michael Reese Hospital Chicago, Ill*. The effect of various degrees of thermal stimulation of the brain on the blood pressure response was tested in 248 trials on 59 turtles. After removing the calvarium a silver wire attached to a water reservoir was inserted into the brain. Various regions were explored for temperature sensitivity as indicated by blood pressure changes.

With large temperature differences (15°C) between brain and wire, consistent responses were not seen when the wire was in the olfactory lobe, optic lobe, in the medulla or laterally in the cerebral hemispheres. When the wire was in the midline between the cerebral hemispheres, a consistent increase in blood pressure was seen with warming by means of the wire, and a decrease with cooling. With small temperature differences (5°C) between the wire and the brain, the blood pressure response was slow and less marked.

Direct determination of gas tensions by blood
ALBERT ROOS and H. BLACK (introduced by H. L. WHITE) *Depts of Surgery and Physiology, Washington Univ School of Medicine, St. Louis, Mo.* A study was made of conditions affecting the accuracy of direct *in vitro* determinations of gas tensions in blood. Human blood with heparin and NaF added was equilibrated for 30–60 minutes at 37°C in a large flask containing known gas mixtures. It was then anaerobically transferred to a small tonometer containing mercury and a small gas bubble, which was mechanically agitated at 37°C . The total bubble tension was determined with a special capillary manometer, keeping the bubble volume constant, after which the bubble was analyzed in a Scholander apparatus. Calculations showed that, if complete equilibration were accomplished with the bubble volumes and gas mixtures used, the blood oxygen tension should not change by more than 1–4 mm Hg. However, although the oxygen tensions of blood and bubble approached each other rapidly during the first 10 minutes of equilibration, the exchange slowed down considerably between 10 and 15 minutes in such a way that the bubble tension after 15 minutes still differed from that of the blood by 5–10 mm Hg, when the initial blood-bubble difference was 10–20 mm Hg. Satisfactory results were obtained only when the initial bubble tension was within 5 mm Hg of the blood tension. In bloods with oxygen tensions of 100 mm Hg and above the tension values fell progressively on repeated determinations, which could not be ascribed to the action of mercury amalgam or stopcock lubricant. This fall was less marked at lower tensions.

Effect of immersion hypothermia on cardiac output
FELIX R. ROSENHAIN (by invitation) and K. E. PENROD *Dept of Physiology, Boston Univ School of Medicine, Boston, Mass.* Cardiac output has been determined by the Fick method in dogs immersed in cold water. Observations were made before immersion and at rectal temperatures of 35° , 30° , 25° and, where spontaneous breathing continued, 20°C . In 3 experiments early signs of shock developed as evidenced by a precipitous fall in blood pressure and O_2 consumption. These were eliminated from the aver-

ages. Data from the other 9 experiments appeared to indicate 1) between 35° and 25°C rectal temperature the average cardiac output, pulse and O_2 consumption fell approximately linearly, 2) a peak in the O_2 consumption curve at 35°C , associated with shivering, was reflected in the cardiac output curve, but not in the pulse curve, 3) individual variation in stroke volume was considerable but in most instances there occurred a slight increase with decreasing body temperature.

Histamine as a possible mediator for cutaneous pain
SOL ROY ROSENTHAL *Univ of Illinois College of Medicine, Chicago, Ill.* Thin slices of freshly obtained human skin were studied *in vitro* by a method described by Rosenthal and Mennard. Such stimuli as punching, pricking, electrical and burning yielded a profusate which when reinjected into the skin of the donors elicited pain responses, wheal, and flare directly proportional to the degree of stimulus and corresponded closely to histamine injections. The anti-histamine drug N-dimethyl-amino-2-propyl-1-thiodiphenylamine (RP3277) when administered to humans in 40 to 80 mg single doses resulted after 3 to 4 hours in a diminution of the responses to pain as well as wheal and flare to histamine.

Measurement of sympathetic denervation in man by sweating patterns after sympathectomy.
GRACE M. ROTH and WINCHELL MCK. CRAIG (by invitation) *Section on Clinical Physiology and Section on Neurologic Surgery, Mayo Clinic, Rochester, Minn.* Over a 10-year period, 1937 sweating tests were carried out by the cobalt chloride method on 1022 patients 8 to 14 days after surgical interruption of sympathetic fibers in treatment of hypertension or peripheral vascular disease. The sweating test immediately after such operations on the sympathetic nervous system demonstrated irregular patterns of sweating in a large group of patients. This observation indicates incomplete denervation of the region which the operation was expected to denervate rather than regeneration. This incompleteness of denervation probably can be explained by the unpredictable lack of uniformity of the distribution of the sympathetic nerves. Tests five to six months after operation in some instances demonstrated that the region of anhidrosis was less extensive than it had been immediately after operation. After this interval of six months, however, the region of anhidrosis remained practically the same for sixteen to twenty years.

In-vitro histamine release from blood cells of sensitized rabbits—relationship to blood-coagulation mechanisms
L. W. ROTH, FLOYD C. MCINTIRE (by invitation), and R. K. RICHARDS *Depts of Pharmacology and Biochemistry, Abbott Research Laboratories, North Chicago, Ill.* The *in-vitro* addition of antigen to whole blood of

sensitized rabbits causes histamine to be released from the cells into the plasma (Katz, *Science* 91 221, 1940) It is well known that coagulation of blood also causes the release of histamine from the blood cells In a study of the mechanism of the 'anaphylactic' *in-vitro* histamine release from rabbit blood cells, the possibility of participation by various components of the blood coagulation system must be considered Our investigations indicate that thrombin, prothrombin and at least part of the 'thromboplastin complex' probably are not involved in the *in-vitro* 'anaphylactic' histamine release because 1) the quantity of heparin required to inhibit only partially the histamine release by antigen is many times the amount required to prevent blood coagulation under identical conditions, 2) extreme prolongation of the blood-clotting time of sensitized rabbits by dicumarol treatment does not impair the 'anaphylactic' histamine release, 3) soybean trypsin inhibitor (Kunitz, *J Gen Physiol* 30 291, 1947), which is a potent 'anti-thromboplastin' (MacFarlane, *J Physiol* 106 104, 1947), fails to inhibit the histamine release by antigen However, a similarity between the mechanism of histamine release by antigen and the blood-coagulation mechanism is suggested by the fact that the histamine release by antigen is completely inhibited by 0.06 molar sodium oxalate or 0.06 molar sodium citrate and is markedly inhibited by 0.3 molar magnesium chloride or 0.2 molar calcium chloride The citrate inhibition is only partially reversed by recalcification

An indication that the ovulating hormone release inducing action of progesterone is an indirect one IRVING ROTHCHILD (introduced by D C SMITH) *Bureau Animal Industry, Beltsville, Md* Progesterone induces ovulation in the intact domestic hen, but is completely ineffective in the absence of the pituitary (Rothchild and Fraps, *Endocrinology* 44 1949) In order to determine whether it acted directly on the pituitary, 0.012-0.018 cc of either 10.0 mg/cc or 1.0 mg/cc progesterone in propylene glycol was applied to the surface of the pituitary, exposed as for hypophysectomy, in birds carrying a mature follicle, and either left in the drill hole or removed after 1.0 or 3.0 minutes Out of 10 birds treated with 10.0 mg/cc progesterone (not removed) 6 ovulated, but only 5 out of 17 treated for 3 minutes, and only 3 out of 8 for one minute ovulated No birds ovulated following treatment with the 1.0 mg/cc progesterone for 3 minutes Practically the same results were obtained when the progesterone (10.0 mg/cc) was applied either to the partially drilled, or completely undrilled, bone over the pituitary Two out of 3 birds in which the progesterone was left, and 2 out of 10 treated for 3 minutes ovulated These findings indicated a relation between pas-

sage of progesterone through a systemic pathway and effectiveness in inducing hormone release from the pituitary Additional weight to this possibility was given by the fact that 7 out of 10 birds ovulated following application of 3 drops of the 10.0 mg/cc progesterone to the thigh muscle for only 3 minutes

Some factors influencing the exchange of ions across nerve membranes MORTIMER A ROTHENBERG (introduced by DAVID NACHMANSOHN) *Dept of Neurology, College of Physicians and Surgeons, Columbia Univ, New York City* The ion exchanges across the nerve membrane have been measured by direct determination of K^{42} and Na^{24} penetration into the axoplasm of the giant axon of Squid Previous results indicate that at rest the ionic composition in these nerves is in dynamic and not static equilibrium with that of their outer environments Employing the same technique, several physical and chemical factors influencing this equilibrium have now been studied Changes in temperature showed a small Q_{10} supporting the assumption that at rest there are no important chemical reactions involved other than a Donnan equilibrium Electrical activity markedly increased the rate of Na^{24} penetration Anticholinesterases, in general, gave an overall result similar to that obtained with nerve activity Exposure to diisopropyl fluorophosphate increased the rate of Na^{24} penetration by about 50% and decreased the K^{42} penetration by about 35% The increase in Na^{24} penetration produced by eserine was slightly smaller Unlike the anticholinesterases, cocaine had a very small effect on the membrane permeability Exposure of nerves to high doses of X-rays produced a marked increase in Na^{24} penetration and a marked decrease in K^{42} penetration in normally conducting nerves This observation indicates an early phase of possible damage to the intact nerve cell by irradiation in spite of apparently normal function

Action of thyroxine on protein metabolism J RUPP (by invitation), K E PASCHKERS and A CANTAROW *Jefferson Medical College, Philadelphia, Penna* Thyroid hormone is usually looked upon as stimulating protein metabolism However, certain well-known facts, such as the dwarfed stature of cretins and the resumption of growth following thyroid therapy, indicate that thyroid hormone may exert a protein anabolic effect The following experiments were performed in order to determine under what circumstances thyroid hormone would be a 'protein anabolizer' All experiments were performed on force fed rats receiving a constant food intake Thyrotoxicity resulted in N loss, this was observed as a N balance effect, thereby precluding unspecific negative N balance due to proprotein catabolism Daily injection of 5 to 10 mg of thyroxine (pharmaceutical grade) was followed by N retention

bolic effect) in the thyroidectomized rat, but had no effect in the intact rat. Large doses (50–100 μ g) caused N-loss (catabolic effect) both in intact and in thyroidectomized rats. In hypophysectomized rats neither the anabolic effect of small, physiological amounts, nor the catabolic effect of large doses of thyroxine could be obtained. Pituitary function appears to be necessary for the effect of thyroid hormone on protein metabolism in fed rats. Experiments are under way to investigate further the interaction of thyroid and pituitary in protein metabolism.

Cinéf luorography of the normal human heart, teaching application (motion picture) R. F. RUSHMER (introduced by T. C. RUCH) *Dept. of Physiology and Biophysics, Univ. of Washington, Seattle, Wash.* A training film demonstrating the anatomical relations of the normal cardiac silhouette in the postero-anterior, right anterior oblique, and left anterior oblique positions is presented. Heart models, teleroentgenograms and cinéf luorograms are arranged in sequence to facilitate visualizing the fluoroscopic appearance of the heart.

Kinetics of cholinesterase of erythrocytes in pernicious and other anemias JEAN CAPTAIN SABINE (introduced by CHARLES I. WRIGHT) *Experimental Biology and Medicine Inst., National Insts. of Health, Bethesda, Md.* Cholinesterase activity was determined manometrically over a wide range of concentrations of acetyl choline in 4 cases of pernicious anemia and 5 cases of anemia from hemorrhage. The data were subjected to the analysis previously made of normal human erythrocytes (*J. Pharm. & Exper. Therap.* 93:230, 1948). The theoretical limiting velocity, V , expressed per unit volume of red blood cells (or per cell), was increased by a factor of 1.5 to 2.5 in anemia from hemorrhage and in pernicious anemia in relapse and during treatment. The values of the apparent dissociation constants K_1 and K_2 of the active and inactive enzyme-substrate complexes were normal in anemia from hemorrhage. In pernicious anemia in relapse the experimental value of K_1 was increased about 3-fold and that of K_2 decreased by the same amount. During three weeks or more of treatment the experimental values of these constants gradually shifted to normal. This shift is probably not attributable to a direct action of the therapeutic agent on the enzyme-substrate system. The high value of V was maintained until the red count and hematocrit approached the normal levels and then fell off. Discussion will include the interpretation of the changes found in pernicious anemia, interpretation of previous findings in pernicious anemia in the light of the present investigation, and a correlation which has been found between clinical condition of severely anemic patients in general

and increased enzymic concentration in the red cells.

Analytic study of effects of various patterns of X irradiation on survival GEORGE A. SACHER, JOHN SACKIS (by invitation) and AUSTIN M. BRUES *Biology Division, Argonne National Lab., Chicago, Ill.* Sixteen hundred female CF-1 mice were treated with 250 kv total-body X radiation according to the following dosage patterns: 400 r in a single dosage, or in 10 or 40 daily treatments; 800 r in 10 or 40 treatments; 1200 r in 40 treatments; 5 r daily throughout life; 800 r in 40 treatments superimposed upon 5 r daily throughout life. There were 1400 control mice. Gross mortality rates have been computed as a function of time, in addition to rates associated with certain prominent types of pathologic change. As a general rule, all patterns of treatment increase the incidence of all types of pathology at all ages. The effect of radiation on rates of mortality is best expressed by means of the Gompertzian diagram (logarithm of rate of mortality against time). In this form, all categories of pathology in control animals show a linear increase with age, as is well known in the case of many human diseases. A dosage administered to young adult mice within a short interval (weeks) displaces these lines upward by a constant amount. The incidence of lymphoid tumors is phasic and constitutes an exception to this rule. The effect of daily small exposures throughout life combined with a heavy exposure early in life is discussed in terms of gross mortality excluding lymphoid tumors. To a first approximation, the results are additive on the Gompertzian diagram. The results indicate lines along which a calculus of radiation mortality may be developed for arbitrary time patterns of exposure.

Turnover rates of acid-soluble phosphorus compounds of liver JACOB SACKS and SHEPHERD STIGMAN (by invitation) *Biology Dept., Brookhaven National Lab., Upton, L. I., N. Y.* Methods have been worked out for the separation of various acid-soluble phosphorus compounds of liver and applied to tracer experiments on rats in the post-absorptive state. The time-course of the P^{32} levels of plasma inorganic-P and the organic-P compounds of liver has been studied. The highest P^{32} level in the plasma P was found $\frac{1}{2}$ hour after subcutaneous injection of the tracer phosphate. The level fell rapidly for the next 1.5 hours, and then progressively more slowly. The maximum P^{32} levels in the P compounds of liver were generally found 1.5 hours after injection of the tracer. The labile P of ATP and ADP showed a higher turnover rate than any other organic P compound studied. The time-course of the P^{32} content of the intracellular inorganic P of liver was found to run parallel with that of the labile P of ATP and ADP,

and to show no such relation to that of plasma P^{32} . This indicates that the former compounds serve as the principal means of transferring inorganic phosphate from the extracellular phase to the interior of the liver cell. Data on the relative turnover rates of ATP-ADP and of glucose-1- and glucose 6- phosphates are presented.

Interplay of light and heat in bleaching rhodopsin ROBERT C. C. ST. GEORGE (introduced by GEORGE WALD) *Biological Lab of Harvard Univ., Cambridge, Mass.* The pigment of rod vision, rhodopsin, is destroyed by heat with an apparent activation energy for this process of about 45,000 cal/mol at pH 5-9 (Lythgoe and Quilliam). At lower temperatures it bleaches in white light by a process independent of the temperature (Hecht). In the present experiments an attempt was made to study the transition between these situations by irradiating rhodopsin with red light, quanta of which are in themselves too small to supply the entire energy of activation. It was found that at pH 7 the bleaching of rhodopsin by light has become appreciably temperature-dependent at 650 $m\mu$. As the wave length is increased further the temperature coefficient of bleaching continues to rise. The apparent thermal energies of activation found at these long wave lengths, added to the energy content of the light itself, appears to approximate 45,000 cal/mol. That is, when less than 45,000 cal are supplied by the absorbed light quanta, the deficit in activation energy is made up from the internal energy of the molecules themselves. About 45,000 cal appear to be required for bleaching rhodopsin, whether supplied as light or heat or as a combination of both. Measurements could not be made at wave lengths longer than 715 $m\mu$, due to the very low absorption of rhodopsin in this region. These observations suggest however that at 1050 $m\mu$, where rod vision still persists (Griffin, Hubbard and Wald) and where the energy content of the radiation is only about 27,000 cal/mol of quanta, about 18,000 cal probably come from the internal energy of the rhodopsin molecule.

Phosphate turnover in the mammalian nervous system A. J. SAMUELS (by invitation), L. L. BOYANSKY (by invitation), B. LIBET, R. W. GIRARD, M. BRIST (by invitation) *Dept. of Physiology, Univ. of Chicago, Chicago, Ill.* Radio active phosphorus 300 microcuries as $NaH^{32}PO_4$ was injected intraperitoneally into guinea pigs in a single dose or spread over 2 weeks in daily doses. Animals were killed at intervals from 6 hours to a week over a 5 week span and the brain, spinal cord and sciatic nerves analyzed for acid soluble phospholipid, phosphoprotein and nucleoprotein phosphorus following Schneider and actively measured

P in plasma or tissue at a given time, indicate rates of penetration or turnover. The uptake of phosphate by nervous tissue from plasma is slow, R (for tissue acid-soluble P against plasma) reaching unity (an arbitrary value) in about 22 days for brain, 30 days for nerve and 36 days for spinal cord. Turnover rates were also slow. Bracketed numbers below give average mg % P of tissue fraction indicated R (against tissue acid-soluble P) for phospholipid reaches unity in 32 days in brain (166) and spinal cord (341), 37 days in nerve (254), for phosphoprotein, 30 days in spinal cord (26), 36 days in brain (21), 40 plus days in nerve (26), for nucleoprotein, 16 days in cord (16), 40 plus days in brain (24) and nerve (17). R for phosphoprotein rises faster in proximal segments of nerve than in distal ones. Our evidence favors the view that protein moves continuously from cord to periphery at a rate of about 3 mm/day. A mathematical method has been developed for describing certain simple cases of the uptake and leakage of radioactive materials by tissues.

Hormones and hormone-like substances in the treatment of (experimental) peptic ulcer DAVID J. SANDWEISS and HARRY C. SALTZSTEIN (by invitation) *Harper Hospital and Dept. of Surgery, Wayne Univ. College of Medicine, Detroit, Mich.* This report deals with the results obtained from 37 control (untreated) Mann-Williamson dogs and 12 other series of similarly operated animals treated with various hormones and hormone-like substances, totaling in all 282 animals. All preparations were administered parenterally. In addition, enterogastrone and the anterior pituitary-like hormone (Ant S) were also administered orally in two other series of Mann-Williamson dogs. The average post-operative survival time of the control series was 63 days. All control animals died of ulcer. The longest survival time was 135 days. Eight % of the ulcers showed microscopic evidence of healing (epithelization). Results in the treated series of animals are as follows: 1) The extracts obtained from human urine (pregnant women, normal women and normal men) were of definite benefit. Extracts obtained from the urine of ulcer patients had some, but very much less activity. Pregnancy urine extract administered orally did not prolong the life of the animals but it did stimulate epithelization in a significant number of the ulcers. 2) The gonadotropic hormone from pregnant mare's serum (Gonadotropin) was definitely beneficial. 3) Progesterone (Proluton) showed some beneficial effect in that it definitely stimulated epithelization. 4) Estrogen (Thelin) was of no value. 5) The posterior pituitary extract (Pituitrin) produced little effect, except that of Pituitrin

the enterogastrone concentrate we employed had no beneficial effect

Concerning relation of the pituitary gland to enterogastrone and urogastrone D J SANDWEISS, J KAULBERSZ, T L PATTERSON and H C SALTZSTEIN (by invitation) *Depts of Physiology and Surgery, Wayne Univ College of Med and Harper Hosp, Detroit, Mich* Enterogastrone and urogastrone were prepared from the intestines and urine of normal dogs and hypophysectomized dogs by the usual methods. The procedures were identical for both series of experiments. The effect of these extracts on gastric secretion was studied by the double-histamine tests on fistula and Heidenhain pouch dogs. The extracts were always administered during the first period of the test. Approximately an equal degree of inhibition of gastric secretion was observed with both normal enterogastrone (12 exper, 4 dogs, dose 50 mg) and hypophysectomized enterogastrone (8 exper, 3 dogs, av dose 23 mg). Normal urogastrone (32 exper, 7 dogs, dose 1 to 2 mg) and hypophysectomized urogastrone (16 exper, 5 dogs, dose 3-10 mg) also produced a comparable degree of inhibition in response to histamine as an immediate effect. However, while no after-effect could be detected in the experiments with normal urogastrone a more striking inhibitory effect in comparison with the immediate effect was noted 5 to 9 hr after administration of the hypophysectomized urogastrone. These effects were found to be statistically significant. It appears, therefore, that hypophysectomy does not significantly alter enterogastrone formation. However, the characteristics of urinary extracts are changed by hypophysectomy in a very striking manner as noted above and as described more fully in another presentation (see Patterson *et al*)

The effect of spinal anesthesia on circulating blood volume S J SARNOFF, E S BUCKLEY, JR, J L WHITTENBERGER and J G GIBSON, II (introduced by W H FORBES) *Dept of Physiology, Harvard School of Public Health, and Dept of Medicine, Harvard Medical School, Boston, Mass* In preliminary clinical studies it was demonstrated that spinal anesthesia benefited the patient in pulmonary edema. Subsequent clinical studies confirmed these early observations. It was thought that one of the associated phenomena that accompanies spinal anesthesia might be a reduction in the volume of blood in the pulmonary vascular bed. As a result of plethysmographic and oncometric studies in which the volume of the extremities and the viscera is found to be increased during spinal anesthesia, it is assumed that the volume of blood in the systemic circuit is increased during this type of sympathetic block. It was decided therefore that if, under spinal anesthesia, the total blood volume remained unchanged, the

volume of blood in the pulmonary bed should be diminished. Total circulating plasma volumes were determined by the T-1824 method and red cell volumes were determined by the radioactive red cell technique just before spinal anesthesia and 30 and 60 minutes after it was started. Spinal anesthesia was administered through an indwelling ureteral catheter previously introduced through the atlanto-occipital membrane. Spinal anesthesia adequate to obliterate the tendon reflexes and cause lower intercostal paralysis was maintained by intermittent injections of 2% procaine hydrochloride. The total circulating plasma volume was found to be unchanged in 6 dogs under the influence of spinal anesthesia.

Effects on the circulation of electrophrenic respiration and positive pressure breathing during high spinal anesthesia STANLEY J SARNOFF, JAMES V MALONEY, JR and JAMES L WHITTENBERGER (introduced by WILLIAM H FORBES) *Dept of Physiology, Harvard School of Public Health, Boston, Mass* It was demonstrated that intermittent positive pressure depresses the circulation of the dog when spinal anesthesia is carried high enough to cause respiratory paralysis. Under these conditions, the sympathetic vasoconstrictor outflow is totally blocked and increased intrathoracic pressure, even though it be intermittent, retards the return of blood to the heart. Proportionality was established between the pressure used during insufflation and the depression of the blood pressure. Electrophrenic respiration was accomplished by applying a cyclically undulating potential to one or both phrenic nerves so that the diaphragm was made to contract and relax in a smooth manner. When electrophrenic respiration was applied, the effect was a stimulation of the circulation in the same animals whose blood pressure was depressed by positive pressure breathing. Changing from positive pressure breathing to electrophrenic respiration in the dog under total spinal anesthesia widens the pulse pressure and elevates the mean arterial blood pressure between 25 and 40 mm Hg. Changing from electrophrenic respiration to positive pressure breathing depresses the blood pressure by the same amount. Under the conditions of the experiment, in which there is a total sympathetic block, elevations or depressions of blood pressure are taken to reflect similar qualitative changes in cardiac output.

Effect of pitressin and desoxycorticosterone in low dosage on excretion of water and sodium OTTO W SARTORIUS and KATHLEEN ROBERTS (introduced by R F PITTS) *Dept of Physiology, Syracuse Univ College of Medicine, Syracuse, N Y* In a series of experiments on 3 normal female dogs in which a standard 2-hr urine flow curve was obtained following the oral administration of 40 cc of water/kg, the intravenous injection of 0.8 mU of pitressin/kg produced an antidiuresis of

30-45 minutes duration. The antidiuresis so obtained was substantially modified by intravenous injection of small doses of homogenized desoxycorticosterone in sesame oil and water, administered 15 minutes prior to the pitressin and at the same time as the water load. All experiments were performed under similar fasting conditions with all control urine flows ranging between 0.2 and 0.5 cc/min. Urine collections were obtained by catheter at 15-minute intervals according to standard technique. Sodium excretion, determined in all experiments, and within limits of 5 and 25 μ Eq/min in the control periods, decreased to about one-third during water diuresis returning to the original level 2-3 hrs later. Pitressin in a dosage of 0.8 mU/kg produced a 10-fold increase in sodium excretion with the peak excretion occurring 45 minutes after its administration. By 90 minutes, sodium excretion had fallen below the control water diuresis level. The effect of pitressin on sodium excretion was modified or abolished by 10 to 100 γ /kg of desoxycorticosterone. It is probable that pitressin and desoxycorticosterone are antagonistic in physiological dosage both as to water excretion and sodium excretion.

DFP and reflex activity in salamander larvae
CHARLES H. SAWYER (introduced by J. E. MARKEE) *Dept of Anatomy, Duke Univ., Durham, N. C.* Di-isopropyl fluorophosphate (DFP) which irreversibly inhibits cholinesterase (ChE), also severely impedes reflex activity in salamander larvae (Boell, 1946). The present experiments were devised to determine what degree of correlation existed between the amount of enzyme inhibition and the extent of interference with reflex motility. Fifty larvae of *Amblystoma punctatum* (Harrison's stage 46) were stimulated individually with a hair 60 times at one-second intervals and the number of each animal's reflex responses was recorded. Twenty-five larvae were then immersed in solutions of DFP (5.5×10^{-5} M) for intervals varying from 5 minutes to several hours. At the end of its DFP period each experimental larva was stimulated and scored as above, washed, and assayed for ChE activity. Controls were clutch-mates of the DFP animals and reared under identical conditions but for the anti-cholinesterase; each control had a reflex-response score closely similar to that of its experimental counterpart prior to the latter's immersion in DFP. A high degree of positive correlation was found to exist between the residual reflex capacity (expressed as percentage of the pre-inhibition response score, range, 0-100%) and the residual ChE activity (expressed as percentage of the control's enzyme content, range, 0-99%). The coefficient of correlation, $r = 0.93$. It is concluded that the effect of DFP on reflex motility is attributable entirely to inhibition of ChE and that a

quantitative relationship exists between reflex capacity, during a given developmental stage, and the amount of cholinesterase available to the neuromuscular apparatus.

Quantitative estimation of capillary passage of fluorescein in rabbit skin
ALFRED A. SCHILLER *Dept of Physiology, Univ of Ill., College of Medicine, Chicago, Ill.* The fluorescence of minute concentrations of sodium fluorescein in the skin can be measured photometrically in long wave UV. The time and intensity relations of the appearance and disappearance of the dye from wheals made with vasoactive drugs have been found to be sufficiently graded to establish quantitative response patterns characteristic of the drug action at a given concentration. Serial dilutions of histamine phosphate, epinephrine HCl, and histamine + epinephrine dissolved in 0.9% NaCl solution were used to make the wheals in the abdominal skin of the rabbit. Controls were performed with the solvent alone. Fluorescein was either injected intravenously or incorporated in the wheal solution (0.1 ml intradermally). The results were as follows: the intensity of fluorescence increased and the disappearance rate of the dye decreased with increasing concentration of histamine over a range of 10^{-3} to 10^{-7} . Epinephrine concentrations from 10^{-3} to 10^{-6} prevented fluorescence in the wheals. The diameters of the non-fluorescent wheals were increased with drug concentration. By using various ratios of simultaneously injected mixtures of histamine and epinephrine it was found that histamine 10^{-3} + epinephrine 10^{-5} produced the same response as the saline control. This method may be of use for the study of capillary permeability when correlated with data supplied by other techniques.

Reduced evaporative water loss from the lungs of certain desert mammals
BODIL SCHMIDT-NIELSEN (introduced by LAURENCE IRVING) *Dept of Physiology, Stanford Univ., and Arctic Research Lab., Stanford, Calif.* It was shown that desert rodents of the family Heteromyidae are able to economize with water by excreting a highly concentrated urine. The question arises if they have other mechanisms for saving water. The evaporation from the animal was determined simultaneously with the oxygen uptake. When the water evaporated is calculated on the basis of the simultaneous oxygen consumption it is found that the water evaporation is approximately one half of what it is in other rodents of the same size (white rat and white mouse). The water evaporation from the Heteromyids breathing completely dry air is on the average 0.45 mg water/ml oxygen taken up. The amount of water formed by metabolism of food in the body is approximately 0.65 mg water/ml oxygen consumed. Therefore water is gained in the metabolism, even when dry air is

inspired (See also abstract by Knut Schmidt-Nielsen)

Efficiency of the excretory system in desert mammals KNUT SCHMIDT-NIELSEN (introduced by LAURENCE IRVING) *Dept of Physiology, Stanford Univ, and Arctic Research Lab, Stanford, Calif* The critical factor in the water metabolism of mammals living in deserts is that the expense of water required for urine, saturation of the expiratory air, and heat regulation cannot easily be covered by the limited supply of water available. Reduction of any of these three main sources of water loss will be of advantage to the organism. Desert rodents of the family Heteromyidae live without access to drinking water. We found that their renal excretory system is well adapted to conserve water by a very concentrated urine (1 normal salt and 4 molar urea). To prove that high urine concentrations can be maintained and are not only occasional, we gave sea water (ca. 0.58N) for drinking. In view of the capacity for concentrating urine it might be expected that the animals would tolerate sea water, which other mammals cannot because of its large salt content. It was found that the Heteromyids do tolerate drinking sea water, and are even able to excrete simultaneously excessive amounts of non-electrolytes (urea). (See also abstract by Bodil Schmidt-Nielsen)

'Impedance' variation in the nerve membrane during passage of the natural action potential OTTO H. SCHMITT *Dept of Zoology and Physics, Univ of Minnesota, Minneapolis, Minn* Extension of a previously described method of analysis permits direct and semi-quantitative calculation of the characteristic impedance-source function for the active nerve membrane during the passage of a normal impulse without superposition of any testing current. This total function can be separated into two parts, a passive component which represents the quiescent physical-chemical properties of the membrane, and an active or 'live' component which includes the energy-yielding mechanism of the impulse. Using experimental results quoted in the literature supplemented by data from new experiments, it has been possible to evaluate, with relatively low accuracy, the passive part of the function, and from this to work back to an approximate numerical evaluation of the active part of the function. By measuring all of the critical quantities involved in the analysis for a single type of nerve, or preferably for an individual specimen, it should be possible to improve greatly the quantitative accuracy of the results. Accurate values for the impedance-source function should prove useful in testing thermodynamically adequate theories for the conversion of biochemical energy to bioelectrical energy.

Action potentials of the cerebellar cortex

elicited by stimulation of olivo-cerebellar fibers GORDON M. SCHOEFFLE *Dept of Physiology, School of Medicine, Washington Univ, St Louis, Mo* Cerebellar action potentials are recorded by means of a unipolar lead (microelectrode) at various levels within the cortex of the dog in response to single shock stimulation of olivo-cerebellar fibers. The stimulating needle electrodes are thrust into the brain stem from above in the region of the inferior olivary nucleus. Voltage changes associated with post synaptic responses when recorded between folia or in the molecular layer of each folium tend to be manifest as several successive positive deflections. At regions containing the Purkinje cell layer, granular layer, or central white lamina the successive post synaptic responses exhibit pronounced negative components. In fact the first post synaptic action potential may show no initial positivity. Such a series of results is reproducible from folium to folium as the recording needle electrode is progressively thrust deeper into the cortex at an acute angle with the plane of the brain surface. Measurements of electrode position taken in connection with histological examination of the cortex appear to be precise to within approximately three tenths of a millimeter. The phase reversals observed are consistent with considerations of volume conductor theory which include the concept of neurones as polarized cylindrical structures which, however, do not undergo depolarization in terminal regions at any time during activity. No phase of any particular response persists for more than a few milliseconds and no underlying slow potential waves are apparent.

Rapid spontaneous variations in blood pressure HENRY A. SCHROEDER *Dept of Internal Medicine, Washington Univ School of Medicine and Barnes Hospital, St Louis, Mo* When blood pressure in the brachial artery of man is measured by Hamilton's optical manometer, spontaneous variations occur from beat to beat which may be of relatively considerable magnitude. Fifty records of blood pressure of normotensive and hypertensive subjects and those with coarctation of the aorta were examined for these variations. In half the difference in systolic pressure from one beat to an adjacent one was 10 mm Hg or more. The average systolic difference was 10.3 mm Hg, the average diastolic 7.0. Various patterns were seen, in some cases respiratory variations were marked, in others two or three beats with high pressure were followed by two or three with lower. In several cases similar variations were found in records made of pressure in the femoral artery. Hypertensive patients on the whole showed greater variations in beat to beat pressure than did normotensive ones, and there was usually little respiratory variation. These changes could also be detected by

the auscultatory method when the pressure cuff was inflated exactly to systolic pressure

Absence of antifibrillatory effect in quaternary nitrogen compounds JOHN E. SCHULTS (by invitation) and JOSEPH R. DiPALMA *Depts of Physiology and Medicine, Long Island College of Medicine, Brooklyn, N. Y.* The activity of a compound as an antifibrillatory drug was determined by means of a thyatron stimulator set at 600 impulses/min and connected through a rheostat to the right auricle of an anesthetized open chest cat. The minimal amount of current, in milliamperes, necessary to induce fibrillation was determined before and after the administration of a drug. The degree of difference was the index to the activity of the compound. The drug to be tested was administered in a dose of 5 mg/kg i.v. and the fibrillation threshold determined immediately and at 10- and 20-minute intervals. Dawes (*British J. Pharm. & Chemotherapy* 1:90, 1948) noted in testing various quinoline derivatives for antifibrillatory activity that, when their tertiary nitrogen was changed to a quaternary one, these compounds lost their potency. A recently synthesized compound, N-methyl-N-(3,4-dimethoxybenzyl)-(4-methoxyphenyl)-ethylamine hydrochloride, is a potent antifibrillatory agent. When the tertiary nitrogen of this compound is converted to a quaternary one by the addition of a methyl and halogen groups the activity is lost and in some respects the compound now resembles muscarinic drugs. Other quaternary nitrogen compounds such as tetraethyl ammonium chloride, dibenamine and mecholyl, some of which are related to antifibrillatory drugs, were tested by this method and found to have no antifibrillatory activity and some even lowered the fibrillation threshold.

A method for determination of the volume of distribution of metabolized substances IRVING L. SCHWARTZ, MURRAY RABINOWITZ and DAVID SCHACHTER (introduced by HOMER W. SMITH) *Dept. of Physiology, New York Univ. College of Medicine, New York City.* Following an intravenous injection of mannitol, Newman, Bordley and Winternitz (*Bull. Johns Hopkins Hosp.* 75:253, 1944) utilized the relationship between 1) renal clearance, 2) volume of distribution, and 3) decrement in plasma concentration with time to determine 1) and 2). It is now known (*J. Lab. & Clin. Med.* 32:192, 1947; *Proc. Soc. Exptl. Biol. & Med.* 66:62, 1947) that mannitol is metabolized. In order to measure the total clearance (renal and metabolic), the rate of infusion (IV) required to maintain a constant plasma level was determined, thereafter the infusion was discontinued and the decrement in plasma concentration with time was observed. Under conditions of constant total clearance, O , the volume of dis-

tribution, V_m , was calculated as
$$V_m = \frac{O(t' - t)}{\log \frac{P}{P'}}$$

where P and P' are the plasma concentrations at times t and t' respectively. It is assumed that P and P' represent the concentration throughout the entire volume of distribution. In 3 experiments on 2 dogs, the volume of distribution of mannitol determined simultaneously with that of inulin (*Proc. Soc. Exptl. Biol. & Med.* 68:507, 1948) was as follows:

Mannitol Space		Inulin Space	
cc	cc/kg	cc	cc/kg
4,750	206	4,850	210
4,775	201	4,900	208
3,230	199	3,310	203

Transcapillary exchange of inulin in man IRVING L. SCHWARTZ, DAVID SCHACHTER and NORBERT FREINKEL (introduced by HOMER W. SMITH) *Dept. of Physiology, New York Univ. College of Medicine, New York City.* The movement of inulin between plasma and interstitial fluid was studied in each of 3 normal men utilizing T_{1824} space as a measure of plasma volume, and inulin space (*Proc. Soc. Exptl. Biol. & Med.* 68:507, 1948) as a measure of extracellular fluid. Following a single intravenous injection of inulin serial samples of venous blood and urine were collected for 24 hours. The amount of inulin (Z) in the interstitial space at any time (t) following administration may be calculated from the injection dose (D), total cumulative urinary excretion at t , (UV), plasma water inulin concentration (P_{wIn}) and plasma water volume (P_{wv}) by the expression $Z = D - UV - P_{wIn}P_{wv}$. The volume of interstitial fluid (I_v) is $ECF - P_{wv}$, hence Z/I_v represents the virtual concentration of inulin in interstitial fluid if it were mixed evenly throughout that compartment. The simultaneous plasma and interstitial fluid concentrations plotted against time demonstrate: a) early rapid movement of inulin from plasma to interstitial fluid, b) maximal concentration of inulin in interstitial fluid in 15 to 20 minutes at a value below the plasma concentration, c) identity of plasma water concentration (P_{wIn}) and mean interstitial fluid concentration (Z/I_v) 40 to 50 minutes after injection, d) Z/I_v remains higher than P_{wIn} thereafter because of the gradient between interstitial fluid and plasma. Two important implications of these studies are: 1) a single injection of inulin should not be used to determine the volume of distribution since true equilibrium never occurs and mean virtual equilibration of interstitial fluid with plasma water occurs at only one instant in time, 2) reversal of flow of inulin between plasma and the most avail-

able phases of the interstitial fluid occurs before the point of mean equilibration

Potential of the prolactin effect ERWIN SCHWENK (by invitation), SIBYLLE TOLKSDORF (by invitation), ROY McCULLAGH (by invitation) and RICHARD TISLOW *Biological Research Lab and the Chemical Research Div of Schering Corporation, Bloomfield, N J* An increase in the effect of anterior pituitary lactogenic hormone preparations (Prolactin) has been observed when Sørensen's phosphate buffer (pH 7.4) was added to the aqueous solution of the hormone. The assay used was the crop sac weight response in white Carneau pigeons according to the method of Riddle and Bates. The effect was observed both by subcutaneous and intramuscular routes of administration. When averaging repeated assays of the International Standard and Schering Substandard of the lactogenic hormone, the increase in response to the two standards in the presence of buffer was approximately 2-fold. The effect was noted with Prolactin fractions of various purity and potency ranging from starting material of crude pituitary extracts with a potency of 0.4 IU/mg to highly purified fractions containing 35 IU/mg. It was observed with phosphate buffer of 0.1, 0.2, 0.4 and 0.6 molarity. The effect was only noticed when the hormone was injected in the buffered solution, and not upon separate injection of buffer and hormone solutions. The effect was not observed when phosphate-buffered Prolactin was dialysed free of buffer, precipitated and redissolved in water. The increased response appeared again upon addition of buffer to the solution. Other buffers, such as acetate and arginine, produced a similar effect.

Response of individual pacinian corpuscles to external stimulation DONALD SCOTT, JR (introduced by FRANK BRINK, JR) *Johnson Foundation, Univ of Pennsylvania, Philadelphia, Penna* Single Pacinian corpuscles can be dissected from the mesentery of the cat with the single sensory nerve fibre and circulation uninterrupted. The electrical activity can then be studied by lifting the corpuscle on to a fine cotton wick electrode connected to recording equipment. The corpuscle will respond to steady pressure by a glass rod with a series of impulses, the average frequency of which can be altered by increasing or decreasing the pressure. The threshold value for this form of stimulation lies below 5 mg. The corpuscle is much more sensitive to vibratory stimulation as applied by a fine vibrating glass rod or a pure tone. Treatment of the corpuscle with citrate will increase the excitability to the point of spontaneous activity. Analysis of the length of interval between adjacent impulses after the scheme of Brink, Bronk and Larrabee (*Ann New York Acad Sci* 47: 457, 1946) shows that the corpuscle responds in the

manner found by these authors where a fundamental unit interval was shown to separate many impulses while others were separated by simple multiples thereof. The length of the fundamental interval of the corpuscle is 4-5 msec. Careful examination of photographic records where two or more fundamental intervals separate adjacent impulses shows small oscillations of the base line between impulses which have the same period as the fundamental interval of the conducted impulses. This suggests the existence of a continuous oscillation of the excitability of the corpuscle.

Anaphylactic release of histamine and heparin from the isolated dog liver A. E. SCROGGIE (by invitation) and L. B. JAKES *Dept of Physiology, Univ of Saskatchewan, Saskatoon, Sask, Canada* The anaphylactic reaction of the livers of dogs sensitized to horse serum was studied by perfusing the organ with the antigen both *in situ* and isolated. The assay of perfusates for histamine and heparin, before and after perfusion with antigen, demonstrated: 1) When the liver was perfused *in situ* the release of large amounts of these substances occurred in 2 of 6 animals. 2) When the livers were isolated, perfusion with antigen in 'silicone' blood caused a smaller but significant release of histamine and heparin in 6 of 9 animals. 3) When isolated livers were perfused with antigen in Tyrode's solution no release of histamine or heparin occurred. Subsequent perfusion with blood and the addition of antigen to this blood also caused no release of the anaphylotoxins. 4) When antigen was perfused in Tyrode's solution through the isolated livers of 2 dogs which had received two sensitizing doses of antigen, as compared with the single sensitizing dose given to the dogs of 1), 2) and 3), a release of histamine and heparin was obtained, of the order of that in 2). From these results, it is concluded: 1) it is possible to demonstrate the Schultz-Dale phenomenon with canine tissue, by using the isolated liver and measuring the release of histamine and heparin, 2) this reaction is assisted by using 'silicone' blood in place of Tyrode's in the perfusion. This is probably due to blood maintaining normal function of liver cells, rather than entering into the anaphylactic mechanism resulting in the liberation of the anaphylotoxins.

Metabolism in *Colpidium campylum* GERALD R. SEAMAN (introduced by CHARLES G. WILBER) *Biological Laboratory, Fordham Univ, New York City* *Colpidium campylum* rapidly utilizes acids of the tricarboxylic acid cycle. The addition of pyruvate results in an increased oxygen consumption of 120%, α -ketoglutarate of 102%, fumarate of 90%, malate of 97%, oxaloacetate an increase of 85%. Added citrate causes no increase in oxygen consumption. Malonate inhibits the oxidation of pyruvate in *Colpidium*. Fumarate, succinate, and

α -ketoglutarate release the inhibition Citrate, however, does not release the malonate inhibition Malonate inhibition in the presence of citrate is released by fumarate to approximately the same extent as when citrate is absent Fumarate and α -ketoglutarate are recovered when colpidia are incubated with pyruvate The addition of fumarate to pyruvate increases the recovery of α ketoglutarate by 140% Pyruvate, fumarate, and lactate are recovered when oxaloacetate is the sole substrate As a result of fumarate release of malonate inhibition, there is an added utilization of pyruvate and an added recovery of succinate The amounts of cytochrome C, DPN, and glutathione in *Colpidium* are (in γ /mg dry weight of cells) respectively 0.17, 0.82, 1.08 The ATPase activity is 4.7 γ inorganic P liberated/15 min/mg dry weight of cells Transamination reactions in *Colpidium* will be reported

Effect of 933F on the action of dibenamine

JOHN C SEED AND ELIZABETH A MCKAY (introduced by J H WILLS) *Pharmacology Section, Medical Division, Army Chemical Center, Md* Inasmuch as 933F (piperidinomethyl-3-benzodioxane) and dibenamine (dibenzyl- β -chlorethylamine) both block the pressor action of epinephrine, it was felt that the two drugs might act by way of the same mechanism If this were true, one should be able to prevent the action of dibenamine with 933F Five hours after the administration of 5 mg/kg of 933F to the nembutalized dog, 5 μ gm/kg of epinephrine causes a marked pressor response Five hours after the administration of 10 mg/kg of dibenamine, 5 μ gm/kg of epinephrine causes a marked depressor response Five hours after the administration of 5 mg/kg of 933F followed in 3 min by 10 mg/kg of dibenamine, 5 gm/kg of epinephrine causes a marked pressor response which is of the same magnitude as if dibenamine had not been given at all

Electrolyte metabolism following massive glucose, mannitol, urea infusions in normal subjects during hydration and dehydration DONALD W SELDIN AND ROBERT TARAIL (introduced by DONALD H BARRON) *Dept of Internal Medicine, Yale Univ School of Medicine, New Haven, Conn* Following glucose or mannitol infusion, serum sodium concentration fell sharply, indicating movement of water out of cells and perhaps transfer of sodium into cells Increased Na excretion could not account for the fall, since the concentration of Na in urine was well below that in serum The fall in serum Na concentration was not sufficient to prevent some rise in osmotic pressure of serum When these changes occurred, the excretion rate of Na increased 2- to 6-fold In contrast to glucose and mannitol, urea, although excreted in comparable amounts, neither increased Na excretion nor significantly altered serum Na concen-

tration Increased Na excretion following glucose and mannitol infusions may be due to expansion of extracellular volume rather than to an osmotic effect in the renal tubules alone, since comparable urea infusions neither expand the extracellular volume nor augment Na excretion The metabolism of K, though closely linked to cellular metabolism, is also affected by osmotic distortions of internal environment Infusions of mannitol, glucose or urea had some effect on K excretion, producing slight initial increases during dehydration and definite decreases during water diuresis Serum -K concentration was not significantly affected by expansion or contraction of extracellular volume The single striking change in serum K occurred following glucose infusion during water ingestion when serum concentration fell by 25%, associated with transfer of 15 meq of K into cells This occurred when extracellular volume was contracting and glucose utilization was accelerated, and was probably related to glucose metabolism

Graded arterial pressure decrement and renal clearance of PAH, creatinine, and sodium EWALD E SELKURT, PHILIP W HALL (by invitation), and MERRILL P SPENCER (by invitation) *Dept of Physiology, Western Reserve Univ Medical School, Cleveland, Ohio* When arterial pressure is gradually decreased by tourniquet action on the dorsal aorta just above the left renal artery in the dog, the clearance of creatinine decreases at first gradually from about 150 to 90 mm Hg, then more rapidly to cease entirely at about 50 mm Hg The relationship of clearance to pressure is thus curvilinear, concave to the pressure axis The clearance of PAH declines more slowly than that of creatinine, remaining near the control value until about 80 mm Hg, then decreasing abruptly to extinction as glomerular filtration ceases and anuria ensues When clearance of PAH is related to pressure it shows curvilinearity with even greater concavity than that of creatinine The more rapid decline of creatinine clearance signifies a decrease in filtration fraction as pressure is decreased Renal tubular reabsorption of sodium was found to remain essentially complete throughout the range of glomerular filtration rates studied Nevertheless, even though urinary excretion of sodium was small, there was a definite tendency for it to decrease further as glomerular filtration was reduced, and at approximately 50% of the control filtration rates urinary excretion of sodium ceased almost entirely

Histo-physiology of the adrenal cortex HANS SELKE and HELEN STONE (by invitation) *Inst de Médecine et de Chirurgie expérimentales, Univ de Montréal, Montréal, Canada* Experiments on the rat reveal that—in addition to mere stimulation or inhibition of secretion—the adrenal cortex can respond in a highly specific manner to certain

agents Chronic treatment with lyophilized beef-anterior-pituitary tissue (LAP), and to a lesser extent with extracts of various necrotic tissues (e g , Walker rat tumor, rat kidney), cause myeloid metaplasia of adrenal cortical cells, with the formation of both leucocyte and erythrocyte forming elements Testoid compounds (e g , methyl-testosterone) cause metaplasia of adreno-cortical into ordinary fat cells, exhibiting the typical 'signet ring' appearance and containing a single, large fat vacuole Simultaneous treatment with LAP and methyl-testosterone cause hemopoiesis in a fat cell-containing stroma This may result in the complete transformation of fasciculata and reticularis into typical bone-marrow tissue, while the glomerulosa remains intact Even acute and intense overdosage with purified corticotrophin fails to cause such severe loss of adrenal cortical lipid granules as is produced by systemic stress during the alarm reaction Indeed, pretreatment with corticotrophin inhibits the loss of cortical lipid granules which is normally produced by alarming stimuli or folliculoid hormones Hence, in addition to increased corticotrophin secretion other factors must prevail during the alarm reaction to effect the usual intense discharge of cortical lipids Experimental procedures will be discussed which permit the production in the adrenal cortex of specific structural changes having hitherto received little attention The most important among these are the formation of intracellular colloid bodies, cytoplasmic 'chromatin-like' basophilic granules, fibrinoid degeneration of adreno-cortical cells, megakaryocyte-like giant cells and of "pseudolumina" with holocrine secretion of adreno-cortical cells The functional significance of these lesions will be discussed

The pattern of localization in the motor cortex of the rat P H SETTLAGE (by invitation), W G BINGHAM (by invitation), H M SUCKLE (by invitation), A F BORGE (by invitation) and C N WOOLSEY *Depts of Anatomy, Surgery and Physiology, Univ of Wis, Medical School, Madison, Wis* The findings of Woolsey and LeMessurier (*Federation Proc*, 1948), that the pattern of cutaneous representation in somatic area I (post-central homologue) of the rat's cerebral cortex consists of "a somewhat distorted picture of a rat with its various parts related to one another in much the same manner as in the actual animal", have led us to make a detailed study of the pattern of localization in the motor cortex of this rodent The motor area lies medial and rostral to the tactile cortex and corresponds approximately to Krieg's areas 4, 6, 10 and 8 The axial musculature from tail to neck is represented along the medial edge of the motor area in a caudo-rostral sequence The limbs are represented more laterally, with their apices meeting the apices of the limbs of the

tactile pattern The motor area for the head fills the rostral part of the field and extends laterally to the rhinal sulcus The whole motor pattern is essentially a mirror image of the 'postcentral' tactile pattern Together the tactile and motor systems of this animal show a clear and simple plan of organization which may prove fundamental to an understanding of less diagrammatic arrangements in other forms

Polyphenoloxidase activity of blood serum BERNARD SHACTER (introduced by MICHAEL B SHIMKIN) *Lab of Experimental Oncology, Univ of Calif Medical School, San Francisco, Calif and the National Cancer Inst, National Institutes of Health, U S P H S, Bethesda, Md* The phenoloxidase activity of human blood serum was investigated in the Warburg manometer In the presence of serum the oxidation of catechol is initially retarded and then accelerated beyond the rate of auto-oxidation of the substrate Adrenaline is acted upon in a similar fashion The activity is apparently enzymatic since it is completely abolished by cyanide and by heating the serum to 80° for one hour The rate of catechol oxidation increases as the temperature is raised from 25° to 43° and as the pH is increased from 6.6 to 7.8 Under fixed conditions of pH and temperature the enzymatic activity is proportional to the amount of serum used Tyrosine, p-cresol, and resorcinol remain unoxidized when added to serum, while the auto-oxidation of hydroquinone is retarded p-Aminobenzoic acid and sulfanilamide in 0.1 M concentration inhibit the oxidase activity The data indicate the presence in blood serum of a polyphenoloxidase, specific for ortho diphenols

Electrical phenomena in the squid giant axon ABRAHAM M SHANES *Dept of Physiology and Biophysics, Georgetown Univ School of Medicine, Washington, D C and Marine Biological Lab, Woods Hole, Mass* The effects of calcium, potassium, veratrine, yohimbine, procaine, cocaine and DDT, on 3 components of the action potential following single spikes, have been studied oscillographically after localized application to axons from *Loligo pealii* One component, a damped high frequency oscillation present in all fibers and dying away usually in less than 10 milliseconds, is closely related to the very similar subthreshold response The negative after-potential (NAP), present in 50% of the untreated axons, has a maximum amplitude of 100-200 microvolts and disappears exponentially within 200 milliseconds, with veratrinization all fibers exhibit NAP, but this declines in two exponential stages, viz, an initial rapid one terminating in about 100 milliseconds and a slower phase lasting about one second A positive after-potential, evident only after yohimbine, reaches a maximum of less than 50 microvolts within 75-100 milliseconds and lasts about one second Cocaine and

procaine (01–02%) reversibly depress the oscillations and NAP whereas veratrine and lowered potassium enhance them. Calcium acts on the oscillations as described by Arvanitaki, it depresses the NAP when veratrine is absent, but increases it in the latter's presence. Only veratrine altered the time constant of NAP decline. DDT was completely inert. The oscillations grow in veratrine but decline in yohimbine during repetitive stimulation. These observations, and others to be described later for crab and frog nerve, appear explainable largely in terms of potassium transport near the fiber surface as governed by electrochemical factors and a labile permeability and metabolism.

Production of adeno-carcinoma of breast in Wistar rats by the gastric instillation of methylcholanthrene HARRY SHAY, ERNEST A. AEGERTER (by invitation), MARGOT GRUENSTEIN (by invitation) and S. A. KOMAROV *Samuel S. Fels Research Inst. and the Dept. of Pathology, Temple Univ. School of Medicine, Philadelphia, Pa.* Wistar strain rats placed on Zucker's calcium-free diet are known to readily develop ulcerations of the antrum of the stomach (*J. Nutrition* 30: 301, 1945). By instilling methylcholanthrene (melting point 180° corrected) in 0.5 cc olive oil by stomach catheter (Shay and Gruenstein, *J. Lab. and Clin. Med.* 31: 1384, 1946), we hoped to create suitable conditions for induction of carcinoma in the ulcerated stomach. Such experiments were performed on 5–7 weeks' old rats weighing 60–70 gm and methylcholanthrene administered in 2 mg doses (6 days a week) for 6–14 months. No gastro-intestinal tumors resulted but breast adeno-carcinoma developed in 15 out of 28 females. Calcium deficiency was apparently not essential for development of these tumors since they occurred in 4 out of 10 methylcholanthrene treated females on colony diet. The shortest period for development of tumors was 143 days and the longest 418 days. No tumors were observed in 12 similarly treated castrated females and 43 males, 24 of which were castrated. The high incidence of tumors induced distally by methylcholanthrene is significant, since in 15 years with an annual turnover of 2500 animals no breast adeno-carcinoma occurred spontaneously in our colony of Wistar strain rats. Keston (Conference on Ageing, Wistar Institute—June 1946) found no breast adeno-carcinoma in 630 autopsies on Wistar rats (mean age 810 days, S. D. 210 days), 164 of which had other tumors.

Isotopic studies of potassium exchange rates between cellular elements and plasma of human and canine blood *in vitro* C. W. SHEPPARD and W. R. MARTIN (introduced by ALEXANDER HOLLANDER) *Biology Division, Oak Ridge National Lab., Oak Ridge, Tenn.* Fresh heparinized blood is maintained in an apparently viable state for 8

to 10 hr *in vitro*. Hemolysis is generally at or below the lower limit of visual detection. Hematocrits are routinely determined and lie within 2½% of the mean. There is some evidence of occasional systematic increases of potassium concentration in the plasma late in the experiments, the total change being about 15%. In human blood the concentration of isotope (K^{42}) in the plasma decreases fairly rapidly. Equal specific activities of cells and plasma are reached at about 18 hr. The curve is a smooth exponential with a single half value time corresponding to an exchange rate of about 10^{-12} millimol of potassium/hr/cell. In the dog the curve shows an initial rapid exponential decline to an elevated base line which slowly declines at about 1%/hr. Removal of a fraction of the cells containing leukocytes, platelets and probably young erythrocytes eliminates the rapid exponential phase. The slow decline is evidently characteristic of the exchange of potassium in mature canine erythrocytes, the rate being about 4×10^{-16} millimol/hr/cell.

A fluorometric method of determination of adrenalin in blood WM. W. SHINGLETON and H. M. BAKER (introduced by K. S. GRIMSON) *Dept. of Surgery, Duke Univ., Durham, N. C.* Numerous chemical and biological methods planned for determination of adrenalin in blood have yielded variable results and failed to consistently determine small amounts of adrenalin which might be present in the circulating blood normally or during abnormal circumstances. Three reported chemical methods have been tested. The colorimetric method of Shaw, its modification by Raab, and the fluorescent method of Lehman and Michealis yielded results which were not consistent or capable of repetition. A new method has been developed employing the selective adsorption of adrenalin by $Al(OH)_3$ at pH 8.3 and a fluorescent determination of this adsorbed adrenalin. Fluorescence of the material oxidized by 25% NaOH was measured using a Coleman Electronic Photofluorometer equipped with special excitation and transmission filters. This method was tested many times using freshly drawn heparinized human venous blood. Plasma obtained by centrifuging was divided into three equal samples. Known equal amounts of adrenalin chloride were added to two of the samples. One cc of formaldehyde was then added to one of the two adrenalin treated samples. Twenty-four hrs later fluorescence of each sample was determined. The fluorescence of the sample without adrenalin and that with adrenalin plus formaldehyde was approximately equal and low. Fluorescence of the third sample containing adrenalin alone showed a high end point approaching 90% of the fluorescence of a sample of water with the same quantity of adrenalin and similarly treated. Using this method, preliminary experi-

ments have been performed which indicate that relatively small amounts of adrenalin injected into dogs or rabbits are detectable

Hepato-renal factors XXVII Biological fractionation of humoral VEM and VDM in renal hypertension (dogs) EPHRAIM SHORR and BENJAMIN W ZWEIFACH *Dept of Medicine, Cornell Univ Medical College and The New York Hospital, New York City* The initial acute stage of experimental renal hypertension in dogs is associated with the appearance in blood of the renal vaso-excitor, VEM. During the subsequent chronic hypertensive stage, the blood progressively reverts to a neutral reaction, as judged by the rat meso-appendix test, presumably due to the presence of both VEM and the oppositely acting hepatic vaso-depressor, VDM, in physiologically equivalent amounts. More detailed analyses have since been made of the sequence in which these vasotropic principles appear in the development of the hypertensive syndrome, utilizing procedures which permit the separate quantitation of each factor when both are present. Development of antisera to VDM (ferritin) provided a tool for selectively inactivating the VDM components of hypertensive blood, and unmasking VEM. Conversely, the ability of normal kidney slices to inactivate VEM aerobically, without affecting VDM, permitted quantitation of the VDM titer. Untreated blood samples, from dogs with renal hypertension (Goldblatt clamp) gave vasoexcitor (VEM) rat tests in the first few weeks after clamping, during the period of rising blood pressure, and thereafter were neutral. Utilizing these fractionation procedures, VDM was found to appear as early as 7-14 days after clamping, during the period of VEM predominance. VDM titers then rose progressively until they masked humoral VEM in the rat meso-appendix test. Thereafter, there was a shifting balance between VEM and VDM, with transitory predominance of each. In animals with fatal malignant hypertension, the terminal stage generally was associated with a shift in equilibrium, with progressive predominance of VEM.

Effect of hypothermia on cardiodynamics WILLIAM J SHRIBER (introduced by A H HEGNAUER) *Dept of Physiology, Boston Univ School of Medicine, Boston, Mass* Dogs weighing 9-15 kg were anesthetized by intravenous pentothal. The right auricle was catheterized through the right external jugular vein, and the left ventricle was catheterized via the left carotid artery. Intracardiac pressures were recorded with critically damped electrical strain gauge manometers together with simultaneous electrocardiograms. The animals were then cooled in 2-4°C water baths. Serial photographic records were taken until death. Left ventricular systolic pressure remained fairly constant until rectal temperatures of 25-23°C and

then fell rapidly. Right auricular systolic pressure remained relatively constant to similar thermal levels and then rose rapidly. These sudden changes in pressure appeared to be correlated with the cessation of respiration. The fall in the left ventricular pressure probably occurred as a result of an inadequate pulmonary circulation and this in turn resulted in an inability to maintain arterial blood pressure. The sudden increase in right sided pressure was again probably attributable to pulmonary circulatory resistance which led to right heart failure. Conceivably, hypoxia of the cardiac muscle might be a factor in the failure of the heart.

Diet and the activity of the insulin-inactivating system of rat liver BENJAMIN SIMKIN (by invitation), ROBERT H BROH-KAHN and I ARTHUR MIRSKY *The May Institute for Medical Research of the Jewish Hospital, Cincinnati, Ohio* Our previous investigations revealed a marked diminution of the content of the insulin-inactivating system (insulinase) per gram of rat liver during a 72-hr fast and a restoration of the content of this system to normal values following a 48-hr period of re-feeding a balanced diet. The present investigation is concerned with the influence of various types of food-stuffs on the restoration of insulinase activity during such re-feeding after a 3-day fast. Re-feeding a high (85%) carbohydrate diet restored the insulinase content to values above those obtained by re-feeding a balanced diet containing 50% carbohydrate. Re-feeding a high (85%) fat diet, on the other hand, restored the insulinase content to values significantly below those obtained by refeeding with the balanced or the high carbohydrate diet. Inasmuch as the diets were iso-caloric and the rats gained approximately equal weights during the period of their re-feeding, it seems probable that the administration of a high fat diet definitely diminishes the content of the insulin-inactivating system in the liver. In other experiments, rats were force-fed by tube with increasing amounts of high carbohydrate and high fat diets. The various results will be presented and their significance briefly discussed.

A quantitative comparison of Goldberger's augmented unipolar limb leads (aV) with Wilson's original V-leads ERNST SIMONSON and ANCEL KEIS *Laby of Physiological Hygiene, Univ of Minnesota, Minneapolis, Minn* Goldberger (Unipolar Lead Electrocardiography, Philadelphia, 1947) considers his two-terminal (aV) electrode to be identical with Wilson's three-terminal (V) 'unipolar' electrode except for a 50% larger amplitude, so that both electrodes may be used interchangeably. Among electrocardiographers preferences for the aV or the V-electrode vary but no quantitative comparison has been made. In 20 normal men and in 6 patients with abnormal axis deviation, the amplitudes of the QRS-complex and

the T-wave in the three limb leads were taken with Goldberger's two lead and Wilson's three lead terminal. In the arms leads the mean augmentation obtained with Goldberger's method agrees fairly well with the predicted 150% of the Wilson leads, but in lead aV_F the mean is 184 (QRS-complex) or 187% (T-wave). The finding in aV_F was confirmed in 22 other subjects. Moreover, in all leads the variability of augmentation between individuals is so great that prediction of the ratio aV/V for the individual is impossible. The results show that Wilson's three lead terminal and Goldberger's two lead terminal are not interchangeable, and that standards for one method are not applicable to the other method. The results have some bearing on the degree of validity of Einthoven's triangle hypothesis.

Diurnal variations in glomerular activity in normal man and in patients with congestive heart failure. JONAS H SIROTA, DAVID S BALDWIN and HERMAN VILLARREAL (introduced by HOMER W SMITH) *Dept of Physiology and Medicine, New York Univ College of Medicine, New York City*. Renal clearances (4- to 6 hr periods) of insulin, p-aminohippurate and endogenous creatinine were determined throughout 24 hr in 13 normal males and in 5 patients with congestive heart failure with persistent edema. Test substances were administered by a constant delivery pump through a plastic intravenous catheter. Urine was collected by spontaneous voiding in normals and by catheterization in heart failure patients. Blood was drawn at the midpoint of each period. With few exceptions the subjects were at bed rest for at least 24 hr prior to as well as during the period of observation and slept approximately from 8 P.M. to 6 A.M. No change in the dietary regimen was made during the test day. Waking clearances were compared with those during sleep and expressed as night to day ratios (N/D). In the normals the average N/D ratio for C_{IN} was 0.94, for C_{TAH}, 0.97, and for endogenous creatinine clearances, 0.97. In patients with heart failure the average N/D ratio for C_{IN} was 1.18, for C_{TAH}, 1.14, and for endogenous creatinine clearances, 1.16. The endogenous creatinine/insulin clearance ratio averaged 0.92 in the normals, and 0.75 in patients with heart failure. It is concluded that glomerular activity is typically unchanged or decreased during sleep in normal subjects, whereas it typically increases during sleep by a significant amount in heart failure. The endogenous creatinine clearance is not a reliable index of filtration rate in subjects with heart failure.

Augmentation by catechol and p-cresol of the oxidation of proteins by mushroom tyrosinase. IRWIN W SIZER and CLYDE O BRINDLEY (by invitation) *Dept of Biology, Massachusetts Institute of Technology, Cambridge, Mass.* In previous

experiments we have shown that certain proteins can be oxidized by tyrosinase and, in the case of invertase, this oxidation is accompanied by a decrease in invertase activity. Since the oxidation of certain substrates by tyrosinase is accelerated by the addition of an activator (See Nelson, J. M. and Dawson, C. R., *Adv. in Enzymol.* 4: 99, 1944) we have tested such activators on the tyrosinase protein systems. As little as 1×10^{-4} M catechol or p-cresol greatly accelerates and renders more extensive the action of tyrosinase on certain proteins. In the action of tyrosinase on invertase the phenolic activator seems to be coupled to the system like a co-enzyme. The reduced and oxidized form of the phenolic activator constitute a reversible oxidation-reduction system, i.e., catechol \rightleftharpoons o-benzo-quinone (see Nelson and Dawson). A side reaction is the slow formation of quinone polymers from the oxidized forms of the phenolic activators. These intermediary and final oxidation products of catechol had no effect on invertase activity in the absence of tyrosinase. Insulin oxidation by tyrosinase is greatly accelerated by the presence of traces of catechol, as indicated by a decrease in the ultraviolet absorption spectrum and a decrease in biological activity. Similar results are obtained with crystalline pepsin where tyrosinase in the presence of catechol produced a marked change in the ultraviolet absorption spectrum of pepsin and a decrease in peptic activity. Indications have been obtained that tyrosinase in the presence of certain phenolic compounds will oxidize many other proteins containing a high percentage of tyrosyl groups.

Comparison of transmission spectra of blood in the human ear flushed by histamine with those obtained after heat flushing. WILLIAM SLEATOR, JR., JAMES O. ELAM, D. J. KILIAN, W. N. ELAM, JR. (introduced by H. L. WHITE) *Dept of Physiology and Dept of Surgery, Washington Univ School of Medicine, St. Louis, Mo.* Using the monochromator and amplifier arrangement already described (Neville *et al.*, *Federation Proc.* 1949), the percentage transmission of the ear lobe or pinna of a number of normal subjects was measured as a function of wave length from 5000 Å to 11,000 Å. Curves were taken with the subjects breathing air, 100% O₂, and 12% O₂, and with the ears flushed by means of histamine administered electrophoretically, or by means of heat (which has been the principal arterializing agent used in oximetry). For both heat and histamine the percentage transmission when the subject breathed pure oxygen was, on the average for λ less than 7800 Å, about 15 to 20% higher than when the subject breathed 12% O₂, for λ greater than 7800 Å the 100% O₂ curve was 3 to 10% lower than the 12% O₂ curve. With histamine the spectrum when the subject breathed room air followed the 100% O₂ spectrum.

closely through the whole wave length range, rarely differing by as much as 4%. This would be expected if the ear were really arterialized since normal arterial O_2 saturation is known to be above 95% on air and 99–100% on pure O_2 . With heat, however, the room air curves were markedly (10–20%) below the 100% O_2 curves, but followed the 12% O_2 curves very closely (3–6%). The presence of a significant fraction of venous blood (O_2 saturation 75–80%) in the heated ear would account both for the large difference between 100% O_2 and air, and the small difference between air and 12% O_2 .

Peripheral vascular reactions in the wing of the bat after total-body X irradiation DOUGLAS E SMITH, GEORGE SVIHLA (by invitation) and HARVEY M PATT *Biology Division, Argonne National Lab, Chicago, Ill*. A number of peripheral vascular reactions (erythema, hyperemia, increased capillary fragility, hemorrhage) have been described in man and laboratory animals following exposure to X radiation. In the hope of obtaining a definitive picture of the development of these phenomena microscopic observations and cinema microphotographs were made of vascular beds in the wing of the living, intact bat (*Myotis lucifugus*) before and at various intervals after total-body X irradiation. Studies were made on several hundred animals during the late autumn and winter. Until high dosages (10,000–60,000 r) were reached no peripheral vascular changes were observed. Within an hr after such dosages large numbers of leucocytes, many of which stuck to the vessel walls, were apparent. This phenomenon which was most marked during the first 24 hr after irradiation persisted for several days. Early after irradiation the blood seemed to be less viscous than normal only to become more viscous at the end of 24 to 36 hr and remain so until death. Consistent changes in vessel diameter, blood flow and venomotion were not observed, nor were any indications of hemorrhage. Toxicity experiments suggested that the bat might be highly resistant to X irradiation, for in these experiments life span was shortened only after dosages of 15,000–60,000 r. Significantly, only such high dosages produced the peripheral vascular changes noted above.

Observations of the action of the cardiac, pulmonic and aortic valves of the beating heart (motion picture) H L SMITH (by invitation), HIRAM E ESSEX, and E J BALDES *The Mayo Clinic and Mayo Foundation, Rochester, Minn*. Perfusion of the isolated heart of the rabbit or dog makes possible direct observation of the action of the cardiac, aortic and pulmonic valves. Observation of the mitral and tricuspid valves is facilitated by incising and retracting the atrial walls. With proper illumination, the pulmonic valves can be observed by cutting and retracting the walls of

the common pulmonary artery. The observation of the aortic valves requires a special cannula and an apparatus for providing peripheral resistance to the flow from the left ventricle. Certain observations are discussed.

Effect of salt-sugar solution and DCA on lactation THOMAS C SMITH (introduced by F L HISAW) *The Biological Labys, Harvard Univ, Cambridge, Mass*. Four-month-old rats were adrenalectomized and maintained with 1% NaCl, 1% glucose in the drinking water. Three days after adrenalectomy, they were paired with normal males. At parturition, some were continued on salt and glucose, while others were given tap water to drink and injected with DCA in 2 ml sesame oil daily during lactation. The ability to lactate was measured by the growth and survival of the litter, reduced to 4 young, the growth of comparable litters of normal mothers served as controls. Salt and sugar therapy enabled adrenalectomized mothers to maintain normal pregnancies and undergo normal parturition, but in no case was lactation normal. Similarly, lactation was not normal in animals given 0.1 or 0.5 mg DCA daily. One mg DCA resulted in significantly improved lactation, with all litters, though underweight, surviving until weaning. Two mg DCA gave normal lactation. Nearly normal body weight and carbohydrate stores have been maintained in adrenalectomized rats with 1% salt solution or 0.5 mg DCA (Anderson and Herring, *Proc Soc Exp Biol & Med* 43:363, 1940). The present results show that salt and sugar or DCA (0.1, 0.5 mg) will not maintain lactation but larger doses of DCA (1.0, 2.0 mg) are effective.

Rate of elimination of sulfadiazine from the blood in the newborn FRANKLIN F SNYDER *Depts of Obstetrics and Anatomy, Harvard Medical School, Boston, Mass*. Sulfadiazine was found in the blood for a prolonged period in premature, newborn, and early suckling infant rabbits in contrast to adults, following subcutaneous injection of a standard dose, 0.5 gm/kg. Thus, the blood was negative in adults in less than 12 hr, while in premature animals of 30 days the blood contained 6 mg/100 cc 24 hr after injection. At 18 hr, the blood level in full term animals of 32 days was 14 mg, in suckling infants of 1 week it was 5 mg, and at 2 weeks or older the blood was negative. Much higher concentration of sulfadiazine was noted in the blood of the newborn than in that of adults despite uniform dosage. At one hour following injection, the blood level in adults averaged 22 mg while in prematures it was twice as great, averaging 47 mg, in the newborn at term it was 33 mg, in suckling animals at one week it was 33 mg, and at 4 weeks was 27 mg. At 6 hr after injection, the blood level in the newborn averaged 35 mg in contrast to 7 mg in adults. In all samples

obtained from 150 rabbits, determinations were made before and after hydrolysis using Marshall's method. The foregoing results are of particular interest in connection with the clinical observation of increased hazard for the child associated with sulfadiazine therapy at the time of birth or preceding it.

Ratio between tension and total electrical activity in human skeletal muscle. R. SNYDER (by invitation), C. F. S. FISHER (by invitation), and A. C. BURTON. *Dept. of Biophysics, Univ. of Western Ontario, London, Ontario, Canada.* Most electromyography has used needle electrodes and recorded action currents of local single muscle units. General opinion has been that since different units were out of phase, the total integrated electrical activity, recorded from surface electrodes at the end of the muscle, would not correlate well with total tension. This is an error in statistical theory, and we find, using the flexor muscles of the arm and rectifying the output of an electromyograph, that there is a reproducible relation between tension and output. With the upper arm vertical, the elbow supported, and forearm horizontal, increasing loads are hung on the wrist. The output increases with increasing load, with a tendency for the slope of the graph to increase at high loads. Resistance of electrodes is not a disturbing factor. With a constant load, the output remains constant until fatigue sets in, when the electrical activity steadily rises. The ratio of tension to electrical output (T/E) is very different in the flexor arm muscles of different individuals, being much greater where the muscle is larger and presumably 'trained'. The measurement of the ratio offers a way of following the development of muscles by training and after disease, and of studying the process of fatigue. A consistent theory would be that in training and in fatigue, the electrical output of the single units is relatively constant, while the tension exerted is variable.

Pressure responses of the cerebrospinal fluid to diffusion respiration and carbon dioxide inhalation. JOSEPH N. SPENCER (by invitation), ELI S. GOLDENSOHN (by invitation), RICHARD W. WHITEHEAD, ROBERT F. GROVER (by invitation) and WILLIAM B. DRAPER. *Dept. of Physiology and Pharmacology, Univ. of Colorado Medical Center, Denver, Colo.* Simultaneous observations on cerebrospinal fluid pressure, systemic arterial and venous blood pressures, and heart and respiratory rates were recorded continually from dogs by means of strain gauge manometers. Venous blood pH was determined at intervals. Observations were made during diffusion respiration and with various concentrations of CO in O₂ administered by inhalation under light thiopental sodium anesthesia. With diffusion respiration and the various concentrations of carbon dioxide administered, the cerebrospinal

fluid pressure was seen to rise consistently to at least twice the control value, and to an average of 3½ times the control value. The maximum elevation is frequently observed within 10 minutes. During diffusion respiration, cerebrospinal fluid pressure rose slowly to 3½ times the control value after about 15 minutes. The pressure then declined from its peak but remained elevated. During inhalation of 40% CO₂ in O₂, the maximum rise in cerebrospinal fluid pressure was usually reached in 5 minutes and rose to 4½ times the control pressure. Changes in heart rate, respiration, and arterial pulse pressure were reflected in the cerebrospinal fluid pulse waves. However, neither these factors nor variations in arterial blood pressure showed close correlation with the observed rise in cerebrospinal fluid pressure. Studies on the effects of inhalation of 10, 20 and 30% CO₂ in O₂ will be reported.

Spectral deviations found in hemolyzed erythrocytes of rabbits fed a purified diet. S. S. SPICER, J. G. WOOLEY, and ARIEL M. CLARK (introduced by WILLIAM J. BOWEN). *Experimental Biology and Medicine Inst., National Institutes of Health, Bethesda, Md.* Rabbits maintained on a purified diet containing leached alcohol extracted casein, sucrose, Wesson oil, cellophane, minerals and pure vitamins have survived for a period of months to years. Some of these animals have shown an unusually dark color in the suspension of their washed red cells. Below 5800 Å, the absorption spectra of hemolysates from purified diet rabbits appeared normal. However, the 1/20 dilution of washed red cells of these rabbits in saponin buffer revealed abnormally high absorption between about 5900 and 6500 Å. This absorption was evident in the oxygenated hemolysate whether buffer of pH 6.2, 7.9, or 9.0 was used. Subtracting the mean molar (equivalent) extinction coefficient of hemolysates (pH 7.4) from several stock diet rabbits from that of the purified diet animals showed a maximum at 6200 Å in the oxygenated and 6150 Å in the CO treated solution. The optical density of the CO derivative exceeded that of the oxy form by about 40%. In hemolysates of the rabbits fed purified diet, when the hemoglobin was converted to methemoglobin at pH 8.6 or to metcyanhemoglobin there was slightly increased density occurring uniformly without the presence of an absorption band between 5800 and 6500 Å. The band in the oxygenated hemolysate was noted spectroscopically in 6 of 16 animals maintained on purified diet and in none of 15 rabbits on the laboratory stock diet for 1 to 3 years.

Electroshock studies. (a) Convulsant effect of microwaves. (b) Changes of current during stimulation. E. A. SPIEGEL, P. H. WILSON (by invitation), and A. J. LEE (by invitation). *Dept. of Experimental Neurology, Temple Univ. School of Medicine.*

Philadelphia, Pa , and Traverse City State Hospital, Traverse City, Mich a) The convulsant effects of repeated rectangular microwaves were studied on guinea pigs using a generator with pulses of 5 to 40 microseconds at frequencies from 60 to 800 per second and Offner's brief stimulus apparatus with pulses from 50 microseconds upwards at frequencies of 60 and 120 per second. The electrodes were applied bitemporally on the intact skull. Generalized epileptiform convulsions outlasting the stimulating current could be produced at a minimum duration of the individual pulses of 10 microseconds. Quantity-duration curves for the component pulses (abscissa msec, ordinate microcoulombs) obtained with Offner's stimulator (120/sec) showed that the necessary pulse Q's decrease as the total duration of the stimulation is increased from 0.1 second to 1.0 second and also as the duration of each pulse is decreased from 0.5 msec to about 0.05 msec. Temporal summation at this frequency apparently is sufficient to reach the convulsive threshold even when relatively few neurones are stimulated with each pulse, thus decreasing the possibility of supramaximal stimulation of some of the neurones.

(b) Cathoderay oscillograph records of voltage and current during stimulations with 60 cycle A C or halfwave rectified A C revealed a definite (up to 40%), regionally unspecific increase of current within the first 0.1 second. The decrease of resistance appears also on subthreshold stimulation. It is still marked 10 minutes after cessation of stimulation. The resistance to a large extent depends on the voltage applied, it is considerably lower with high than with low voltage.

Hemorrhagic diathesis in leukemia MARYLOO SPOONER (introduced by W J MEEK) *Dept of Physiology, Univ of Wisconsin Medical School, Madison, Wis*. Few studies concerning the cause of the hemorrhagic tendency in leukemia have appeared in the literature. A group of tests including coagulation time, clot retraction, prothrombin time, antithrombin titer, bleeding time, capillary fragility test, vitamin C levels of blood and plasma, and fluorescein test for capillary permeability was run on 33 patients with clinical diagnoses of various types of leukemia. Patients with Hodgkin's disease and lympho sarcoma were used as controls making a total of 50 patients. An attempt was made to correlate the findings with the presence of hemorrhagic signs. Results show that a correlation does not necessarily exist between positive laboratory data and the presence of hemorrhagic signs. In the group of patients with acute leukemia, there was a better correlation between positive laboratory data and clinical evidence of hemorrhage. Increased capillary fragility did not correlate with increased capillary permeability as determined by the fluorescein test. Lower vitamin-C

levels were found in the control group, which rarely showed hemorrhagic signs, than in the leukemic group which had a higher incidence of hemorrhagic signs. The data do not indicate whether or not hemorrhages might occur at some future time. As in other laboratory tests, a series of the same determination or a group of different determinations will yield more information than a single test.

Changes in blood pressure and renal function in hypertensive dogs following abscess production J STAMLER (by invitation), S ROBBARD and L N KATZ *Cardiovascular Dept, Medical Research Inst, Michael Reese Hospital, Chicago, Ill*. The production of an abscess in hypertensive dogs leads to a fall in blood pressure which usually begins within 24-48 hr of the tissue injury and persists for periods up to 3 weeks. This fall in blood pressure was studied in 9 dogs, it occurred when either subcutaneous tissue implantation or intramuscular carbon tetrachloride injection was used to incite abscess. As part of an extensive project investigating the mechanism of this phenomenon, renal clearance studies were done on these dogs prior to and following abscess production. These studies showed that abscess formation is also associated with a significant increase in renal blood flow, which usually begins within 24-48 hr and persists for as long as three weeks. This was more marked and prolonged in those dogs showing a greater, more sustained blood pressure fall. The increase in renal blood flow was not always synchronous with the fall in blood pressure. No significant changes in glomerular filtration rate were noted, the filtration fraction was decreased, renal vascular resistance was significantly lowered. The data suggest that the fall in blood pressure after abscess production may not be causally related to the increased renal blood flow (relief of renal ischemia) observed in these hypertensive dogs.

Further direct experiments on the relation between ballistocardiograms and systolic velocity curves ISAAC STARR, R L MAYOCK (by invitation) and O HORWITZ (by invitation) *Depts of Therapeutic Research and Medicine, Univ of Pennsylvania, Philadelphia, Penna*. We have continued the experiments of the type reported at the last meeting. Using cadavera lying on the ballistocardiograph at necropsy, fluid has been injected into the aorta alone, the pulmonary artery alone, and into both together. The amount of the injection was optically recorded at each instant. Aortic blood pressure was secured by a Hamilton manometer in the later experiments, and a normal diastolic pressure was attained by connecting a pressure bottle with a femoral artery. 'Normal' injections produced normal pressure pulse waves. We have secured good records of about 100 'systoles' in 6 cadavera. The deflection of the ballistocardiogram

is caused by the acceleration of the injected fluid and not by its velocity per se. The contour of the ballistic record follows that of the calculated curve of acceleration, lagging a little behind it. The 'J'-wave follows the contour of the first part of the acceleration curve with a fidelity which has surprised us, the latter part of the acceleration curve is not so accurately reflected in the ballistocardiogram unless the initial impacts are very small. Evidently after-vibrations from large initial impacts distort the latter part of the curve in more 'normal' records. In these experiments the 'I'-wave was smaller than is found during life, probably because the shape of the cannulae used did not reproduce the movement of blood within the heart during systole. For a given acceleration fluid injected into the pulmonary artery causes less initial deflection of the ballistocardiogram than fluid injected into the aorta.

Interrelation of effects of intraarterial injections of histamine and acetylcholine on gastric secretion. GEORGE W STAVRAKY *Dept of Physiology, Faculty of Medicine, Univ of Western Ontario, London, Canada*. As previously reported, acetylcholine injected into a quiescent stomach through the gastrosplenic artery causes an alkaline secretion which is free from pepsin (G M Morton and G W Stavrazy, *Federation Proc* 7: 82, 1948). Similar intraarterial injections of histamine in 1/300,000 dilution (100-200 cc/hr — 17 kg dog) are found to evoke a secretion of highly acid gastric juice of low digestive power. More concentrated solutions of histamine inhibit the secretion of hydrochloric acid. Addition of acetylcholine (1/100,000 concentration) to the solution of histamine lowers the acidity of the gastric juice but markedly activates the secretion of pepsin. Also, if acetylcholine is injected before histamine (causing an alkaline secretion without pepsin) a subsequent injection of histamine evokes a reduced secretion of acid but the gastric juice secreted in response to histamine now contains large quantities of pepsin. When atropine is injected before a combined administration of acetylcholine and histamine, it precludes the secretion of pepsin. However, interposed between the injections of acetylcholine and histamine, atropine does not prevent histamine from causing a secretion of gastric juice of high digestive power despite the fact that before atropinization acetylcholine caused a secretion which contained no pepsin. The results of these experiments seem to warrant the conclusion that a preexisting or combined cholinergic stimulation of the glandular elements of the gastric mucosa is necessary in order that histamine may cause a secretion of pepsin.

Pressure and activity recordings in the pylorus and rectum of man. F R STUEGGEND and W C CLARK (by invitation) *Dept of Physiology, Univ*

of Illinois, Urbana, Ill. By means of a pressure recording pick-up, consisting of a small water capsule at the end of a rubber catheter connected to an electronic amplifier, a continuous record of pressure changes can be made in various locations along the gastro-intestinal tract. In preliminary observations it was noted that there were very obvious differences in pressure and activity when the water capsule was believed to be passing from the stomach through the Pyloric Sphincter region and on into the small intestine. That these changes in activity and pressure recordings are consistent has been verified in experiments on 4 different individuals. Furthermore that this change in activity and pressure is found only in the Pyloric Sphincter region is confirmed by X-ray examinations. The actual pressure in the Pyloric region was 5-10 cm of water higher than on either the stomach or intestinal side of the sphincter area and the activity showed a tonic rhythm of 11 contractions per minute, an observation very different from that recorded in the stomach or intestine. When comparative studies of pressure and activity were made between the rectum and sigmoid colon, differences were also recorded.

Effect of coffee on the growth of white rats. ARTHUR H STEINHAUS and AARON SPITZER (by invitation) *George Williams College Laby for Physiologic Research in Physical Education, Chicago, Ill*. A coffee infusion interfered markedly with the growth of white rats when this was added to a milk diet fortified with iron and copper. Rats fed the fortified milk plus coffee infusion from the time of weaning averaged a gain of 49 gm in body weight in 9 weeks. Litter mates consuming identical quantities of milk plus tap water or milk plus 5% tannic acid in water, both equivalent to volume of coffee infusion, averaged a gain of 87 gm in the same period. When the diets of the 'coffee rats' and 'tannic acid' rats were switched at about 13 weeks of age, the direction of the growth curves was modified as expected, but not to as great an extent. This observation exonerates the tannic acid in coffee as the growth interfering factor. Studies now underway are designed to determine more accurately the 'coffee effect' at different ages and whether the observed growth failure is due 1) to the diversion of food to sustain a greater amount of spontaneous activity or 2) to some interference with its utilization.

Metabolism of pigeons in extreme cold. E STRFICHLER (by invitation), D B HACHEL (by invitation), W LEFISCHMAN and G L BULGER (by invitation) *Physiology Section, Medical Division, Army Chemical Center, Md*. The ability of fasting pigeons to survive up to 75 hr in extreme cold (-35°C) has been demonstrated (Hatch et al *Science* 107: 171, 1948). Further studies have been carried out to elucidate the metabolic factors

involved in this long survival time. The metabolic rates of pigeons weighing between 275 and 625 gm have been studied by means of an apparatus consisting of a desiccator jar of known volume containing a CO_2 absorber. The metabolic rate is estimated simply by determining the change in O_2 content of this chamber. At room temperature (24°C) the range for the basal metabolic rate is from 105–125 C/kg/24 hr. This rises to about 375 C/kg/24 hrs after 4 hr at -40°C , and varies between 300 and 500 C/kg/24 hr until the terminal drop in body temperature. Blood glucose remains within the normal range up to 72 hr, although the liver glycogen is almost depleted by the end of 24 hr.

Effect of cold adaptation on highest and lowest temperatures at which turtle heart continues beating. D. E. STULLKEN (by invitation), F. M. WHITE (by invitation) and W. A. HIESTAND *Laby of Animal Physiology, Purdue Univ., Lafayette, Ind.* Turtles were kept at 4°C as long as 10 weeks to allow 'cold adaptation'. The hearts of these turtles were placed in Ringer solution with 'control' hearts of non-cold adapted turtles. The temperature of the Ringer bath was slowly lowered and the temperatures at which the beating ceased was recorded. After both hearts had stopped beating at the lower of the two temperatures the bath was then heated and the temperature raised slowly until both hearts resumed beating and continued until each stopped at its respective high temperature. The results were as follows. With falling temperature the cold adapted hearts stopped beating at an average temperature of 11.3°C (av. of 8 hearts) while the non-cold adapted hearts stopped at an average temperature of 10.3°C (av. of 8 hearts). With rising temperatures the cold adapted hearts stopped at an average temperature of 38.9°C (av. of same 8 hearts) while the non-cold adapted hearts failed at 36.2°C (av. of same 8 hearts). Evidence shown by the cardiograms indicates that the sinus tissue is apparently affected by cold adaptation more than the myocardium. It is surprising that the cold adapted heart failed at a higher temperature than did the non-cold adapted heart with falling temperatures. Also paradoxically the cold adapted heart continued beating at a higher temperature than did the non-cold adapted heart with rising temperatures.

Centers governing temperature selection and the frequency response to temperature in the brain of trout. CHARLOTTE SULLIVAN (by invitation) and KENNETH C. FISHER *Univ. of Toronto, Toronto, Canada.* Trout in a gradient of temperature normally remain at, that is, select, a comparatively narrow range of the temperatures available. This response to temperature disappears when lesions are made in the forebrain of these organisms

but not when the dorsal portion of the cerebellum is destroyed. The frequency with which spontaneous movements occur in trout changes with temperature in a complex but reproducible manner. The general nature of this response is unaffected by damage to the forebrain but may be completely changed by destroying the dorsal part of the cerebellum.

Effect of posterior pituitary extract on man. F. SUNAHARA (by invitation), D. DUNCANSON (by invitation) and O. G. Edholm *Dept. of Physiology, Univ. of Western Ontario, London, Canada.* Forearm blood flow was measured before and after the intravenous injection of 4 units of histamine-free posterior pituitary extract into healthy male volunteers. Measurement of cutaneous blood volume was made with a Millikan oximeter ear piece. Immediately following the injection, the subjects' faces become ashen in color, the pallor persists for approximately 60 minutes. The oximeter readings on the ear showed a definite diminution in ear blood volume, but these readings returned to normal before normal color returned to the face. The forearm flow shows a slight increase immediately after the injection and thereafter is within normal limits. The subjects complained of nausea and abdominal discomfort and a desire to defecate. In several cases there was slight headache. These symptoms and the appearance of the subject are strikingly similar to those observed after fainting as a result of haemorrhage.

Effects of low environmental atmospheric pressure on the blood of turtles. LOUIS A. SUSCA (introduced by CHARLES G. WILBER) *Biological Laboratory, Fordham University, New York City.* Susca and Wilber (1948) reported changes in the cholesterol content of the blood of turtles of the variety *Pseudemys* sp. after subjecting them to pressures equivalent to various altitudes. Further pressure studies indicate that changes occur also in the glucose and phospholipid content of the

	CHOLESTEROL (PREVIOUS REPORT)	GLUCOSE	PHOSPHO- LIPID
Normal	38.2	51.8	14.4
18,000	108.5	110.2	14.0
33,000	118.5	129.4	21.8
48,000	87.2	219.6	12.6
64,000	70.1	188.2	50.9
90,000	49.2	205.6	9.1

blood of turtles. Estimations for glucose in the blood, using the Somogyi filtrate, were made for each of 10 untreated animals, and also, for 10 turtles subjected individually to pressures equivalent to altitudes of 18,000, 33,000, 48,000, 64,000, and 90,000 ft respectively. Estimations for the phospholipid content of the blood of turtles similarly treated also were made. The accompanying ta-

ble gives the mean value, in mg/100 cc of blood, for each of the substances estimated. Each value represents the mean of 10 readings, each of which is for the blood of a different animal.

Considerable variation occurs at different pressures, but changes within a single group of 10 animals are of the same order of magnitude. Emphasis in the changes is noted at pressures equivalent to altitudes of 33,000 and 48,000 ft respectively.

Effects of various concentrations of potassium cyanide on *Pelomyxa carolinensis* LOUIS A. SUSCA (by invitation) and CHARLES G. WILBER *Biological Lab., Fordham Univ., New York City*. A detailed study of the effects of 0.01M, 0.05M and 0.5M KCN on *Pelomyxa carolinensis* indicates that the cellular structures of the amoeboid organism are affected. Changes in the number, size, and shape of nuclei occur after the organisms are placed in the various solutions for more than 24 hr. In a 0.01M solution, the large number of nuclei characteristic of the organism is not affected, in a 0.05M solution, very few nuclei appear, in a 0.5M solution, a very small number of nuclei is observed. Sizes of the nuclei vary from 12 micra to 14 micra in diameter in organisms placed in 0.5M KCN, to 24 micra to 28 micra in organisms placed in 0.01M KCN. In 0.01M KCN, nuclei are spherical or elongated in shape, in 0.05M KCN, nuclei are ellipsoidal in shape, in 0.5M KCN, nuclei are either spherical or elongated in shape. The number of contractile vacuoles is largest in organisms placed in 0.5M KCN. Many contractile vacuoles are observed in organisms placed in 0.01M KCN, and very few are noted in organisms placed in 0.05M KCN. The distribution and spherical shape of refractive bodies in the organisms are not affected by any of the solutions. Food vacuoles form at frequent intervals in organisms from each solution, but fewest are formed in organisms placed in 0.5M KCN. The bipyramidal and truncated crystals are 3 micra in width in organisms placed in 0.01M KCN, mostly 1.5 micra in width in 0.05M KCN, and 1.5 micra to 3 micra in width in 0.5M KCN.

Free amino acids in the plasma of Eck fistula, meat intoxicated, and hepatectomized dogs MURIEL H. SIEG (introduced by SMITH FREEMAN) *Dept. of Experimental Medicine, Northwestern Medical School, Chicago, Ill.* Sixteen free amino acids were measured in the plasma of fasting dogs by microbiological assays. Alanine and glycine were determined chemically. Normal ranges were established on series of 12 to 36 dogs for each of 13 amino acids. Following Eck fistula formation, 4 of 5 dogs showed an average reduction of 10 μ g. of free leucine/ml. of plasma. This decreased value remained within the previously established normal range. Twelve other amino acids showed no consistent changes. Five of 6 meat intoxicated Eck fistula dogs showed definitely increased plasma histidine

and arginine, 8 of 9 showed increased methionine and tyrosine. Five other meat-intoxicated Eck fistula dogs showed slightly elevated cystine and leucine levels. The remaining 6 amino acids studied varied inconsistently or not at all. Two normal dogs and 7 or 8 portal-obstructed dogs on the same horse meat diet did not show these changes. In a series of 15 dogs, during an 8 to 16 hr. period following hepatectomy, plasma methionine dropped markedly in 12, sometimes disappearing completely. These animals also showed a definite elevation of the histidine level. Tryptophan decreased in all of 8 dogs studied. Three out of 4 animals showed increased alanine and lysine. Determinations of glutamic acid in 5 dogs and glycine in 4 showed definite increases in plasma levels post-operatively in all animals. These changes were distinct from those of the control period during which the animals to be hepatectomized received by vein the amount of glucose to be given post-operatively.

Castration and the mobilization of nitrogen during fasting CLARA M. SZEGO and ABRAHAM WHITE *Dept. of Physiological Chemistry, School of Medicine, Univ. of California at Los Angeles, Los Angeles, Calif.* The mobilization of nitrogen from lymphoid tissue, liver and carcass of castrate male and female mice of the CBA strain was studied during a 48-hr. fast. Castration in both sexes inhibited the decline in the ratio of lymphoid tissue to body weight which normally accompanies the stress of total inanition. Loss of nitrogen from lymphoid tissue was concomitantly depressed. The composition of the liver tissue of fed castrate mice differed significantly from that of intact controls. Higher concentrations of liver lipids and greater liver weights were observed in the gonadectomized animals. Utilization of liver nitrogen during subsequent fasting, however, was unaffected by castration. The mechanism by which castration inhibits the fasting involution of lymphoid tissue is being investigated in relation to adrenocorticotrophic and gonadotrophic activity of the adeno-hypophysis.

Measurement of renal plasma flow in man by the slope method without urine analysis HALL S. TACKET (introduced by C. RILEY HOLT) *Divisions of Medicine and Physiology, Univ. of Tennessee, Memphis, Tenn.* The rate of disappearance from the plasma of a substance, previously administered by a single intravenous injection, represents the amount of that substance removed by the kidneys per unit time, assuming no extra renal loss. That rate, expressed as a fraction (S) of the total substance available for excretion, multiplied by the volume in which the total substance is distributed (V_d), gives the volume of fluid that contains the amount removed by the kidneys per unit time, or the renal clearance (C). Thus $C = V_d S$.

We have determined para-aminohippurate clearance by this method in 12 experiments on 10 patients with no evidence of renal impairment, comparing results with simultaneously measured urine clearances (Before colorimetric analysis, all plasma filtrates and diluted urines were hydrolyzed in 0.4N HCl at 97°F for 3½ hours to free PAH conjugated by the liver) Slope clearance based on the volume of distribution of PAH gave values that were consistently too high However, using empirically the volume of distribution of mannitol, the quotient of the slope clearance and the urine clearance averaged 0.91 with a range from 0.65 to 1.08 In 8 of the 12 experiments, the quotient averaged 1.005 with a range 0.95 to 1.08 The reason for the failure to obtain completely consistent results is not yet evident, but good correlation was observed more frequently than can be explained fortuitously We failed to find satisfactory correlation between the slope clearance and the urine clearance of mannitol The explanation for this discrepancy, likewise, is not yet apparent

Reabsorption of potassium by the kidney
ROBERT TARRILL and DONALD W SELDIN (introduced by DONALD H BARRON) *Dept of Internal Medicine, Yale Univ School of Medicine, New Haven, Conn* From a survey of the literature one may surmise that a ratio of the concentrations of urine potassium to serum potassium below one has not been reported Concentration ratios (U/P) below one would signify formation of renal tubular reabsorbate with a concentration of potassium greater than that of extracellular fluid Such concentration ratios were observed during the following conditions: water diuresis, glucose diuresis during ingestion of water, and during recovery from the shock of 'dolphine' intoxication When urine flows were 948 and 1008 cc/hour during water diuresis, in 2 normal subjects, urine potassium was 2.5 and 2.8 mEq/l per liter, and concentration ratios were both 0.68 During glucose diuresis and water ingestion, urine potassium was 1.3 mEq/l, concentration ratio was 0.38, and urine flow was 1462 cc/hour Such high rates of urine flow are not a necessary prerequisite for a concentration ratio below one A ratio of 0.84 and urine potassium of 3.3 mEq/l when the urine flow was only 135 cc/hour occurred in a patient sustained on fluids without potassium during recovery from peripheral vascular collapse Despite the capacity of the kidney to produce a urine with a concentration ratio below one, this is rarely found during the development of potassium deficiency in man Conditions affecting efficient reabsorption of potassium during low rates of urine formation have not been clearly defined, but probably include the state of hydration and metabolic factors

Repetitive studies of intra-arterial pressures after resection for coarctation of the aorta in man

BOWEN E TAYLOR (by invitation), O THERON CLAGETT (by invitation), HOWARD B BURCHELL and EARL H WOOD *Section on Physiology, Mayo Foundation, and the Division of Surgery, Mayo Clinic, Rochester, Minn* Continuous and simultaneous intraradial and intrafemoral arterial pressures and electrocardiographic data obtained from 17 patients before and after resection for coarctation of the aorta have indicated that, postoperatively, the cardiovascular dynamics were altered toward, but did not attain, normal values Additional studies have been carried out to determine whether or not additional changes occur over longer periods after operation In 10 patients studied preoperatively, radial and femoral arterial pressures averaged 199 systolic and 101 diastolic and 117 systolic and 84 diastolic, respectively During the studies conducted an average of 3 weeks after operation, radial and femoral arterial pressures averaged 158 systolic and 77 diastolic, and 128 systolic and 74 diastolic, respectively When these patients were again studied an average of 7 (3 to 11) months after operation, the radial arterial pressure had decreased on the average from the first postoperative study 16 ± 5 mm Hg systolic and 9 ± 3 mm Hg diastolic, although the average change in femoral systolic pressures was not significant The average femoral diastolic pressure decreased 5.2 ± 2.5 mm Hg Significant improvement in the femoral/radial systolic pressure ratio and the femoral/radial pulse pressure ratio probably indicate that the decrease in cephalic blood pressure was related to a continued improvement in the blood flow through the descending thoracic aorta Evidently, additional changes in the cardiovascular dynamics toward normal may continue over a period of months after resection for coarctation of the aorta

Relationships between the blood pressure increases in exercise, carbon dioxide inhalation and the cold pressor test
HENRY LONGSTREET TAYLOR, AUSTIN HENSCHEL, JOSEF BROZEK and ANCEL KEYS *Laby of Physiological Hygiene, Univ of Minnesota, Minneapolis, Minn* The blood pressure responses to 3 standardized situations which increase blood pressure were studied in 379 normotensive healthy males There were 159 subjects 18 to 26 years old and 220 men 45 to 54 years old The tests were rigidly standardized as to time of events, relation to meals and psychological atmosphere Blood pressures were recorded during a preliminary period with the subject supine The hand was then immersed in ice water for one minute Five minutes later, the subject breathed 6% carbon dioxide for 5 minutes After a 5-minute recovery period, the subject walked on a motor driven treadmill at 3 miles/hour and 5% grade Blood pressures were observed at frequent intervals during each test by the auscultatory method

The greatest increase above the control level in the systolic blood pressure in each test was used as an index of the response. The product moment correlations for the following pairs were all less than 0.2 in both age groups: work systolic blood pressure increment and the cold test increment, work systolic blood pressure increment and the CO₂ test increment and cold test systolic blood pressure increment and the CO₂ test increment. There was no relationship between the resting systolic blood pressure and the systolic blood pressure increment in work or in the CO₂ test but the correlation between the resting systolic blood pressure and the systolic blood pressure increment in the cold test were 0.52 in the younger and 0.44 in the older age group.

Anti-diuretic substance in the urine after fainting. N. B. G. TAYLOR (by invitation) and R. L. NOBLE, *Dept. of Medical Research, Collip Medical Research Lab., Univ. of Western Ontario, London, Canada.* The present study was made on 14 normal young adult human male subjects, 7 of whom fainted and 7 of whom did not faint. They were bled by venesection of amounts from 270 to 1250 cc. There is no correlation between amount of blood loss and fainting. Urine specimens were obtained, by voiding, before and after fainting. These were extracted by the method of Noble *et al.* (1939) and tested for anti-diuretic activity by the rat method of Burn (1937). It has been established that the urine secreted after a faint contains an anti-diuretic substance in readily detectable amounts. No anti-diuretic activity is found in urine from 1) normal subjects, engaged in routine duties about the laboratory (24-hour specimens), 2) experimental subjects before venesection, 3) experimental subjects after venesection, if they did not faint. Urine has also been obtained from 2 subjects who fainted spontaneously, i.e. psychic, not due to venesection, both these cases show anti-diuretic activity in the urine after the faint and not at other times. Evidence will be presented that the anti-diuretic substance found is the secretion of the posterior lobe of the pituitary gland.

Origin of renal vasoconstriction in tourniquet shock. R. D. TAYLOR and IRVINE H. PAGE, *Research Division and Bunts Institute of the Cleveland Clinic Foundation, Cleveland, Ohio.* We have previously reported on the renal vascular effects of application of tourniquets to the hind legs of dogs and concluded that the renal vasoconstriction which occurs is predominantly of humoral origin. The demonstrations of Trueta, Barclay *et al.*, impelled us to re-examine this conclusion. Observations were made in dogs in which afferent stimuli from the hind legs were blocked by section of the spinal cord at L₁. The data thus made available for comparison consist of observations on renal circulation after application of leg tourniquets in

1) normal dogs, 2) in dogs with the kidneys denervated and 3) in dogs with spinal cord section causing deafferentation. In contrast to normal dogs or dogs with denervated kidneys, dogs of the third group (deafferented) do not show significant changes in renal vascular resistance or urine flow either during the time the tourniquets are in place or after their removal. Consequently, we conclude that the primary stimulus to renal vasoconstriction during the onset of tourniquet shock is neurogenic and arises at the site of tourniquet application. The presence of renal vasoconstriction in dogs with denervated kidneys suggests that the ultimate effector mechanism of this vasoconstriction is partly humoral.

Postural reflexes in deafferented limbs of the cat. R. D. TEASDALL (by invitation) and G. W. STAVRAKY, *Dept. of Physiology, Faculty of Medicine, Univ. of Western Ontario, London, Canada.* Sensitization of spinal neurones to chemical stimulating agents and to impulses reaching them via pyramidal tracts was demonstrated by Draks and Stavrakys (*J. Neurophysiol.* 229:11, 1948) and by Teasdale and Stavrakys (*Federation Proc.* 7:123, 1948). The present study of postural reflexes carried out by means of moving picture recordings showed that these reflexes though present in intact cats are not as distinct as in decerebrate animals being modified by voluntary movements and by a tendency of the forelimbs to stay in partial flexion. Within 1-3 weeks after deafferentation of one limb by means of posterior root section, postural reflexes, as noted by Bremer (*Annales de Physiologie* 4:750, 1928), become very active and brisk. The responses are not only of the extensor type but also active flexion of limbs becomes prominent. Furthermore, in the supine position with labial cleft at 45° above the horizontal plane ('minimal attitudinal extensor position' of Magnus, *Lancet* 211:531 & 585, 1926) the deafferented forelimb assumes a flexed position while the intact paw is extended at the radio-carpal joint. Ventroflexion of the head causes extension of the deafferented forelimb but usual flexion of the intact limb. Similarly, placement of the animal in the prone ('minimal') position increases the extensor tone of the deafferented forelimb. It is felt that these are modified positional and attitudinal reflexes brought about by impulses from the labyrinth and neck proprioceptors acting on spinal neurones sensitized by deafferentation. A differential study of these reflexes is in progress.

Metabolism of rat testis in vitro. J. A. T. 111

than it does in the presence of glucose, whereas the mature gland exhibits a markedly diminished Q_{O_2} in the absence of glucose. These data may signify that a metabolic difference exists between the tubular and endocrine portions of the gland, since the adult organ contains a much larger proportion of functioning tubular tissue than does the immature one. To test this hypothesis, 11 adult rats (mean weight 287 gm) were rendered unilaterally cryptorchid. Between 23 and 28 days after transplantation of the right testis, when histological examination showed marked regression of the tubular system, Q_{O_2} and representative dry weight determinations were made on the transplanted glands (mean weight, 155 mg/100 gm of rat). The scrotal glands (mean weight, 495 mg/100 gm of rat) from the same rats were used as controls. The results were as follows:

	Q_{O_2}	
	No substrate	200 mg p c glucose
Transplanted (11 rats, 11 vessels)	6.24 S.E. \pm 0.14	5.36 S.E. \pm 0.20
Control (5 rats, 13 vessels)	3.95 S.E. \pm 0.12	6.78 S.E. \pm 0.12

It is suggested that the tubular and interstitial components of adult testis may be differentiated by the metabolism of the surviving tissue as described herein.

Reorganization of sensory function in amputation stumps two-point discrimination HANS-LUKAS TEUBER (by invitation), HOWARD P. KRIEGER (by invitation) and MORRIS B. BENDER *Psychophysiological Laboratory, Dept. of Neurology, New York Univ. College of Medicine, New York City*. Thresholds of tactile discrimination of two points, simultaneously applied, were obtained in 36 men with unilateral amputations above the knee. All measurements were made with a touch compass applied to the skin of the stump and to homologous areas of the sound limb. Longitudinal thresholds were obtained by placing the compass along the proximo-distal axis of the limb, transverse thresholds by placing the points at right angles to this axis. The method of constant stimuli was used. The threshold was defined as that distance, between the two points of the compass, at which 50% of the responses were correct. Each threshold was determined by 140 trials. The thresholds for the stump were consistently below those obtained for homologous areas of the sound limb in 34 of the 36 subjects. The average transverse thresholds of the stump for all subjects were 24 mm compared to 36 mm on the sound limb. The average longitudinal thresholds were 29 mm on the stump and 42 mm on the sound limb. These differences were significant

at the 1% level of confidence. Similar, though smaller, differences were found between the stump and sound limb on testing proximal parts of the stump, the differences decreasing with a definite gradient as the hip was approached. There were no indications that superiority in two-point discrimination of the stump was due to the stretching or folding of the skin or concomitant distortion of the peripheral patterns of its innervation. The results are considered as presumptive evidence for central readjustments which impart to the stump some of the functional characteristics of the amputated limb.

Ascorbic acid and acclimatization of animals to cold environment M. THÉRIEN (by invitation) and L. P. DUGAL *Dépt. d'Acclimatation, Institut de Biologie Humaine, Univ. Laval, Québec, Canada*. It has been shown in our previous studies, that the white rats, which are able to synthesize ascorbic acid, do react to a long exposure to cold by an important increase in the ascorbic acid content of their tissues if adaptation to the new surrounding takes place, if not, the contrary occurs, namely a significant decrease in the content of that vitamin. Following that observation it was found that guinea-pigs, which are unable to synthesize ascorbic acid, need more and more of that vitamin as the temperature of the surrounding is being lowered. Among interesting topics that have been observed, it may be said that a) there is a retention of ascorbic acid in the cold, as indicated by analysis of the tissues and of the urine, b) a direct relation between ascorbic acid content of the adrenals and adaptability to low temperature, c) a preventive effect of ascorbic acid towards the normal hypertrophy of the adrenals in the cold.

Effect of vitamin deficiency, thyroid and adrenal hormones on oxidation enzymes of rat tissues SAMUEL R. TIPTON and FRANCES M. COLVIN (by invitation) *Dept. of Zoology and Entomology, Univ. of Tennessee, Knoxville, Tenn.* We have extended our earlier work on the relationship of vitamins, hormones and respiratory enzymes (succinic dehydrogenase and cytochrome oxidase) to include adenosinetriphosphatase and d-amino acid oxidase of rat liver and kidney. Albino rats maintained on casein-sucrose-vegetable oil plus the necessary supplement of commercial vitamin B factors have somewhat lower liver and kidney phosphatase and oxidase activities than do rats maintained on Rockland Rat pellets. Administration of desiccated thyroid or thyroxine for 10 to 20-day periods results in an increase in the activity of both enzymes. After adrenalectomy there is a somewhat larger decrease in amino acid oxidase. Treatment with lipo adrenal extract prevented the decrease in oxidase with a possibly significant rise in adenosinetriphosphatase. In vitamin B deficiency there is a small increase in liver phosphatase.

while that of kidney is not significantly altered. There is a decrease in the activity of amino acid oxidase in both tissues. When the B-deficient animals are treated with thyroid powder there is a marked rise in liver phosphatase, the rise being considerably greater than that in hyperthyroidism in rats on stock diet. Kidney phosphatase does not show this B-deficiency effect. The stimulation of oxidase activity by the thyroid powder is depressed by B deficiency.

Syringe oxygen cathode for measurement of oxygen tension in solution and in respiratory gases JULIAN M. TOBIAS *Dept. of Physiology and Toxicity Lab., Univ. of Chicago, Chicago, Ill.* If the ends of a syringe plunger be removed, one has a hollow tube which fits tightly into the syringe barrel. It is then a simple matter to insert an insulated platinum wire and a pencil-type calomel cell so that their tips just protrude from the end of the opened plunger. The plunger is then filled with paraffin or a cement. With leads from the other ends of the electrodes the platinum wire can be made a cathode 0.8 v. negative to the calomel anode. If a solution be aspirated into the syringe, using the electrode fitted plunger as the piston, the current which flows can be shown to be a linear function of the oxygen tension of the solution. Precision has been studied using Ringer's solution. In a 0.5 cc. volume one can measure a change of 0.019 cmm. of O_2 with a standard error of 0.0001 cmm. With the volume reduced to 0.05–0.02 cc., using a 1-cc. tuberculin syringe, sensitivity is extended to 0.0008 cmm. of O_2 with a standard error of 1.7–2.3%. The instrument can also be used for measuring pO_2 in gas mixtures by taking in a small amount of Ringer and some of the gas. After equilibration, the pO_2 of the Ringer is measured. The instrument is not as precise as the Haldane analyzer, and at pO_2 values from 185–75 mm. Hg it may differ from the Haldane by 3.2 mm. Hg, on the average. An analysis takes a few minutes. The instrument has not yet been successfully used with blood.

Coagulation decelerating action of hemophilic plasma on normal plasma L. M. TOCANTINS and R. T. CARROLL (by invitation) *Division of Hematology, Dept. of Medicine, Jefferson Medical College, Philadelphia, Penna.* A coagulation decelerating action of hemophilic plasma on normal plasma can be demonstrated if the bloods are collected swiftly, over suitable surfaces, centrifuged at high speed at 5°C. and the tests carried out in collodion or silicone coated tubes. This property of hemophilic plasma varies among hemophilic individuals

while it is being reduced in the lower layers. Dilution, aging and exposure to glass impair and eventually destroy clot decelerating and anticephalin activity. Normal plasma to which a lipid antithromboplastin (*Proc. Soc. Exptl. Biol. & Med.* 68: 110, 1948) has been added, acquires clot decelerating action on intact normal plasma, and behaves on dilution, and incubation with cephalin, like hemophilic plasma.

Effects of anticonvulsants upon properties of frog sciatic nerve J. E. P. TOMAN, M. D. GREENHALGH (by invitation), J. S. CARLSON (by invitation) and G. H. BJORKMAN (by invitation) *Depts. of Physiology and Pharmacology, Univ. of Utah, College of Medicine, Salt Lake City, Utah.* Frog sciatic nerves were treated for 5 or more hours with solutions of various hydantoins, acetylureas, barbiturates, and oxazolidine-2,4-diones in Ringer's solution at pH 7.3 and room temperature. Those compounds having a 5-phenyl group and no substitution in the 3-N position were effective in concentrations of 1 mM or less in preventing the following normal phenomena: prolonged lowering of threshold and production of 'ultrasupernormality' and 'rebound spikes' following supramaximal stimulation, lowering of threshold and appearance of repetitive discharges after immersion in neutral isotonic phosphate solution. These phenomena are similar in some respects to the convulsive behavior of pyramidal neurones described by Adrian and Moruzzi (*J. Physiol.* 97: 153, 1939). Diphenylhydantoin, the most effective compound studied ($ED_{50} = 0.04$ mM), had little effect on other aspects of excitability and responsiveness. In occasional experiments there was a moderate increase in threshold and an abolition of the positive overshoot following a conducted spike. Diphenylhydantoin had no consistent effect upon membrane time and space constants, membrane potential, action potential amplitude, conduction velocity and recovery following submaximal stimulation. Although diphenylhydantoin did not alter the depolarizing and conduction blocking effects of KCl, it prevented the increased exchange of radioactive K and Na ions in nerves pretreated with phosphate solution. The results indicate that some anticonvulsants may prevent abnormally induced hyperexcitability and high frequency discharges without impairing the normal function of neurones.

Effect of amino acids on neuro-muscular func-

tion characteristic for this disease, a maintenance of the amplitude of the action potential during indirect stimulation. To ascertain whether this beneficial effect was due to supplying an essential amino acid to muscle and nerve single amino acid deficiencies were established in mice to ascertain whether the deficient animal shows a myasthenia gravis-like impaired neuro-muscular function. Single amino acid deficiencies were established by administering crystalline amino acids, vitamins, fat, and carbohydrate as suggested in diets based on the work of Rose. Well fed mice, mice starved for 3 days, and mice kept on low rations of mixed food for 6 weeks served as controls. Mice starved for 3 days did not show an impaired neuro-muscular function as measured by electromyography. Mice kept on low food rations for 6 weeks showed some impairment of the neuro-muscular function. Mice on the diet containing the 10 essential amino acids showed a similar neuro-muscular function as mice on chronic starvation. Mice fed with diets containing 9 essential amino acids did not show an impaired neuro-muscular function after 2 weeks, showed some impairment after 4 weeks if the diet was lacking either leucine, or phenylalanine, lysine, histidine, threonine, methionine, and showed a marked impairment of neuro-muscular function after 6-8 weeks. Glutamic acid deficiency did not induce marked impairment of the neuro-muscular function.

Effect on the action potential of nerve and muscle of 2-methyl naphthoquinone. CLARA TORDA and HAROLD G. WOLFF. *New York Hospital and the Depts. of Medicine and Psychiatry, Cornell Univ. Medical College, New York City.* The effect of 2-methyl naphthoquinone on the function of nerve and muscle *in vitro* and *in vivo* was investigated to ascertain whether or not this potent inhibitor of choline acetylase impairs the function of nerve and muscle. In the presence of the naphthoquinone in concentrations of $1 \times 10^{-5}M$ the conduct on time of isolated nerve preparation increased and the area and amplitude of action potential decreased, the conduction time of nerve-muscle preparation (*in vitro* and *in vivo*) increased and the area and amplitude of action potential decreased. The muscle function on direct stimulation was not significantly impaired. Since naphthoquinone, in the concentrations used, is known to inhibit only choline acetylase, the dysfunction observed suggests that acetylcholine is necessary to maintain an optimal function of nerve, myoneural junction and the excitatory mechanism of muscle.

Gastric distention as a factor in the satiation of thirst. E. J. TOWBIN (introduced by E. F. ADOLPH). *Physiology Dept., Univ. of Rochester, Rochester, N. Y.* Since dogs stop drinking before any appreciable amount of water enters their

blood, this study was designed to determine some of the factors in the satiation of thirst. Thirsty dogs with esophageal fistulae were allowed to sham-drink at hourly intervals. In some experiments a stomach-balloon was inflated with water before the dogs drank. The volume sham-drunk was two to three times in excess of the real water deficit, and water introduced into the stomach reduced sham-drinking (corroborating Bellows, 1939). Water introduced into the stomach-balloon was equally effective in partially inhibiting sham-drinking. Supra-diaphragmatic vagotomy abolished this effect in 3 of 5 dogs. Inflation of the balloon increases the interval between sham-drinks when there is free access to water. In other experiments, intact dogs had free access to water. Each drink was automatically recorded. Following supra-diaphragmatic vagotomy (2 dogs) the mean drink-size increased, in one dog it almost doubled. Bilateral splanchnicotomy and sympathectomy (D3 through D12), in 2 dogs, was followed by a reduction in the mean drink-size. Subsequent to vagotomy the drink-size rose above control values. To date, attempts to pick up vagal afferent impulses stimulated by gastric distention have been inconclusive. It is believed that the filling of the stomach initiates afferent impulses which are partial cues in gauging the amount of water drunk. Either these impulses ascend the vagal trunk or the atony subsequent to vagotomy interferes with the initiation of 'distention' impulses.

Thrombin formation in presence of proteolytic enzymes and platelets. BURTON L. TRAVIS (introduced by JOHN H. FERGUSON). *Dept. of Physiology, Univ. of North Carolina, Chapel Hill, N. C.* The plasma protease (Loomis *et al.*, *Arch. Biochem.* 12, 1, 1947), said not to activate prothrombin, clot ovalated plasma or purified fibrinogen (Loomis and Seegers, *Science* 104, 461, 1946), has been found 1) to accelerate the clotting of recalcified plasma and 2) to speed up the rate of thrombin formation from purified prothrombin activated in the combined presence of calcium (optimal), 'accelerator globulin' (AcG), and washed platelet suspensions. Small amounts of crystalline pancreatic trypsin showed apparently analogous effects, but these, unlike the 'fibrinolysin' actions, were inhibited by crystalline trypsin-inhibitor from pancreas and by the 'antifibrinolysin' of Loomis. It is unlikely, therefore, that the fibrinolytic protease itself is responsible for these accelerator effects. There is no thrombin demonstrable in the 'fibrinolysin' and it shows no accelerator effect in the absence of platelets. It does not accelerate prothrombin activation by tissue thromboplastin in the presence or absence of AcG.

Metabolism of radioactive iodine in severe anoxic anoxia. L. VAN MIDDLESWORTH. *Dept. of Physiology, Univ. of Tennessee College of Medi-*

cine, Memphis, Tenn Several authors have shown that 40-50% reduction in oxygen consumption occurs in rats exposed to severe anoxic anoxia. This observation suggested the present investigation regarding the effect of anoxia on the thyroid activity as indicated by the alteration of radioactive iodine metabolism in anoxic rats. Fifteen young rats were placed on a low iodine diet and after 8 weeks 8 of the animals were exposed to 268 mm Hg barometric pressure (simulated 27,000 ft altitude) for 12 hours. All 15 of the rats were then injected with 10 microcuries of I^{131} and the anoxic rats were returned to the low barometric pressure. After 24 additional hours all of the animals were killed. The total I^{131} was determined in the thyroid gland, urine and a sample of plasma. The plasma I^{131} was fractionated by trichloroacetic acid into 'bound' and 'unbound' I^{131} . Significant findings include a pronounced reduction in 'protein bound' I^{131} in the plasma of the anoxic rats. The average values (expressed as percentage of plasma I^{131}) for the male animals were as follows: trichloroacetic acid filtrate of plasma of control rats, 26%, and of anoxic rats, 91%; trichloroacetic acid precipitate of the plasma of the control rats, 74%, and of the anoxic rats, 8%. In these experiments the I^{131} content of the thyroid glands and urine was not significantly altered by the hypoxia.

Trial of enterogastrone in Mann-Williamson dogs FRANK E VISSCHER and STANLEY C LYSTER (by invitation) *Labs of the Upjohn Company, Dept of Pharmacology & Endocrinology, Kalamazoo, Mich*. Two groups of six Mann-Williamson dogs were prepared, one group was injected intramuscularly five times weekly with 100 mg enterogastrone, the other group was uninjected. Animals were maintained on a diet of specially prepared food containing 25 parts ground pancreas, 25 parts ground liver, and with the remaining 50 parts consisting of commercial dog food, bread, cod liver oil and milk. One gram of ferrous sulfate was added per day to the diet of each dog. Four of 6 injected animals survived in good condition, one of two deceased animals died of a perforated ulcer, the average survival time of the living animals was 8 months and of the deceased animals, 9 months. Three of 6 uninjected animals survived in good condition, 2 of 3 deceased animals died of a perforated ulcer, the average survival time of the living animals was

PHILIP F WAGLEY (by invitation), IRWIN W SIZER, LOUIS K DIAMOND (by invitation) and FRED H ALLEN (by invitation) *Dept of Biology, Massachusetts Inst of Technology, and Blood Grouping Lab, Children's Medical Center, Boston, Mass*. Observations have been made of the serological, physical and biochemical effects of peroxidase plus H_2O_2 on human serum containing anti-Rh agglutinins of specificity anti-D and anti-C (anti-Rh₀ and anti-Rh¹) active in both saline and albumin. Peroxidase activity against antibodies was considered of interest because it has recently been shown to oxidize certain proteins (Sizer, *Fed Proc* 6, 202, 1947) and because of the presence of this enzyme in phagocytic cells and its apparent absence in lymphocytes. There was significant diminution in titer of anti-Rh agglutinins after exposure for 6 hours at 37°C (0.1 ml serum) with as little as 300 µg of peroxidase (having a P Z activity of 167) in the presence of H_2O_2 (0.3%). Hydrogen peroxide alone in concentrations as high as 0.3% had no observable effect. Manometric studies during the reaction revealed oxidation of serum proteins induced by the peroxidase- H_2O_2 system. Ultra-violet absorption spectra obtained before and after such inactivation of the antibodies by peroxidase showed changes in the absorption range of proteins. This suggests an oxidation by peroxidase of such constituent amino acids in protein as phenylalanine, tyrosine and tryptophane. These apparent peroxidase effects were not accounted for by any catalase present in the serum. Dialysis of serum against 0.85% saline at 6°C for 72 hours prior to incubation with peroxidase did not prevent the effects of the latter on the antibodies. Purified anti-Rh globulin showed comparable changes induced by peroxidase when studied by serological, ultra-violet and manometric techniques. However, these peroxidase effects are not considered specific because anti-A and anti-B activities of human serum were also decreased by incubation with peroxidase in the presence of H_2O_2 . Furthermore, serum containing no Rh antibody during exposure to peroxidase- H_2O_2 showed manometric evidence of oxidation and alteration in ultra violet absorption spectra of certain serum proteins.

flashes were 5 seconds or more apart. With a 2-second interval, the occurrence of a response created a bias against a response to the second flash. In a series of paired flashes of equal intensity separated by 2 seconds, with 10 seconds or more between successive pairs, responses occurred much less frequently to the second flash of the pair than to the first. This is true even of flashes following those flashes to which there had been no response, although the depression of sensitivity was less than when the first flash elicited a response. By varying the interval between flashes in the pairs, and by holding the intensity of the first flash constant while varying that of the second, it was found that within an interval up to about 1 second the effect of a subliminal flash summed with that of a flash following it. This was then followed by a condition of decreased sensitivity, the sensitivity reaching a minimum at 2 seconds (1 to 2 log units below normal) and returned to normal by 5 seconds. Following a flash that had elicited a response, there was loss of sensitivity which disappeared in about 5 seconds.

Renin concentration of the kidney in experimental renal hypertension. G. E. WAKERLIN, JOHN MARSHALL (by invitation), R. O. BURNS (by invitation) and BESS G. OSGOOD (by invitation). *Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago, Ill.* During the past several years in our study of experimental renal hypertension we have determined the individual renin concentrations of 500 dog kidneys. The renin concentration of the normal dog kidney is relatively constant at approximately 2.0 dog (Goldblatt) units per gm. of fresh tissue. Renin disappears from the contralateral kidney 20 days after unilateral renal artery constriction, remains absent for at least 100 days, and returns to normal concentration 10 days following homolateral renal artery constriction. Significant atrophy of the constricted kidney, injection of certain hog renal extracts containing renin, and extreme morbidity from distemper and/or pneumonia favor the return or presence of renin in the contralateral kidney. Unilateral nephrectomy appears not to change the renin concentration of the remaining kidney. The renin concentration of the kidney is probably normal 1-3 months following renal artery constriction and is increased by injections of purified hog renin. Prolonged renal artery constriction (5-78 months) tends to decrease the renin concentration of the kidney and significant atrophy may enhance this tendency, whereas distemper and/or pneumonia favor an increased renin concentration. The renin concentration of the constricted kidney of experimental malignant hypertension varies from subnormal to supernormal, is not changed by injections of crude hog renal extracts containing renin but is increased by injections of purified hog renin. There was no correlation

between the renal renin concentration and the level of hypertension, chronic or malignant. Because of their limitations, these findings neither directly support nor oppose the renin-hypertension hypothesis of the pathogenesis of experimental renal hypertension.

Treatment and prophylaxis of experimental renal hypertension with renal extracts. G. E. WAKERLIN, J. E. BOURQUE (by invitation), JOHN MARSHALL (by invitation), R. O. BURNS (by invitation), BESS G. OSGOOD (by invitation) and LIONEL SCHOUR (by invitation). *Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago, Ill.* We previously reported that crude hog renal extracts containing renin were highly effective in the treatment and prophylaxis of experimental renal hypertension in dogs. During the past four years we found various crude hog renal extracts and extract fractions to be variably and less effective. We now know that this difference is importantly due to the fact that our early extracts were prepared from hog renal cortex and our later less effective extracts were prepared from whole hog kidney. Thus all dogs of a group recently treated prophylactically with crude hog renal extract containing renin and prepared from cortex were protected against experimental renal hypertension, whereas no dogs treated with the same extract prepared from whole kidney were protected. Similarly, the same cortex extract was much more effective against experimental malignant hypertension than whole kidney extract. Purified hog renin (70 dog units/mg. N) was found to be effective in the treatment of renal hypertensive dogs. Purified hog renin was likewise effective in prophylaxis against hypertension but was definitely less effective than crude cortex extract in protecting against renal insufficiency and death following renal artery constriction to produce chronic hypertension. The results here reported are definitely favorable to antirenin as the mechanism of these therapeutic and prophylactic effects, but the question is still open and requires work now under way. The factor in renal medulla responsible for the inhibitory effect on the therapeutic and prophylactic potency of hog renal cortex and renin is also under investigation.

Enzymic reduction of the retinenes to the vitamins A. GEORGE WALD. *Biological Labs. of Harvard Univ., Cambridge, Mass.* Retinene, formed by the bleaching of the photosensitive pigment of rod vision rhodopsin, is subsequently converted to vitamin A₁ by a reaction in which reduced coenzyme (DPN-H₂) acts as coenzyme. Two hydrogen atoms are transferred from DPN-H₂ to retinene, reducing its aldehyde group to the primary alcohol group of vitamin A₁ (Wald and Hubbard, *J. Gen. Physiol.* Jan. 20, 1949). The system which performs this reduction has now been isolated and fractionated into its components: the coenzyme DPN-

H₂, the substrate, synthetic retinene₁ (vitamin A₁ aldehyde) prepared by the oxidation of crystalline vitamin A₁, and the apoenzyme, retinene₁ reductase, extracted with dilute salt solutions from homogenized frog or cattle retinas. The apoenzyme is non-dialyzable, is precipitated by half saturation of its solutions with ammonium sulfate, and is destroyed by heating to 100°C within 30 seconds. Its p_H optimum lies at about 6.5. The rods of freshwater fishes contain a parallel system which reduces retinene₂, formed by bleaching porphyropsin, to vitamin A₂. This also can be put together from its isolated components in solution: retinene₂ reductase, extracted with dilute salt solutions from homogenized retinas, DPN-H₂, and as substrate synthetic retinene₂ prepared by the oxidation of vitamin A₂. These enzyme systems bring a member of the vitamin B complex, the anti-pellagra factor nicotinamide contained in cozymase, into the chemistry of rod vision. They introduce also a novel vitamin relation—a B vitamin participating directly in the regeneration of the vitamins A.

Pancreatic secretion in response to mechanical stimulation of the duodenum. J. M. WALDRON (by invitation), J. L. THOMAS and L. P. TRACZ (by invitation). *Dept. of Physiology, Jefferson Medical College, Philadelphia, Penna.* Three healthy adult dogs were provided with gastric and duodenal tubulated fistulas of the type described by Thomas (*Proc. Soc. Exp. Biol. & Med.* 46: 260, 1941). The duodenal fistula was placed opposite the main pancreatic duct and therefore the pancreatic duct could be easily cannulated with a fine glass cannula. The accessory pancreatic duct had been severed and doubly ligated. After complete recovery from the operation a total of 78 experiments were made on these 3 dogs to determine the effect of mechanical stimulation of the duodenum on pancreatic secretion. In the principle type of experiment fasting juice was collected for 1 hour, then the dog was fed 200 gm. of Purina Dog Chiekers and pancreatic juice collected for 5 hours. In each dog experiments of this type were done with rubber tubes in the stomach and duodenum as the mechanical stimulus and without these tubes in place. Other experiments were performed on each dog to investigate the possibilities that increased HCl secretion or patent pylorus or specific properties of the rubber tubing accounted for our results. These experiments conclusively demonstrate that mere mechanical stimulation of the duodenum changes the rate of pancreatic secretion in the fasting animal usually increasing it. Furthermore the presence of mechanical stimuli in the duodenum alters the response of the pancreas to food.

Effect of temperature on the response of rat muscle to indirect repetitive stimulation. SURI-PARD M. WALKER. *Dept. of Physiology, Washing-*

ton Univ. School of Medicine, St. Louis, Mo. Isometric myograms were obtained from the triceps surae (in situ) of male rats under sodium barbital (300 mg/kg) anaesthesia. Electrograms were recorded simultaneously from the gastrocnemius. The muscle temperature was reduced by refrigeration (10°C) of the animals for 1 to 2 hours. At body temperature (36.5°C) the peak tension induced by double shocks is 200 to 220% of peak twitch tension. The peak of added tension resulting from the second shock increases from 140 to 160% of peak twitch tension as the interval between shocks is increased beyond the refractory period to the early portion of the falling phase of the first response. Reduction of the muscle temperature to 26°C induces a twitch with peak tension 180% and peak time 300% of normal. The peak tension produced by double shocks is only 133% of twitch tension in the cool muscle. The peak of tension added by a second response increases from 40 to 65% of twitch tension as the interval between shocks is increased beyond the refractory period to the peak time. The tension induced by tetanic stimulation at 125 shocks per sec. is slightly greater in warm muscle. Duration of the action potentials is prolonged in warm and in cool muscle by shocks repeated at short intervals. The results show that the cool muscle summates twitch tension less effectively than warm muscle both in double responses and in tetanic responses, although it shows a marked increase (180%) of twitch tension.

Effects of pancreatic duct ligation upon the action of secretin and pancreozymin in rabbits. C. C. WANG (by invitation), K. J. WANG (by invitation) and A. C. IYI. *Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago, Ill.* Rabbits with pancreatic ducts ligated for periods ranging from a few hours to two weeks were used. The functional state of the pancreas was assessed by studying the response to secretin and pancreozymin and correlations were made with the histological picture. Within 24 hours, the volume response to a standard secretin stimulation was clearly decreased and the enzyme content was lowered. Histopathologically, there were protoplasmic changes in the acinar cells and the ducts became dilated. On the fourth day, the effect of pancreozymin began to disappear and the secretin effect was greatly reduced. The enzyme content of the juice was very low. The corresponding tissue sections showed the progressive degeneration of the parenchyma and an infiltration of fibrous tissue. The response to secretin continued to fall progressively as the period of ligation was lengthened. Finally, on the 14th day the parenchyma was mostly replaced by fibrous tissue and the secretin action was also gone. The secret juice obtained showed absence of amylase.

Formation of protein or other aggregates by

reactions linked in series DAVID F WAUGH *Dept of Biology, Massachusetts Inst of Technology, Cambridge, Mass* The intermolecular bonds formed during collision of two single molecules may not be sufficiently strong to afford stabilization against thermal disruption. The association of three or more molecules, either by simultaneous collision or by single molecules colliding with previously associated groups, may be stabilized by the simultaneous formation of several low energy bonds. Although similar bonds may be formed throughout, the reaction course may be examined by assuming two reactions the first of which is termed an initiating (active center forming) reaction and the second the augmentation reaction (elongation reaction in fibril formation). The order of the first reaction indicates the number of molecules necessary to initiate a stable aggregate. The second is expected to be pseudo-first order with respect to unit aggregating surface, i.e., the rate at which 1 cm^2 of surface augments will vary with the concentration of free protein. The total augmenting area of each locus of aggregation may increase with size thus introducing a further kinetic complication. Such considerations give differential equations which have been solved for first, an initiating reaction of order N followed by a pseudo-first order augmentation reaction assuming the aggregate rapidly approaches constant augmenting area, and second, an augmentation reaction alone in which predetermined numbers of active centers of initial size M_0 are introduced into solutions of protein and augment with increase in augmenting area. The general case of linked initiating and augmentation reactions with increase in augmenting area with size has been examined numerically.

Effect of increased renal venous pressure on renal function RENÉ WÉGRIA and (by invitation) WILLIAM D BLAKE, RICHARD P KEATING and HENRY P WARD *Dept of Medicine, Columbia Univ College of Physicians and Surgeons and Presbyterian Hospital, New York City* The effect of increased renal venous pressure on renal function was studied in anaesthetized dogs by means of clearance techniques. Renal function was measured separately and simultaneously in the two kidneys, but the pressure was raised in the left renal vein only, by means of a specially designed clamp. Moderate elevation of the left renal venous pressure from a normal value of around 100 mm saline up to 350 mm saline caused in that kidney a significant decrease in water and sodium excretion without any change in renal plasma flow, glomerular filtration rate, glucose Tm or diodrast Tm. The reduction in water and sodium excretion was due to an increase in the reabsorption rate of these substances by the renal tubule cells. This effect also occurred when the sodium load was low. Greater elevation of venous pressure (up to 550

mm saline) decreased renal blood flow and filtration rate, but there were insufficient results to state whether filtration fraction was significantly altered. The mechanism for the increased reabsorption of sodium and water ascribed to the increase in venous pressure and the implications of these results on the pathogenesis of the edema of cardiac failure will be discussed.

Effects of the 'hyperglycemic factor' found in insulin preparations H F WEISBERG (by invitation), R CAREN (by invitation), B HUDDLESTON (by invitation) and R LEVINE *Dept of Metabolic and Endocrine Research, Medical Research Inst, Michael Reese Hospital, Chicago, Ill* This study was undertaken to evaluate certain aspects of the action of the 'hyperglycemic factor' (H F) present in commercial insulin preparations. 1) Well-fed unanesthetized rabbits given 1-50 U/kg/hr of insulin intravenously over a 6-hour period, demonstrated the presence of H F in all cases. However the degree of effect bore no consistent relation to dosage. 2) Fasting (24 hrs) abolished or greatly reduced the tendency to hyperglycemia. 3) Subcutaneous injections of insulin into well-fed animals, in contrast to the intravenous route, resulted only in hypoglycemia. 4) During the hyperglycemic phases of alloxan action in dogs there was no detectable increase in the H F of serum from the pancreaticoduodenal vein, as measured in vitro by the method of Sutherland and Cori (*J Biol Chem* 172: 737, 1948). 5) The part played by the adrenal medulla in determining the degree of hyperglycemia will be discussed. No physiological significance can, as yet, be attributed to this factor, on the basis of our findings or those in the literature.

Chemical structure and the physiological properties of acetylcholine JOHN H WELSH *Biological Labs, Harvard Univ, Cambridge, Mass* As a working hypothesis to explain the reactions between acetylcholine (Ach) and cellular constituents it has been suggested that Ach acts as a co-enzyme. This requires postulating two classes of enzymes in order to account for the muscarinic and nicotinic actions of Ach. The nature and spatial arrangement of certain groups within the Ach molecule and the enzymes would determine, in part, which enzyme was activated. Pfeiffer (*Science*, 107: 94, 1948) has correlated the spatial relationships of possible 'prosthetic' groups in Ach and certain parasympathomimetic drugs with the muscarinic activities of these compounds. This study is an attempt to determine the properties of the Ach molecule responsible for nicotinic action. The physiological actions of certain simple quaternary ammonium ions on invertebrate nerve and muscle have been observed and it has been found that at least three methyl groups are necessary for maximal nicotinic action. The substitution of 3

ethyl groups for the methyls attached to the nitrogen of Ach produces an effective Ach blocking agent which is stable to insect cholinesterase. In instances where choline esters, tetramethylammonium ions and anti cholinesterases fail to produce excitation while triethylacetylcholine and tetraethylammonium ions excite, it is suggested that the level of active Ach within the tissue may be within the paralytic range and partial blocking of Ach may, in effect, reduce the level until it falls within the excitatory range.

A volumetric apparatus for studies of tissue metabolism. REIDAR WENNERSLAND (introduced by J. FENN) *Arctic Research Lab., Point Barrow, Alaska, and Dept. of Physiol., Stanford Univ., Calif.* A portable microrespirometer has been devised, which offers several advantages over the conventional Warburg type. These are: The volumetric principle, which allows a direct reading of the oxygen consumed, so that calibration of the respirometer vessels is unnecessary, less sensitivity to temperature changes, and a thermobarometer compensation for changes in barometric pressure and humidity. The measuring unit is compact, unbreakable and well suited for transport and field work. It is based on the same general principles as the respirometers worked out by Scholander. A V-shaped manometer and an oxygen chamber are drilled into a rectangular piece of plexiglass, (a material first introduced into microrespirometry by Scholander) and are connected to a respiration chamber of the Warburg vessel type. The second branch of the manometer is connected with a small thermobarometer bottle. The carbon dioxide produced is absorbed in alkali. The decrease in oxygen is indicated by the manometer. A stainless steel plunger is pushed into the oxygen chamber until the manometer is back to the level it had at start and at previous readings. The position of the plunger can be read with a dial indicator or by a simplified micrometer screw device and gives the oxygen consumed between the two readings, when the dimensions of the plunger are known. For a comparative study on tissue respiration by this and the Warburg method see abstract by Peiss and Wenersland.

decompression and anoxia. Following sudden decompression from sea level to 40,000 ft simulated altitude in O_2 , at which altitude the anoxia is insignificant, the slight blood pressure reduction is only of few seconds duration, after which both systolic and diastolic pressures are maintained at near control level while the animal remains at this altitude. The marked and sustained vasomotor change is observed only after sudden decompression to altitudes at which the animal is completely anoxic. We have confirmed the observation that the pulse pressure disappears after explosive decompression to altitudes above 60,000 ft. With relatively slow decompression to such altitudes, however, the pulse pressure is reduced but does not disappear. The systolic pressure may begin to recover during recompression when the animal is still at altitudes of complete anoxia, indicating a recovery from effects of sudden ambient pressure changes. The diastolic pressure recovery is slower and appears to be essentially a function of recovery from anoxia and very similar to records of recovery from 100% N_2 anoxia. This difference in recovery rates results in a temporary increase in pulse pressure above control level. Both systolic and diastolic pressures rise above predecompression levels towards the end of recompression but return to near control levels at end of recompression.

Renal dynamics and salt and water excretion in the dog following expansion of extracellular fluid. LAWRENCE G. WISSON, JR., W. PARKER ANSLOW, JR., LAWRENCE G. RAISZ, ALFRED A. BOLOVRY and MICHAEL LADD (introduced by HOMER W. SMITH) *Dept. of Physiology, New York Univ. College of Medicine, New York City.* Extracellular fluid volume in dogs was increased 5 to 10% of body weight by slow or rapid infusion of modified Loeb's solution and the increase sustained up to 9 hours by the intravenous replacement of NaCl, KCl and water as fast as the components were excreted. Filtration rate (CF), renal plasma flow ($CPAH$), filtration fraction (FF) and urine flow (V) showed a 3 phase response. During *Phase I*, $CPAH$, CF and V increased to peak values well above control values. At the rate of

then fell to a value slightly hypotonic to the blood shortly after the peak value of V was reached. If the control values were high, UCI decreased steadily to the hypotonic level. During Phase III, UCI rose until it attained a stable hypertonic level. In Phase III, renal dynamics, urine flow and urine concentration manifested a marked stability.

Renal tubular action of diuretics. II Effects of mercurial diuresis on glucose reabsorption. R. E. WESTON, J. GROSSMAN (by invitation), I. S. EDELMAN (by invitation), D. J. W. ESCHER (by invitation), L. LEITER (by invitation), and L. HELLMAN (by invitation). *Medical Division, Montefiore Hospital, New York City.* The tubular site—proximal and/or distal—at which mercurial diuretics act has never been precisely localized. Recent investigations have demonstrated that in man salyrgan produces not only decreased tubular reabsorption of electrolytes and water, but also significant depression of TM_{GH} , a renal function presumably limited to proximal tubular segments. Since it is well established that glucose reabsorption is limited to the proximal tubule, the effect of mercurial diuretics on maximal tubular capacity for glucose reabsorption (TM_G) was determined in human subjects without clinical renal disease. Preliminary experiments revealed that intravenous administration (2 cc.) of Mercuzanthin or Thiomerin (Disodium-N-(γ -carboxy methyl mercapto mercuri-B-methoxy) propyl camphoramate) immediately after several ten minute control periods generally resulted in a 40–80% decrease in TM_G , which coincided with maximal electrolyte and water diuresis. As diuresis diminished, TM_G returned toward normal. In these earlier experiments, concentrated glucose-mannitol aqueous solutions were infused, and the combined mercurial-osmotic diuresis produced significant decreases in serum electrolytes and extracellular fluid. To obviate this, infusions were prepared in normal saline. Because prolonged (90–150 minute) administration of glucose in saline has been reported to produce 20–30% decreases in TM_G in occasional subjects, in a second series of experiments, control and post-mercurial glucose TM 's were determined separately at 5–7 day intervals. In these shorter experiments, mercurials were administered prior to glucose infusion and produced similar depression of TM_G . It is concluded that in man mercurial diuretics depress a specific proximal tubular function.

Liberation of histamine by epinephrine. P. WHEELER (by invitation) and F. R. GOETZL. *Inst. of Medical Research, The Permanente Foundation, Oakland, Calif.* Recent reports indicate that in human beings an increase occurs in histamine content of blood plasma following intravenous administration of epinephrine. The observation was interpreted to indicate an ability of epinephrine to bring about liberation of histamine from

tissues. The present *in vivo* and *in vitro* experiments were performed using rabbit's blood. Histamine was assayed according to the method of Barsoum and Gaddum as modified by Code. In 25 *in vivo* experiments epinephrine in doses varying from 0.05 to 1.0 mg/kg was injected intravenously into anesthetized rabbits. Blood samples were obtained from the animals by heart puncture before and at 10 to 15 minute intervals after the injection for a period of 50 to 60 minutes. In blood samples thus obtained histamine was assayed and the leucocytes were counted. For the purpose of control similar experiments were performed in 10 animals which received intravenous injections of physiological saline solution instead of epinephrine. The results obtained from the *in vivo* experiments showed that intravenous injection of epinephrine causes a decrease in blood histamine and in the leucocyte count. In 37 *in vitro* experiments blood was obtained by heart puncture. Histamine was assayed in whole blood and in plasma after addition to the whole blood of equal volumes of epinephrine (0.001 and 0.1 mg/10 cc) and of saline solution, respectively. The results of the *in vitro* experiments showed that addition to whole blood of epinephrine is followed by an increase in plasma histamine, presumably due to liberation of histamine from leucocytes. The observations described are regarded to indicate that the decrease in blood histamine which, under the conditions of the experiments, followed intravenous injection of epinephrine into rabbits, was preceded by a shift of histamine from intracellular to extracellular compartments.

Development of seizure pattern by repetitive minimal cortical stimulation. J. A. WHELDON (by invitation) and A. VAN HARREVELD. *Kerechhoff Labs of the Biological Sciences, California Inst. of Technology, Pasadena, Calif.* Leño described a slowly spreading, transitory depression of the normal cortical activity which could be elicited by stimuli too weak to produce an afterdischarge. During this spreading depression he furthermore observed a cortical activity of a convulsive type. In the present experiments it was found that this convulsive activity is completely absent during the depression produced by the first stimulus after a long rest (1 hour). On repetition of the same stimulus at 5 to 10 minute intervals the convulsive activity developed and grew in intensity and duration. Also it was found that a stimulus of a strength and duration which initially was insufficient to produce an afterdischarge did so after repetition. Both afterdischarge and convulsive activity growing in intensity and duration with further repetition were in some experiments finally found to merge, producing an electroencephalogram typical of an electrically induced seizure. A rest of one hour was sufficient to restore the original situation.

in which a stimulus produced spreading depression without either afterdischarge or convulsive activity. After a wave of spreading depression had passed over the cortex the cortical activity was abnormal (bursts or continuous runs of medium to high potentials, 100–500 micro volts, slow, 2–5/sec waves) even if the stimulation produced neither afterdischarge nor convulsive activity.

The capacitance method for the study of cardiac activity WILLIAM V. WHITEHORN and EDWARD R. PERL (by invitation) *Dept of Physiology, Univ of Illinois College of Medicine, Chicago, Ill.* Studies of capacitance changes accompanying the cardiac cycle in the human have continued. Substitution of A C for D C amplification in the apparatus has increased stability and gain and simplified operation. Techniques and conditions for record taking have been standardized and the relative importance of technical factors determined. Further analysis of records (cardiodiagrams) in relation to simultaneously recorded electrical and mechanical cardiac events supports earlier conclusions that such records are directly related to cardiac volume changes and may provide an approximation of stroke volume. Comparisons of relative stroke volumes calculated from cardiograms of normal subjects under conditions of rest, exercise and performance of the Valsalva maneuver yield results in keeping with expected changes and provide evidence for the relative validity of the method. Absolute calibration of the instrument has been carried out by the introduction of spherical, saline filled balloons of known volume into the condenser field in positions approximating that of the heart. Results derived from such calibration curves cannot, however, be considered conclusively valid and comparison with simultaneous determinations by the direct Fick procedure are now in progress.

Action of dilute trypsin on muscle protoplasm (motion picture) FLOYD J. WIERCINSKI and BRIAN A. COOKSON (introduced by JOHN C. SCOTT) *Dept of Physiology, Hahnemann Medical College, and Dept of Zoology, Univ of Pennsylvania, Philadelphia, Penna.* Recent studies have indicated that the colloidal behavior of muscle protoplasm is similar to the colloidal behavior of blood. The clotting of muscle protoplasm (for example by free Ca ion) produces shortening. Inasmuch as dilute trypsin solutions act like thrombin in causing blood clotting, it was thought to be of interest to determine the effect of dilute trypsin on muscle protoplasm. The trypsin preparation used contained 50% of a magnesium salt, the concentrations reported here are based on the trypsin content. The microinjection of a dilution of 1:1000 and 1:2000 in a calcium-free Ringer solution caused a dramatic clotting of the sarcoplasm but the sarcolemma was unaffected. The interior protoplasm

contracted so sharply that it broke into pieces. A somewhat weaker effect is produced by the injection of a 1:10,000 solution. The myofibrils in the interior of the fiber were seen to break in several places along the longitudinal axis. The cortical region just under the sarcolemma appeared to be unaffected by this concentration of trypsin. A 1:100,000 dilution of trypsin upon injection into the muscle fiber caused very little effect but there was a slight contraction.

Testing the validity of ashing and digestion procedures by isotope dilution WALTER S. WILDE *Dept of Physiology, Tulane University, New Orleans, La.* We analyze for phosphate in the phosphoprotein-nucleic acid residue of Schmidt and Thannhauser. The sulfuric-nitric acid digest (Fiske and Subbarow, *J Biol Chem* 66:377) of intestinal mucosa from the rabbit is analyzed colorimetrically. The procedure is tested by applying the expression, $P_d^*/P_d = P_{Mg}^*/P_{Mg}$. We call the known tracer mixed with the phosphate residue before digesting P_d^* . After digestion an aliquot is neutralized and precipitated as $MgNH_4PO_4$. This is washed thoroughly, redissolved in dilute acid, and analyzed for tracer and for phosphate (colorimetric). This gives the ratio P_{Mg}^*/P_{Mg} . We then calculate for P_d which is compared to a direct colorimetric analysis of another aliquot of the acid digest. Three runs from a common pool are tabulated.

P_d^*	P_{Mg}^*	P_{Mg}	CALC	P_d FOUND
c/sec	c/sec	mg	mg	mg
273	227	0.265	0.318	0.320
204	185	0.294	0.324	0.310
41	28	0.209	0.303	0.282

Although absolute loss occurs with the magnesium ppt. the tracer and non-tracer are lost proportionately so that their ratio remains unchanged and available for a valid calculation. The agreement between the value calculated and that found indicates there is no volatilization heat loss, that the presence of possible chemical interfering substances in the acid digest gives colorimetric results no different from those for the simpler solution of dissolved magnesium ppt., that pyrophosphates, if they do form, are later hydrolyzed.

Toxicity studies on antioxidants—butylated hydroxyanisole and hydroquinone O. H. M. WILDER and H. R. KRAYBILL (introduced by A. J. CARLSON) *American Meat Inst. Foundation, Univ of Chicago, Chicago, Ill.* Butylated hydroxyanisole (BHA) and hydroquinone (HQ) are both excellent antioxidants for use in animal fats. Toxicity studies have been made on BHA and on a mixture of BHA and HQ, using rats, to determine their LD-50s and to see if their long continued ingestion would have

any harmful effect on the animals Six % of lard containing up to 2% of BHA was fed in a good basal ration for periods up to 21 months from weaning. No significant differences were observed in weight gains from weaning time between the controls and any of those getting the antioxidant, nor were there any differences in reproduction and growth of young. Another group of rats was started as soon after weaning as they would consume the ration and fed for 6 months at levels of BHA up to 2% of the ration. The only observed effect was a retarded weight gain at the higher levels. No effect was observed upon autopsy in any of the rats, and histological examination revealed no pathological conditions that could be attributed to the antioxidant at any level of feeding. A mixture of 80 parts BHA, 12 parts HQ, and 8 parts citric acid fed at levels of 0.3 and 2.0% of the ration has shown no effect during the growing period. The LD-50 of BHA dissolved in corn oil is 4.1 gm/kg body weight in the rat. In a water emulsion, on a fasted rat, it is in excess of 5.0 gm/kg body weight.

Pressure and flow of blood distal to a point of reduced arterial bore. ARNOLD H. WILLIAMS (introduced by HENRY A. SCHROEDER) *Dept of Internal Medicine, and the Oscar Johnson Inst., Washington Univ. School of Medicine, and Barnes Hospital, St. Louis, Mo.* The decreases of pressure and flow of blood distal to a point of reduced arterial caliber are of considerable physiological importance. Heretofore studies of these hemodynamic changes, produced by external compression of an artery, have been complicated by obstruction of venous return or have been done without exclusion of collateral circulation. For this reason the changes of the pressure and flow of blood were studied in the brachial artery, distal to an adjustable clamp, in the isolated forelimb of five dogs. Blood was supplied from the animal but a perfusion reservoir was connected to the circuit to obtain pressure-flow curves. In one instance oxygen tension of the muscle was measured simultaneously. Short or prolonged arterial constrictions (up to 25 minutes) did not produce any consistent relationship between the reduction of pressure and the reduction of flow. Neither was this relationship found at the end of 15-second perfusions of the constricted artery. The calculated percentile decrease of flow was not always greater at lower perfusion pressures. Thus arterial constriction produced no consistent change of arteriolar resistance distal to the constriction.

Hepatic vital staining and dye excretion in CCl₄-treated mice. W. LANE WILLIAMS *Dept of Anatomy, Univ of Minnesota, Minneapolis, Minn.* Hepatotoxic centrilobular injury was produced by CCl₄ and concomitant hepatic reactions to the following were studied: 1) two acid diazo dyes, trypan blue (Color Index 477) and chlorazol fast

pink (C I 353), 2) the triphenylmethane fluoran dye (also a xanthene fluoran), rose bengal (C I 779), and 3) the sulfonated triphenylmethane dye, isamine blue (C I 710). Dyes were injected subcutaneously or intraperitoneally as 0.5 to 1% saline or aqueous solutions in amounts of 0.1 to 0.4 cc. The azo dyes were excreted in normal and CCl₄-treated mice by the kidney, Rose Bengal by the liver, isamine blue, by liver and kidney. The azo's and isamine blue were present in macrophages as segregated granules. Rose Bengal was not deposited in macrophages in such a fashion and did not appear in such cells in an observable quantity. The azo's and isamine blue clearly stained the cytoplasm of damaged cells and remained in such cells until lysis ensued. The former dyes were definitely aggregated into large particles during actual necrosis, but the latter did not clearly undergo such a striking alteration. In contrast to the above, Rose Bengal stained the cytoplasm of all hepatic parenchymal cells. At 4-6 hours after intraperitoneal injection normal cells were clear of this dye, while cells in the centrilobular area of damage retained the dye for 8-14 hours depending on the interval (24-48 hours) subsequent to treatment with the hepatotoxic hydrocarbon.

Metabolism of the rat spinal cord functioning in isolation. R. D. TSCHIRGI (by invitation), R. W. GERARD, H. JENNERICK (by invitation), L. L. BOYARSKY (by invitation), J. Z. HEARON (by invitation) *Dept of Physiology, Univ of Chicago, Chicago, Ill.* The perfused isolated spinal cord lends itself admirably to combined functional and metabolic studies. A technique has been developed for removing the rat thoracic cord supported on vertebral bodies, fixing in plaster, and perfusing (after Galli-Manini) with Tyrode plus glucose (100 mg %) plus purified egg albumin and, sometimes, washed beef erythrocytes, at regulated temperature, pH, CO₂, CCO₂, arterial, and pulse pressure. Flow averaged 1 cc/gm/min. Reflex activity, tested by stimulating a dorsal root and recording ventral root potentials, continued undiminished in experiments lasting over 3 hours. One minute asphyxia or 2 to 4 minutes perfusion without glucose abolished reflex response. Recovery was complete within 2 minutes on restoring oxygen or glucose, even after more than 30 minutes deprivation. Average glucose utilization, 7.5 mg/gram wet weight per hour (range 7.1 to 8.0), corresponds to a QO₂ of 5000. Adrenaline increases the reflex response of a failing preparation. The areflexia of aglycemia can be used to test the ability of other substrates to support function. After 15 minutes inactivity without glucose (and later return with glucose) the following were ineffective, although several are easily oxidized: alcohol, acetate, lactate, β -hydroxybutyrate, fumarate, malate, succinate, adrenaline, dl alanine, dl lysine,

l tyrosine, dl aspartic, dl cysteine and l cystine. The following, in contrast, restored full function: pyruvate, oxaloacetate (partial restoration), isocitrate, α -ketoglutarate, glutamate and glutamine. Ammonia and glucose appeared in the perfusate with glutamate, glucose with α -ketoglutarate. Seemingly, successful metabolism of a substrate does not guarantee support of function.

Effect of abdominal ice packs on recovery from fatigue. W. W. TUTTLE, W. P. HAPP (by invitation) and M. WILSON (by invitation) *Dept. of Physiology, State Univ. of Iowa, Iowa City, Ia.* It has been reported that cold hip baths were employed in Germany during the last World War, in both industry and the air force, to ward off the onset of fatigue and to hasten recovery from it. It is the purpose of this investigation to gain further information relative to the beneficial effects of the local application of cold on recovery from fatigue. The problem was approached by comparing the maximum amount of work a subject would do on a bicycle ergometer in one minute of maximum effort before and after a 10-minute rest, both without and with an ice pack applied to the abdomen during the rest period. Eleven graduate men ranging in age from 25 to 35 years, and in weight from 130 to 200 pounds, served as subjects. A study of the data showed, that in every case, the drop-off in work following a rest period with an abdominal ice pack was significantly less than when the ice pack was omitted. The mean drop-off in work for the subjects when the abdominal ice pack was omitted from the rest period was 225 kg.M. and when ice was applied the mean drop-off was 69 kg.M. This difference is significant at the 1% level of confidence. It was also observed that for the most part, the application of abdominal ice packs between bouts of strenuous exercise alleviated the symptoms of exhaustion such as dizziness, nausea and muscular weakness.

Residual electrocardiograms. WILLIAM G. TURMAN (by invitation) and JANE SANDS ROBB *Dept. of Pharmacology, Syracuse Univ. College of Medicine, Syracuse, N. Y.* In order to test the adequacy of our recording instruments for another study, various standard extremity, unipolar extremity and direct leads were recorded from turtles. Cardiac tissue was progressively cut away eliminating first the ventricle, then one and finally both atria. With the entire heart excised potentials from two pacemakers were still recorded. Inspection showed movement of the intrapericardial portions of right and left veins. These had to be resected far into the thorax before the galvanometer became quiescent. When but a few millimeters of one vein remained and pulsation of one spot, approximately 1 mm. sq., could be seen with a hand lens, potentials were still recorded from direct electrodes either paired to each other or with each paired to

the central terminal which in turn was connected to 3 extremities. From this small area of pulsating vein, whose anatomical arrangement is relatively simple, Q-, R-, S-, and T-waves were registered. During this procedure there was a surprising maintenance of voltage suggesting considerable neutralization of current in the intact turtle. This study lends support to the view that the heart too has functional 'unit' areas which become activated, contract and produce each of the characteristic electrocardiographic waves. Although injury to some given area of the intact heart may alter the total electrocardiogram by altering summation, it seems unjustified to state that activation of some specified area is the cause of a given wave.

Effect of dinitrophenol on respiration of excised brains of rats at various stages of development. DAVID B. TYLER *Dept. of Embryology, Carnegie Inst. of Washington, Baltimore, Md.* To test the hypothesis that the mechanisms involved in oxygen consumption may differ in brains of different ages, the effect of dinitrophenol, $10^{-5}M$, was determined on the minced brain of rats, ranging in age from new born to adult, by means of Warburg respirometers. The oxygen uptake of the new born pallium (less than 24 hours old) is increased by an average of 28%. During the next 7 days there are indications that the sensitivity to the stimulating effect of this drug is diminished. The pallium of the 3-day-old rat shows only a 15% increase with the same concentration of dinitrophenol. Beginning about the 8th day there occurs a gradual increase in the response to dinitrophenol until the adult level is reached. Under the conditions of these experiments dinitrophenol ($10^{-5}M$) increases the oxygen uptake of the adult pallium by an average of 40%. No significant regional differences in response to dinitrophenol have been found. However, dinitrophenol enhances the depression of respiration produced by malonate or iodoacetate, depending upon the sensitivity of the region to the inhibitor. Thus the addition of dinitrophenol and iodoacetate to a suspension of minced adult medulla results in a lower oxygen uptake than when iodoacetate only is added, whereas a combination of dinitrophenol and malonate results in approximately the same oxygen consumption as occurs if malonate alone is added. In the case of the adult pallium a combination of dinitrophenol and malonate results in a significantly lower oxygen uptake than when malonate alone is used whereas the oxygen uptake of that region in media containing a combination of dinitrophenol and iodoacetate is approximately the same as when iodoacetate alone is used. The results with dinitrophenol support the hypothesis that the growth of the brain of the rat is accompanied by qualitative or quantitative differences in the systems responsible for its oxygen uptake.

Fibrinolysin activation by specific antigen, peptone and polysaccharides GEORGES UNGAR (introduced by A A SCHILLER) *Rheumatic Fever Research Inst, Northwestern Univ, Chicago, Ill* It is known that active proteolytic enzyme can appear when its inactive precursor present in serum is treated with chloroform or streptokinase. Activation has now been found to occur on addition of the antigen to serum from guinea-pigs previously sensitized to egg albumin. Activation was also observed in normal guinea-pig serum treated with peptone, agar, hyaluronic acid, chondroitinsulfuric acid, glycogen, pneumococcal polysaccharides and heparin. Higher concentrations of heparin however inhibit activation. The activating agent was added to serum which was then diluted with distilled water and adjusted to pH 5.4. The resulting precipitate, containing the enzyme, was centrifuged, redissolved and tested for proteolytic activity by a very sensitive fibrinogenolytic method and also by viscosimetry. Activation under the conditions mentioned above differs from the chloroform or streptokinase induced activation by the fact that it does not occur when the activating agent is added to the euglobulin precipitate or to serum previously heated to 56°. The findings reported here may have some bearing on the mechanism of anaphylactic and anaphylactoid reactions and on the functions of some high molecular carbohydrates.

Effects of explosive decompression on blood temperature in the right and left heart EDWIN G VAIL (by invitation) and FRED A HITCHCOCK *Dept of Physiology, Ohio State Univ, Columbus, Ohio* The development of the thermistor makes available an instrument for the precise measurement of small and rapidly occurring temperature changes. Experiments have already been reported in which thermistors were used to record temperature changes in the lungs. In the experiments reported in this communication, thermistors were inserted through the right jugular vein into the region of the right heart and through the right carotid artery into the region of the left heart. The exact location was determined by autopsy. Temperatures were recorded by means of a two channel ink-writing oscillograph. The temperature in the right heart was found to be slightly higher than that in the left heart. There were slight fluctuations in the temperature of the right heart which could be correlated with respiratory movements and may possibly be due to the proximity of the superior vena cava to the respiratory passages. No temperature fluctuations were observed in the left heart. Following explosive decompression to 30 ± 5 mm Hg there was an immediate drop in the temperature of the right heart and two or three seconds later, a drop in the temperature of the left heart. This decrease in temperature was greater in the left than in the right heart. The maximum drop in temperature occurred in about 20 seconds.

Studies on the effect of anoxic anoxia on sheep blood sugar and hemoglobin EDWARD J VAN LIERE, J CLIFFORD STICKNEY and DAVID W NORTHUP *Dept of Physiology, West Virginia Univ School of Medicine, Morgantown, W Va* Sheep were chosen for these experiments because of their known low fasting blood sugar. Two castrate male Corriedale lambs (no 32 23.6 kg and no 53 27.4 kg) were studied in regard to blood sugar and hemoglobin in jugular vein samples drawn after a 24-hour fast under control conditions and before and after 30-minute exposures at weekly intervals in a decompression chamber to simulated altitudes. Blood sugar was determined colorimetrically on Somogyi-Shaffer-Hartmann filtrates and hemoglobin by the Sahl method. The average control blood sugar in the 2 sheep was 52 and 53 mg/100 ml. Sheep 32 responded to a 30-minute stay in the decompression chamber at ground level with an average rise of 10 mg/100 ml in blood sugar in 4 tests. Sheep 53 in 3 tests showed no further rises after the first. The first exposure to 22,000 ft produced rises in blood sugar of 40 and 24 mg/100 ml in sheep 32 and 53 respectively. Subsequent exposures to altitudes from 18,000-28,000 ft produced no significant blood sugar rises in sheep 32. Similar exposures in sheep 53 produced rises of 6-18 mg/100 ml. Blood hemoglobin was also followed before and after exposure to anoxia. Sheep 32 consistently responded with an increase in hemoglobin concentration. Sheep 53 responded at first with small elevations, but later with reductions in hemoglobin concentration.

Pharmacology of N, 1 dimethyl hexylamine (Oenethyl) J H WILLS, *Dept of Pharmacology, Univ of Tennessee, Memphis, Tenn* In the dog anesthetized with pentobarbital sodium, N, 1 dimethyl hexylamine (Oenethyl) has both depressor and pressor actions, the latter predominating. The pressor action appears to have a dose-effect curve of the general form $Y = A + BX$, it is blocked by ergonovine but not by ergotamine or ergotoxin. Oenethyl increases the tonus of both jejunum and uterus *in situ*. The drug produces premature ventricular contractions but fibrillation has not been seen. The rate and depth of respiration may be increased by Oenethyl.

Permeability of rabbit leucocytes to sodium and potassium D L WILSON (by invitation) and J F MANERY *Dept of Biochemistry, Univ of Toronto, Toronto, Canada* Rabbit leucocytes were suspended in buffered solutions, the osmotic pressure of which was largely supplied either by sucrose or ammonium chloride. In the sucrose solution the chloride and potassium concentrations of the cells decreased 80% and 50% respectively. In the ammonium chloride solution the intracellular potassium decreased 90%, the intracellular sodium about 80%. The permeability to sodium was further studied by examining the penetration of Na^{22} .

During the first 75 minutes after exposure to the isotope the specific activity (i.e. counts/min/m Eq) of the sodium inside the cell was 90% of that outside. In addition, data were obtained which show that the external concentration of glucose influences the intracellular sodium and potassium concentrations of leucocytes.

Inhibition of growth of mice on a low protein, choline deficient diet by coramine J. WALTER WILSON and ELIZABETH H. LEDIC (introduced by PAUL F. FENTON) *Brown University, Providence, R. I.* Weanling mice were fed a low protein (4% casein) choline deficient diet, and the same diet with coramine. Without coramine, they grew slowly to nearly adult size and were still gaining slightly at 200 days. They had very fatty livers. With coramine, growth was greatly inhibited. With 1% coramine a gradual loss of weight occurred for 15 days. The mice were then fed 3 mg of choline by stomach tube whenever they showed signs of decline. They were maintained 64 days with no gain of weight. There was no fat in the liver. With 0.5% coramine growth was completely inhibited, but the mice were maintained 84 days without supplementary choline. With 0.25% coramine a slight but varied growth occurred but it did not approach that on the basal diet. The livers contained considerable fat. The basal diet contains a minimum of labile methyl groups. Detoxication of coramine occurs by transmethylation. 0.25% coramine added to the diet leaves just enough labile methyl groups for very slight growth, 0.5%, just enough for maintenance without growth, 1% requires so many that not enough are left for maintenance and the animal declines. This can be corrected by choline. This effect of choline cannot be attributed to its lipotropic action, for on 1% coramine the liver is not fatty. The choline must supply methyl groups. On a low protein diet then, choline may supply methyl groups for the detoxication of excess coramine, thus permitting maintenance during prolonged inhibition of growth.

Effect of antihistaminic drugs upon trained rats CHARLES A. WINTER and LARS PIATIER (by invitation) *Merck Inst. for Therapeutic Research, Rahway, N. J.* Antihistaminic drugs affect central nervous system activity as is evident from the side reactions observed in man, but this effect has not

to climb as do untreated rats, but they appear to be weak and easily become fatigued. Sufficient doses of antihistaminic drugs, on the other hand, make the animal become confused, and unable to decide whether to climb or not. It is therefore tentatively assumed that the results are a demonstration of central action of antihistaminic drugs. The central mechanism involved may not be the same one that was measured in experiments on barbiturate potentiation, because the compounds do not fall in the same order of graded activity in the two experiments. Two compounds having especially strong activity in the rope climbing test are Phenergan, N-(β -dimethylamino- α -methyl-ethyl)-phenothiazine, and Diparcol, N-(β -diethylamino-ethyl)-phenothiazine. Both of these compounds have been reported to be useful in the treatment of Parkinsonism.

Effect of potassium deficiency upon gastrointestinal motility HELEN A. WINTER (by invitation), HEBBERLE HOFF and LESLIE DSO (by invitation) *Dept. of Physiology, McGill Univ., Montreal, Canada.* The motility of the gastrointestinal tract has been investigated in rats during potassium deficiency, and the therapeutic effects of potassium have been shown. Potassium deficiency was induced by reducing the intake of potassium or by injecting desoxycorticosterone. Synthetic diets were given ad libitum or by stomach tube. Observations on motility were made by X-ray and by inspection of the gut and were correlated with studies of potassium metabolism. In potassium deficient rats the tone and rhythmicity of the whole gut were reduced, particularly in the small bowel and stomach. Gas and fluid accumulated in the ileocecal tract. Evacuation of barium was very slow. While normal animals evacuated barium from the stomach in about three hours and excreted more than one half in five hours, deficient rats usually retained some barium in the stomach for a day and often excreted little barium in this time. In some the cecum reached gigantic proportions. In force fed animals tremendous distention developed suddenly which proved fatal in all untreated rats. Therapy with potassium relieved the symptoms in surprisingly short time. In animals injected with potassium rhythmic contractions were initiated in the small bowel within four

Jefferson Medical College, Philadelphia, Penna. All subjects were patients with proved duodenal ulcer. Treatment periods ranged from 11 to 15 months and data are adjusted for a 12-month period. *Series A* 34 patients received orally 12 to 15 gm of dried defatted duodenum substance per day, plus ulcer diet. Average number of 'ulcer attacks' per year were 3.1 before treatment and 0.4 after treatment. X-ray evidence of ulcer healing was not obtained in 4 cases. *Series B* 15 patients received 12 gm enteric coated lactose tablets per day plus ulcer diet. Average number of 'ulcer attacks' per year were reduced from 3.2 to 0.1. All patients showed X-ray evidence of ulcer healing. *Series C* 18 patients received ulcer diet alone. Average number of 'ulcer attacks' per year were reduced from 3.3 to 0.5. One patient gave no X-ray evidence of ulcer healing. It is concluded that, when given daily for a period of 11 to 15 months in doses approaching the maximum tolerated by the patient, dried defatted whole duodenum did not prove to be of benefit to the patient with peptic ulcer.

New evidence on the nature of cutaneous hyperalgesia. HAROLD G. WOLFF, H. GOODELL (by invitation) and JAMES D. HARDY. *New York Hospital and the Depts. of Medicine, Psychiatry and Physiology, Cornell Univ. Medical College, New York City.* Two important types of Hyperalgesia in the skin were identified as regards their special characteristics: 1) That occurring at the site of skin injury characterized by lowered pain threshold, increased sensitivity to noxious and ordinarily non-noxious stimuli and termed for convenience primary hyperalgesia. 2) Secondary hyperalgesia, occurring in areas of undamaged skin characterized by unchanged pain threshold but increased sensitivity to noxious stimulation. Development of secondary hyperalgesia has been observed following prolonged stimulation at or just below the pain threshold. Such hyperalgesia ends immediately on termination of stimulation. Temporary obliteration of secondary hyperalgesia can be accomplished by pin pricks within the zone of secondary hyperalgesia. Spatial summation of noxious impulses has been observed to be a characteristic of painful stimulation in the zone of secondary hyperalgesia. All of the above evidence further supports the view that secondary hyperalgesia is the result of a central excitatory state maintained by a flow of noxious impulses from the periphery.

Effect of altered metabolism on extreme hypothermia in dogs and albino rats. RICHARD C. WOLFF (by invitation) and K. E. PENROD. *Dept. of Physiology, Boston Univ. School of Medicine, Boston, Mass.* Twenty-nine rats fed 0.03% propylthiouracil in the diet for 24 days, 30 fed 0.12% thyroid extract for 9 days, and 28 controls were cooled by immersion to the shoulders in water of 7°C.

Twenty rats receiving 0.03% propylthiouracil for 30 days, 16 receiving 0.50% thyroid extract for 15 days and 20 controls were cooled in a similar manner. The rats which were fed the propylthiouracil diet showed a metabolic depression of 15% while the group fed a 0.12% thyroid diet showed a stimulation of 14% and those receiving the 0.50% diet showed a stimulation of 41%. The first group of 87 rats was cooled to a rectal temperature 15.8-16.0°C. Sixty-nine per cent of the hypothyroid, 75% of the control and 63% of the hyperthyroid rats survived. The second group (56 rats) was cooled to a rectal temperature of 14.8-15.0°C. Forty-nine per cent of the hypothyroid, 50% of the control and 38% of the hyperthyroid rats survived. The rate of cooling to a rectal temperature of 20°C in a bath of 4°C, the time of rewarming in water and air to 31°C and the oxygen consumption were measured on 6 dogs, twice each. Two of the dogs succumbed in 'rewarming death'. The 4 surviving dogs were fed 4 mg/kg thyroid extract for 13 days and again cooled. Only one dog showed an increased metabolism and all showed an increased rate of cooling. In 2 cases the rewarming time was lessened, in 2 increased. It is concluded that during immersion in very cold water the metabolic defenses of the animal are not capable of significant protection.

Rapid methods for enzymatic estimation of nucleoprotein intermediate metabolites by differential colorimetry. W. Q. WOLFSON (by invitation), C. COHEN, R. LEVINE and K. KADOTA (by invitation). *Depts. of Biochemistry and of Metabolic and Endocrine Research, Medical Research Inst., Michael Reese Hospital, Chicago, Ill.* Since the purine intermediates in nucleoprotein breakdown eventually give rise to urate when exposed to suitable enzymes, the methods here proposed consist of measuring the quantitative appearance of urate (colorimetrically) after incubation of the sample with known enzyme solutions. Enzyme purification need only be carried far enough to yield a liquid preparation of satisfactory specificity which gives no blank in the determination and may be stored frozen. The procedures now in use include: 1) *True urate*, estimated from urate disappearance after treatment with beef kidney uricase. 2) *Oxypurine (xanthine plus hypoxanthine)*, estimated as urate appearing after treatment with xanthine oxidase from raw milk. 3) *Purine nucleoside plus oxypurine*, estimated from urate appearing after treatment with xanthine oxidase and nucleosidase from human liver. The latter has adenosine desaminase activity, but has little guanase and no adenase activity. 4) *Purine nucleoside*, calculated by subtracting #2 from #3. 5) *Purine nucleoside, aminopurine and oxypurine*, estimated from urate appearing after treatment with xanthine oxidase, nucleosidase and crude adenase.

gunnase from *Esch coli* 6) *Aminopurine*, estimated by subtracting #3 from #5. Apart from their simplicity, these procedures have the advantage of being relatively free from interference by side-reactions, such as the conversion of methyl-xanthenes to methylurates during xanthine oxidase treatment. An example of the application of one of these methods to physiological problems is given elsewhere in these proceedings.

Metabolism of oxypurines in man preliminary observations WILLIAM Q. WOLFSON (by invitation), CLARENCE COHN and KINU KADOTA (by invitation) *Dept of Biochemistry, Medical Research Inst., Michael Reese Hospital, Chicago, Ill.*

In man, the physiological course of intermediate purine metabolism prior to urate is largely unknown. The liver may be the chief site of urate production since it is believed to be the sole locus of xanthine oxidase, and because extraordinarily low blood urate levels have been reported in acute yellow atrophy. Oxypurines might be produced in the periphery and transported to the liver for conversion to urate, about $\frac{1}{10}$ being excreted by the kidney. If so, a significant increase in serum oxypurine should occur in hepatic, but not in renal, insufficiency. However, a large portion of the oxypurine might be formed as well as metabolized in the liver, and, in this event, oxypurine precursors rather than oxypurine should be retained in hepatic insufficiency. It has also been suggested that gouty hyperuricemia might be associated with a failure of inhibition normally exerted on hepatic xanthine oxidase. Serum and urine oxypurines have been determined by differential enzymatic colorimetry. In 14 normal adults, serum oxypurine averaged 0.30 mg % (S.D. \pm 0.14 mg %). Serum oxypurines were not elevated in pooled sera from patients with liver damage or renal insufficiency, but these data are not sufficiently consistent to permit definite conclusions as yet. The average serum oxypurine in repeated samples from five gout patients averaged 0.11 mg %. This result is consistent with the suggestion that there may be a relative hyperactivity of hepatic xanthine oxidase in gout.

Oximetric measurement of circulation time and arterial saturation time during oxygen breathing in man EARL H. WOOD, BOWEN E. TAYLOR (by invitation) and JULIAN KNUTSON (by invitation) *Section on Physiology, Mayo Foundation, Univ of Minnesota, Rochester, Minn.* Arterial saturation was recorded continuously and simultaneously both at the ear and wrist (abstract J. Knutson et al.) in 6 subjects as the gas mixture breathed was changed from air to 40% O₂, from 40 to 99.6% O₂, and from air to 99.6% O₂. After changing from air to 40% O₂ and air to 100% O₂, the initial increase in saturation (circulation time, Matthes) occurred at 6.2 (3 to 9) and 9.6 (6 to 12) seconds at the ear

and wrist, and the maximal plateau value was attained at 47 (19 to 74) and 46 (19 to 71) seconds, respectively. Time required to increase from 97.9% to the plateau value minus 0.5% (Fowler and Comroe), averaged 25 (11 to 58) seconds at both the ear and wrist, as compared to 19 (14 to 28) seconds (ear) and 25 (18 to 30) seconds (wrist) required to increase from 71 to 97 per cent after termination of breath-holding with helium (abstract J. Knutson et al.). Changing from 40 to 100% O₂ produced an increase in arterial saturation both at the ear and wrist, averaging 0.40 (0.2 to 0.5) and 0.35 (0.1 to 0.7) %, respectively. The approximate arterial O₂ tension calculated from the physically dissolved oxygen (O₂ content—O₂ capacity) averaged 213 and 595 mm Hg when 40 and 100% O₂, respectively, was breathed. The similarity in time and the magnitude of changes in arterial saturation recorded photoelectrically at the ear and directly on the arterial blood at the wrist indicate that an ear oximeter can be used successfully for determination of circulation and arterial saturation times in man.

Passive sensitization of normal dogs by thoracic duct lymph from dogs sensitized to horse serum PARKE H. WOODARD (introduced by K. E. JOCHIM) *Dept of Physiology, Univ of Kansas, Lawrence, Kan.* It is known that normal dogs may be passively sensitized to horse serum by transfusing into them blood from dogs previously sensitized to horse serum and that they will exhibit some of the symptoms of anaphylactic shock upon intravenous injection of horse serum. The symptoms most generally reported have been a fall in arterial blood pressure and changes in blood coagulation time. I have been able to confirm previous work from this laboratory that such passive sensitization is immediate and does not require lapse of time as has been reported by some workers. Not all sensitive animals are capable of passively sensitizing normal animals. In those which are capable it was thought worth while determining whether or not antibodies were distributed in other body fluids than blood. Accordingly dogs were sensitized to horse serum. Each animal was anesthetized with nembutal and the thoracic ducts cannulated and sufficient lymph collected for injection into a second anesthetized dog. In most cases blood was also drawn from the sensitized animal and transfused into a third anesthetized dog. Blood pressure records and blood clotting times were recorded on all animals before and after the administration of shock doses of horse serum. Eight control experiments were run in which the lymph and blood from normal dogs was similarly tested. These showed no evidence of shock. Of 11 experimental animals, 2 were not sensitive, 4 were sensitive but did not passively transfer immunity either through blood or lymph, 1 showed passive transfer through

blood but not lymph, 4 showed evidence of passive transfer by both blood and lymph

Antagonism of adrenocorticotrophic hormone (ACTH) to desoxycortico-costerone acetate (DCA) on electroshock threshold and electrolytes DIXON M. WOODBURY (by invitation), CHI-PING CHENG (by invitation) and GEORGE SAYERS *Depts of Physiology and Pharmacology, Univ of Utah College of Medicine, Salt Lake City, Utah* By the 12th day after implantation of DCA (six 15 mg pellets into each of 14 rats) the electroshock threshold had risen to +17%, 14 controls showed no significant change (+2%) Beginning on the 12th day, 7 of the DCA-implanted rats and 7 of the controls were injected twice daily for ten days with 2 mg of ACTH On the 22nd day the electroshock thresholds of the four groups were as follows I) control, +3%, II) ACTH, +6%, III) DCA, +22%, IV) DCA +ACTH, +5% When ACTH was withdrawn from group IV the threshold rose to +17% by the 32nd day Readministration of ACTH resulted in a threshold reduction to +10% after 9 days, III had an average threshold of +24% at this time (41st day) DCA elevated plasma sodium and reduced plasma potassium and chloride ACTH partially corrected the deranged electrolyte pattern of the DCA-treated rats Whole extract of adrenal cortex restored to normal the elevated threshold of DCA-implanted rats Since ACTH did not influence the elevated electroshock threshold of adrenalectomized rats implanted with DCA, the trophic acts via the adrenals The results are interpreted to mean that DCA in intact animals, produces a relative deficiency of cortical steroids with an O on C-11 by inhibiting the release of ACTH from the pituitary

Early components of contralaterally evoked cortical potentials in the intact unanesthetized rat LOWELL A. WOODBURY (introduced by J. E. P. TOMAN) *Dept of Physiology, Univ of Utah College of Medicine, Salt Lake City, Utah* As a preliminary to a study of the action of central excitant and depressant drugs, control observations were made on the initial components of the electrocorticogram evoked by stimulation of the contralateral hemisphere in ten unanesthetized intact rats Epidural insulated screw electrodes were implanted under anesthesia one or two days prior to each experiment Brief rectangular pulses or condenser discharges were delivered to the visual cortex of one hemisphere, while responses were taken from the symmetrical contralateral area The characteristic initial response to submaximal stimuli consisted of two initially surface-positive spikes and a third surface-negative spike with latencies of 1.8-2.0, 3.4-3.6, and 4.0-4.2 msec respectively A voltage-response study of the large third spike showed a relatively homogeneous response population Strength-duration curves for

this response gave a limiting log-log slope of -0.7 and a chronaxie of 0.18 msec The recovery curve following maximal responses exhibited relative refractoriness to 2.5 to 3.0 msec followed by a period of greatly enhanced excitability with a peak at 4.0 to 6.0 msec Since this phase was absent when conditioning responses were reduced below the amplitude of test spikes, it is attributed to a true supernormal period of previously responding elements rather than to indirect facilitation This phase was followed by subnormal excitability with a minimum in the vicinity of 10-20 msec and further minor oscillations at equal log-time intervals to at least 400 msec

The pattern of localization in the motor cortex of the marmoset (*Hapale jacchus*) C. N. WOOLSEY, P. H. SETTLAGE (by invitation), H. M. SUCKLE (by invitation) and W. G. BINGHAM (by invitation) *Depts of Physiology, Anatomy and Surgery, Univ of Wisconsin, Medical School, Madison, Wis* We have examined in detail the motor area of this primitive new world monkey in order to compare the pattern of organization with the relatively simple plan found in the rat (see abstract by Settlege et al) Certain comparisons are possible, but the main features of the primate arrangement have already been established The relation of the motor pattern to that of the post-central tactile area as defined by the evoked potential method (unpublished data, C. N. W.) will be considered

Actions of dihydro-B-erythroidin and dtubocurarine on neuromuscular and synaptic transmission in frog and crayfish ERNEST B. WRIGHT (introduced by W. O. FENN) *Dept of Physiology and Vital Economics, Univ of Rochester School of Medicine and Dentistry, Rochester, N. Y.* 2 mg/kg dihydro-B-erythroidin (eryth) and 4 mg/kg dtubocurarine (dtubo) paralyze the frog in 20 and 30 minutes respectively, neuromuscular transmission being totally blocked Neuromuscular transmission in the isolated sciatic-sartorius is blocked by immersion in 5×10^{-7} eryth and 5×10^{-6} dtubo with reduction of the endplate potential Action potentials in the crural nerve and contractions of the cruralis muscle with blood supply tied off, elicited by stimulation of the foot skin, knee skin or sciatic nerve show that a) sensory endings and nerve conduction are unaffected by both drugs, b) transmission of flexion reflex is unaffected or slightly enhanced by dtubo, greatly depressed or blocked by eryth The crayfish is unaffected by 50 mg/kg dtubo, but paralyzed in 2 minutes by 0.05 mg/kg and in 10 seconds by 1 mg/kg eryth Sensory endings, nerve conduction and neuromuscular transmission are unimpaired at these concentrations Spontaneous impulses originating in the central system and led off from peripheral nerve are extinguished with remarkable sudden-

ness by eryth, but are unaffected by dtubo. Electrical recordings from the ventral nerve cord and from roots of the abdominal ganglia show that eryth has no effect on giant fiber conduction, blocks transmission from giant fiber to root, depresses the spontaneous activity of the cord somewhat, and depresses greatly or blocks the spontaneous activity led off from the root originating presumably in the attached ganglion. Eryth, unlike dtubo, blocks central synaptic transmission in frog and crayfish, and like dtubo blocks neuromuscular transmission in frog.

Anti-stiffness factor in relation to hearing in guinea pigs. ROSALIND WULZEN and ALICE B. PLYMPTON (by invitation) *Dept. of Zoology, Oregon State College, Corvallis, Ore.* 117 guinea pigs were divided into two groups, one on stock diet, the other on diet deficient in anti-stiffness factor. The stock diet consisted of rolled barley, green feed and straw. The deficient diet was composed of skim milk powder and water, plus an adequate supply of minerals and all known vitamins except the anti-stiffness factor. For the test, animals were exposed to signals from an audio signal generator combined with loud speaker and key. They responded with flicking of the ears to a wide range of auditory stimuli. Only slight deviations were found in repeated readings for any one animal. Limits of normal hearing, judged by the ear flick and expressed as frequency in cycles per second, were established as follows: low range, 200-450, high range, 10000-15000. Of 45 animals on stock diet, 41 proved to have normal hearing, 4 had restricted hearing, none were completely deaf, that is, without ear flick response. Of 72 animals exposed to deficient diet for varying lengths of time, 42 showed normal hearing, 15 had restricted hearing, 15 were completely deaf, that is, without ear flick response.

Fasting serum vitamin A and carotene levels, and vitamin A absorption in males over 40. MARVIN J. YIENGST (by invitation) and NATHAN W. SHOCK. *Section on Cardiovascular Diseases and Gerontology, National Heart Institute, National Insts. of Health, and Baltimore City Hospitals, Baltimore, Md.* The purpose of this investigation was to study age changes in absorption of vitamin A and fasting levels of vitamin A and carotene in a population living under homogeneous conditions. Fasting levels were determined on 121 males ranging in age from 40 to 90 years who were unselected except to exclude any subjects with clinical signs or history of liver disease. All subjects were residents of the Baltimore City Hospitals and Infirmary (Old People's Home) and were receiving an adequate diet. The method of Bessy, Lowry, Brock, and Lopez (*J. Biol. Chem.*, 166, 177, 1946) was used in the determinations. The serum vitamin-A levels ranged from 10 to 88 and per 100

ml with a mean of 49.9. Arranged by decades, the 5 groups from 40 to 90 years of age showed no age differences. The carotene levels ranged from 28 to 231 and per 100 ml with a mean of 113.1 and likewise showed no significant age difference. A low positive correlation exists between the fasting vitamin A and carotene levels ($r = 0.17$, $N = 121$) in the serum. Fifty-four males included in the above group were fed 100,000 U of vitamin A in oil. Blood samples were drawn at 0, 2, 4, 6, 8, 10, 16, and 24 hours after oral administration. Mean values in and per cent for the group at these times were 53.4, 125.3, 150.2, 172.1, 118.9, 96.7, 73.0, and 63.3. There were no age differences in response to oral administration of vitamin A. Serum carotene values remained unchanged.

Effects of DOCA and adrenal cortical extracts on survival of adrenalectomized rats after burning. S. S. YU (by invitation) and E. A. SLIERS. *Dept. of Physiology, Univ. of Toronto, Toronto, Canada.* Rats of both sexes weighing about 150 gm were bilaterally adrenalectomized and maintained on saline for 4-10 days. They were then divided into 3 groups, the first of which constituted a control and continued to receive saline. Desoxycorticosterone acetate and adrenal cortical extract were administered to the second and third groups, which were allowed water ad libitum. A standard burn was produced under ether anesthesia by immersing the backs of all animals in water at 85°C for 30 seconds. This proved lethal in 2-42 hours to the control group (10 animals). The 28 rats of the second group received DOCA by subcutaneous injection in daily doses of 0.25, 0.50 and 1.0 mg for 4 to 7 days prior to burning. At the higher dosage levels DOCA afforded a definite protection, 4 of the 5 animals which received 0.5 mg/day for 7 days survived. All the 9 animals receiving 1.0 mg/day for 7 days survived, while in another group of 9 rats which were given the same dosage for 4 days, 2 died. Adrenal cortical extracts from different sources (Connaught and Upjohn Laboratories) were administered to group 3 in doses varying from 15 to 150 dog U/day (divided doses when the dosage was high) for 4-8 days. Among the 24 rats thus treated only 2 animals which received 60 and 150 U/day respectively survived the burning. The effects of these substances on intact animals subjected to a similar stress will also be reported.

A respirator and perfusion pump with new features and an improved constant injection apparatus (motion picture). W. B. YOUNG. *Dept. of Physiology, Univ. of Oregon Medical School, Portland, Ore.* A piston pump, having new features, and an improved constant injection apparatus have been designed and constructed for this laboratory by the Martin-Hubbard Corp., Boston. The pump will deliver any stroke volume between zero and 1000 cc. A rate continuously

variable from zero to 180/min is obtained by the use of a Graham transmission unit. Either rate or stroke volume can be changed through the entire range without stopping the motor. There are 4 input ports and one output port. All parts are made of metal, the valves consisting of an axle with sectors cut out to permit passage of the gas at the proper phase of the cycle. The piston empties the cylinder at each downstroke regardless of the stroke volume. A pump having the same basic features with a smaller maximum stroke (500 cc) which will deliver against pressures in the range of arterial blood pressure is designed also. Use of the instrument to pump gases directly and indirectly and to pump liquids indirectly will be shown. The constant injection apparatus consists of a Graham transmission unit geared to turn a threaded shaft against the plunger of a syringe of any size up to 50 cc. Any injection rate between zero and 10 cc/min can be obtained without stopping the motor. Uses of the instrument to maintain steady submaximal effects by continuous intravenous injection will be shown.

Bone absorption and relaxation of guinea pig pelvis by prolonged estrogenic injections. WILLIAM C. YOUNG (by invitation) and FREDERICK E. EMERY, *Dept. of Physiology and Pharmacology, Univ. of Arkansas School of Medicine, Little Rock, Ark.* The effects of various estrogens on the pelvis of the guinea pig have been studied in about 60 young guinea pigs of both sexes. The males were injected with 1.0 mg of diethyl stilbestrol in olive oil for a duration of several months. Periodic X-rays were taken throughout the injection period. By palpation and X-ray it was demonstrated that there was a separation of the pelvis varying from little or no separation to almost complete relaxation in some cases. The testes seemed to be dominated by the estrogens, as shown by the fact that the separation at the pubic symphysis was almost as pronounced in males as in castrated males. Female guinea pigs treated in a similar manner with 0.1 mg of stilbestrol twice weekly have shown bone absorption and relaxation. So far complete disappearance of the bony pelvis, like that of the pocket gopher, has not been obtained.

Response of the young cockerel to methyl folic acid and testosterone propionate. M. X. ZARROW and I. B. KORETSKY (introduced by F. L. HISAW), *Biological Labys, Harvard Univ., Cambridge, Mass.* Studies were made on the relationship of folic acid to the response of the testis and comb of the chick to testosterone propionate. A folic-acid-deficient state was produced by the administration of a crude methyl folic acid preparation (courtesy of Dr. E. L. R. Stokstad, Lederle Laboratories) in the diet. Day-old cockerels of the Leghorn strain were divided into 4 groups of 10 each and treated in the following manner: 1) normal con-

trols, 2) 100 γ of testosterone propionate daily, 3) 2% methyl folic acid in the diet, and 4) 2% methyl folic acid in the diet plus 100 γ of testosterone propionate daily. The androgen was dissolved in sesame oil and injected subcutaneously in a daily volume of 0.05 ml. In the first experiment the chicks were treated for 7 days and killed on the 8th day at which time organs were removed for weight determination and histological examination. The testis weights for the 4 groups were 20, 15, 20 and 10 mg respectively or 27, 20, 28 and 16 when expressed as a function of the body weight. The absolute comb weights were 21, 76, 16 and 54 mg and the relative weights were 52, 98, 39 and 83. A second experiment in which 4% methyl folic acid was used in the diet gave essentially the same results for testis response. The comb weights were 34, 83, 24 and 106 mg or 40, 90, 37 and 163 respectively when expressed as a function of body weight. These results would seem to indicate that testosterone propionate was able to produce comb growth in the presence of the antagonist.

Effect of adrenoxyl on blood loss from surgical wounds. J. J. ZAVERNIK and R. F. HAGERTY (introduced by K. S. GRIMSON), *Dept. of Surgery, Duke Univ., Durham, N. C.* Adrenoxyl, a mon-semicarbazone of adrenochrome, reportedly decreases bleeding time of rabbits and man. In our studies in dogs it was determined that intramuscular injection of 10 gamma of Adrenoxyl does produce a marked decrease, effect being maximal by 30 to 60 minutes. A study of ability of the drug to decrease blood loss from surgical wounds was also undertaken. Consecutive apparently similar incisions were made in the liver of dogs estimating bleeding by weight of blood collected. One hour after administration of 10 gamma of Adrenoxyl amount of bleeding decreased markedly. Wounds were also produced by resection of similar portions of a rabbit's two ears. Amount of blood loss was found decreased moderately one hour after administration of 10 gamma of Adrenoxyl. Three similar wounds were produced on opposite ears of the same rabbit 5 days apart. The 3 different wounds produced on one ear before the drug bled respectively an average of 31 gm, 72 gm and 80 gm of hemoglobin, increasing amounts representing the larger or more proximal incisions. Five days later 3 similar wounds were made after Adrenoxyl using the opposite ear. Decrease in bleeding after administration of Adrenoxyl was 44.7%, 3.7%, and 13.3% respectively. Decrease after Adrenoxyl was greater in wounds where bleeding occurred predominantly from small vessels. A less marked decrease occurred from 3 wounds of control rabbits produced 5 days after incisions of the opposite ear and without using Adrenoxyl. Hematologic examinations demonstrated a rise in the platelet count which might explain this observation.

Effect of low protein and plasmapheresis on fluid compartments and glomerular filtration rate MARJORIE B ZUCKER, LOUIS CIZEK (by invitation) and DOUGLAS TOMPKINS (by invitation) *Dept of Physiology, College of Physicians and Surgeons, Columbia Univ, New York City* Weekly measurements were made on 3 dogs on a control diet of Beacon Meal alone, and then after 3 weeks on a diet supplying 16 gm protein/kg/day and 80 Cal/kg/day (50% Beacon Meal, 16% dextrose, 33% Mazola, 0.5% NaCl, 0.2% yeast, 0.4 gm/kg/day Cal-C-Tose, 0.4 drops/kg/day oleum percomorph). Finally, determinations were made for several weeks during which the diet was continued and the dogs plasmapheresed 1 to 4 times. Including a weekly supplement, the NaCl intake approximated 20 mEq/kg/week. Clinical edema was not observed. The first number in following parentheses represents percentage change from the control value in dog 1, the second in dog 2, etc. The diet alone caused weight loss (-17, -13, -4), decreased serum protein concentration (-10, -6, -10), electrophoretic A/G ratio (-, -49, -34), colloid osmotic pressure (COP) (-8, -13, -16), T-1824 plasma volume (PV) (-23, -23, -26), creatinine clearance (GFR) (-26, -30, -46) and, in 2 dogs, reduced thiocyanate space (AF) (-21, -23, -). The latter is contrary to Keys' results in man on a famine diet. The results after plasmapheresis, expressed as percentage change from the values observed on the low protein diet, are given for the week during which the lowest COP was observed, and were determined at least 2 days after bleeding. Plasmapheresis did not alter the weight (-3, 0, 0), but usually reduced the serum proteins (-16, -, -11), A/G (-, +10, -34) and COP (-31, -32, -27). PV increased (+10, +5, +23), a finding which is difficult to explain. The maintenance of the GFR at a low level (-10, -15, +3), is of interest. AF increased (+12, +16, -), indicating that fluid retention occurred although COP was not reduced to values associated with clinical edema.

Hepato-renal factors in circulatory homeostasis XXVI **Effect of adrenalectomy on renal VEM system and hypertension** BENJAMIN W ZWEIFACH and EPHRAIM SHORR *Dept of Medicine, Cornell Univ Medical College and The New York Hospital, New York City* The formation of a vasoexcitor principle (VEM) by kidney appears to depend on the presence of the adrenal cortex, kidneys from adrenalectomized animals failing to produce VEM *in vitro*. The present study deals with attempts to produce renal hypertension in adrenalectomized rats maintained on a high salt diet in apparently good health for periods of 30 to 120 days. Various methods for inducing hypertension were employed, capping of one or both kidneys with gauze-collodion, partial ligation of the renal

artery, or figure-eight loop around the kidney. These experimental procedures, which produced hypertension in about 75 to 85% of the control rats, did not induce any clear-cut hypertension in a comparable series of 45 adrenalectomized rats maintained on salt. Microscopic observations on the mesoappendix of these latter animals showed no vascular hyperreactivity such as occurs in the terminal arterioles and precapillaries of hypertensive rats. There was also no hypertrophy of the blood vessels of the mesenteric capillary bed such as we have found to occur during the development of hypertension in rats. A previous study has shown that kidneys of hypertensive animals develop a metabolic lesion as a result of which VEM production, normally limited to anaerobiosis, now takes place aerobically as well as anaerobically. No such metabolic alteration was present in the kidneys of the adrenalectomized rats in this study. The relation of the content of renin in such kidneys and of the α_2 -globulin substrate in the blood of these animals will be discussed.

Muscular performance during positive radial acceleration LAURENCE E MOREHOUSE *Dept of Aviation Medicine, School of Medicine, Univ of Southern California, Los Angeles, Calif* Performance of pilots in a simulated cockpit on a human centrifuge was photographed by a motor driven high speed movie camera. Deviations in normal performance were studied. During acceleration, even at low levels, errors were repeated by trained subjects which would have marked effect on aircraft control. Physiological measurements of alterations in the functions of the nervous and muscular systems during positive radial acceleration indicate that, even at blackout g levels, normal function is intact. Errors in neuromuscular control, best shown by electromyographic records, suggested that alterations in proprioceptive sensations induced by g forces acting upon the moving segments played the major role in affecting performance.

Action potentials in single muscle fibers W L NASTUK and A L HODGKIN (introduced by M I GREGERSEN) *Dept of Physiology, College of Physicians and Surgeons, Columbia Univ, New York City, and the Physiological Laboratory, Cambridge, England* Graham and Gerard (*J Cell & Comp Physiol* 28: 99, 1946) and Ling (*Federation Proc* 7: 72, 1948) showed that the potential difference across the surface membrane of a resting muscle fiber could be measured directly by transverse impalement of the cell with a microelectrode. We have repeated these experiments using equipment capable of recording both action and resting potentials. Frog sartorius muscle, stretched in order to minimize movement, was stimulated either through its motor nerve or directly. Deflections in the record attributable to contraction were

often seen, but they did not appear until after the spike and under favorable circumstances might be absent altogether. Most of the experiments were carried out with electrodes which were filled with 3M KCl, but results qualitatively similar were obtained with electrodes filled with 0.118M KCl, but results qualitatively similar were obtained with electrodes filled with 0.118M KCl or with Ringer's solution. Electrodes filled with 3M KCl are believed to give the most accurate results since 1) junction potentials should be small, 2) electrode resistances are relatively low, 3) the quantity of KCl diffusing from the electrode during the time required for measurement can be shown to be negligible. In experiments at both 19°C and 7°C it was found that the action potential exceeded the resting potential in magnitude. It has been suggested that the membrane potential is reversed during activity because the membrane becomes selectively permeable to sodium during that period. If this is correct, one would expect that the membrane would cease to reverse its potential during activity if the sodium concentration in the external fluid is reduced to a low value. We have found that the resting potential and the action potential are approximately equal when the external sodium concentration is lowered to 30% of normal, and that over a considerable range the magnitude of the action potential is proportional to the logarithm of the external sodium concentration.

Adenosine phosphorylation by rabbit kidney homogenate DAVID RAPPORT, ATTILIO CANZANELLI, and RUTH GUILD (by invitation) *Dept of Physiology, Tufts College Medical School, Boston, Mass.* Rabbit kidney was homogenized in KCl NaHCO₃, and to the mixture was added MgCl₂ or SO₄ (0.01M), Na Succinate (0.01M), Na F (0.01M), and Sorensen phosphate buffer, (0.02M), the molarities representing final concentration. The final tissue dilution was 1:4. This was incubated aerobically alone or with added adenosine in final concentration of 0.03M, usually for 30 min at 37°. Phosphate and pentose were determined in the acid filtrate and in the barium and alcohol insoluble fractions. The evidence indicates that under our conditions adenosine, or one of its products, was phosphorylated, and that in the course of this phosphorylation, pentose disappeared. The resulting phosphate ester was not a mononucleotide, such as was reported by Colowick, Kalckar and Cori (*J Biol Chem* 137:343, 1941). The failure to find adenylic acid indicates that animal tissue behaves differently in this respect than yeast (Oster and Tersakowec, *Zeitschrift f. physiol Chem* 250:155, 1937). The results suggest that a mechanism similar to that described by Schlenk and Waldfogel (*Arch Biochem* 12:181, 1947) may be operating.

An enzymatic reaction involving amino acids and adenosine triphosphate JOHN M. REINER *Dept of Physiology, Tufts College Medical School, Boston, Mass.* In the search for an intermediate of peptide bond formation, the direct phosphorylation of amino acids by adenosine triphosphate was attempted. When a rat liver homogenate in isotonic KCl is incubated for 15 minutes at 37°C with 10 micromoles of adenosine triphosphate and 10 to 20 micromoles of amino acid, a labile phosphate compound is formed. With more prolonged incubation, the labile phosphate decreases, appearing as inorganic phosphate. A washed 'mitochondrial' fraction of the homogenate contains the activity, requires as a cofactor MnSO₄, the optimal concentration being 0.0016M, and inorganic phosphate. No labile phosphate is formed in the absence of amino acid, in the absence of adenosine triphosphate, or in the presence of only catalytic amounts of the latter. Activity has been obtained with glycine, methionine, arginine, histidine, tryptophan, and aspartic acid. The activity increases with increasing amounts of enzyme and of adenosine triphosphate. Activity is inhibited by 2,4-dinitrophenol. The product is extremely labile at pH 4 it is destroyed in 30 minutes at room temperature, and the hydrolysis is accelerated by molybdate, cold 5% trichloroacetic acid splits it within a minute or two, so that deproteinization must be performed with saturated ammonium sulphate. Negative results have been obtained with Lapmann's hydroxylamine reagent for acyl phosphates. This suggests that an N-phosphoamino acid may be involved, however, since it is not known how the adjacent amino group would affect the reaction of an acyl phosphate with hydroxylamine and the color formation with ferric ion, the alternative possibility cannot be ruled out.

A new blood supply for ischemic hearts, by connecting the aorta with the coronary vessels JOSEPH THOMAS ROBERTS *Univ of Buffalo School of Medicine, Buffalo and Veterans Adm Hospital, Batavia, N. Y.* The concept of revascularizing ischemic hearts by bringing aortic blood to the myocardium through the coronary sinus and veins was introduced earlier by the author. Since then others have confirmed his report that such revascularization inhibited myocardial infarction after ligation of the coronary arteries. This operation devised by the present author is now being applied to human patients suffering from myocardial ischemia. Subsequent studies on the procedure are brought up to date, as are efforts to anastomose the internal mammary artery with the coronary arteries and veins. It is predicted that this operation may become a useful method of treating the coronary artery disease problem. After such anastomosis, myocardial blood is apparently drained by the Thebesian vessels as well as by other routes.

Initial effects of potassium on the mechanical responses of skeletal muscle ALEXANDER SANDOW and ARTHUR J. KAHN (by invitation) *Washington Square College of Arts and Sciences, New York Univ., New York City* The present studies (supported by a grant from the Office of Naval Research under Contract N6on-279, Task order 7) deal with changes in the latency relaxation (LR) and peak tension (T) of isometric twitches of the frog sartorius immediately after immersion of the muscle in K-enriched Ringer's solution. Square-wave, massive, transverse shocks, applied to the muscle immersed in the test solution, are used as stimuli. The LR is recorded by the piezoelectric, cathode-ray method, and T by optical myography. During an initial period of 3 to 4 minutes after placing a muscle in a solution containing 28 mg % K, both the magnitude (R) of the LR and T increase. The alterations are reversed relatively slowly after the initial period, although T does so somewhat more slowly than R. At the end of the initial period, the maximal increases, when compared with responses in normal Ringer's (7.8 mg % K), are approximately R, 100% and T, 25%. Solutions containing some 50 mg % K or greater cause only immediate decreases in R and T, although initial increases, too fleeting to observe, may have occurred. The initial increases observed with the smaller potassium concentrations develop so rapidly, before any appreciable K had accumulated intracellularly, that it is inferred the added K could only have directly affected the behavior of the membrane excitatory mechanism. This view will be discussed in relation to the significance of the LR in the process by which excitation and contraction are linked in the response of the muscle fiber.

Action potentials of giant fibers of the crayfish, *cambarus clarkii* C. A. G. WIERSMA *Kerckhoff Labs of Biology, California Inst of Technology, Pasadena, Calif* Action potentials lead off from partially or totally isolated giant fibers of the central nervous system almost invariably show instead of a single phase under one electrode, a double phased deflection. The first phase is always positive, and depending on leading off conditions, varies in size from very small to as large as the subsequent negative deflection. All trials to account for this positive deflection as an artifact or a pick up of a distant potential by the inactive electrode, have thus far failed. Especially convincing for the independent nature of the positive deflection which occurs under the active electrode were experiments in which both leads were taken from a part of an isolated fiber, completely surrounded by oil. Under these circumstances there will be at first a diphasic potential with a monophasic lead (quadrupole phasic with bipolar leads). After a short time in oil the negative phase becomes smaller and disappears, leaving a large positive variation. On re-submerging of the fiber in physiological fluid and subsequent return to oil this order of events can be repeated several times. In fiber bundles a diphasic deflection remains under these circumstances, only the isolated giant fibers show this phenomenon. As a working hypothesis it may be supposed that these fibers contain two double layers of opposite sign. The inner, which would be responsible for the positive deflections, would use the very fluid interior axoplasm as a conductive medium and would hence not be influenced by the increase in outside resistance caused by oil.

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(For possible corrections in any of the following abstracts see the June issue)

Phenolsulfatase of animal tissues LYNN D ABBOTT, JR and MARY K EAST (by invitation) *Dept of Biochemistry, Medical College of Virginia, Richmond, Va* In order to establish more precisely the optimal conditions for the determination of animal phenolsulfatase activity, we have determined pH-activity curves with rat liver and kidneys and temperature-activity curves with rat liver using *p*-nitrophenylsulfate as substrate. The optimum pH appears to be slightly higher (about 6.6) than that of the phenolsulfatase of takadiastase (6.2). As was previously noted with the takadiastase enzyme, the rate of reaction was much greater at 50° than at 37°C. We have studied the phenolsulfatase activity of the sera and livers of normal rats, carbon tetrachloride-poisoned rats, and rats exposed to CCl₄ but protected by xanthine injection. The data indicate that the serum phenolsulfatase activity of carbon tetrachloride-poisoned rats was greater than that of control animals or of protected animals. The phenolsulfatase activity of the liver of carbon tetrachloride-poisoned rats appeared to be decreased.

Effect of uric acid on the peroxidative detoxification of diphtheria toxin KJELL AGNER (introduced by J W WILLIAMS) *Dept of Chemistry, Univ of Wisconsin, Madison, Wis* It has been previously reported (*Fed Proc* 7:140, 1948) that crude diphtheria toxin preparations are detoxified by peroxidases in the presence of H₂O₂. Purified toxin was not detoxified in experiments performed under similar conditions. The reaction required the presence of a dialyzable substance or substances present in the crude culture filtrate of diphtheria bacilli. It has now been found that the above crude filtrate can be replaced by a dilute solution of uric acid. Verdoperoxidase as well as horse radish peroxidase oxidize uric acid in the presence of low concentrations of H₂O₂. The rate of the destruction of uric acid is increased by the addition of diphtheria toxin. It seems likely that an intermediate oxidation product of uric acid reacts with the toxin to give detoxification.

Antimycin antibiotics KAMALUDDIN AHMAD (by invitation), F MERLIN BUMPUS (by invitation), B R DUNSHEE (by invitation) and F M STRONG *Dept of Biochemistry, College of Agriculture, Univ of Wisconsin, Madison, Wis* A new antibiotic, antimycin A, has been isolated from cultures of a

Streptomyces species. Antimycin A is a nitrogenous phenol of the probable formula C₂₅H₄₀O₆N₂. Alkaline hydrolysis destroys the antibiotic activity, and gives rise to a nitrogenous phenolic acid, a neutral, nitrogen-free product, and two moles of a volatile fatty acid. Antimycin A is highly toxic to many yeasts and other fungi, but is relatively inactive toward bacteria. Its effect on several of the more sensitive organisms is easily observable at concentrations of a few parts per billion. It inhibits the respiration of *Saccharomyces cerevisiae* Y30 and stimulates aerobic fermentation up to the anaerobic rate. It also inhibits the cytochrome oxidase and succinic dehydrogenase activities of rat liver homogenates at concentrations in the range of 10⁻⁶ molar. Paper chromatography of crude extracts of the original culture filtrate reveals the presence of two additional antibiotics, provisionally designated as Antimycin B and C.

Abnormal excretion of tryptophane-like substances in tuberculosis ANTHONY A ALBANESE, L EMMETT HOLT, JR, MARILYN LEIN (by invitation), EMILIE M SMETAK (by invitation) and BETTY VESTAL (by invitation) *Dept of Pediatrics, New York Univ College of Medicine, New York City* By the application of the tryptophane and amino N methods developed by some of us to the urines of patients of both sexes the following data were obtained:

Clin diag	Age group	No. of det.	Tryptophane amino N Total amino N, %
Non tubercular	3 m - 15 yr	55	2.6 ± 1.0
	2-14 yr	20	3.4 ± 0.7
	18-40 yr	48	4.5 ± 0.6
Tubercular	3-13 yr	32	6.7 ± 1.0
	20-62 yr	9	7.1 ± 0.8

Successive measurements on patients over a period of 2-3 years gave the impression that a) the T A N / A N ratios are consistently higher for chronic than for acute forms of tuberculosis, b) critical episodes are characterized by sharp increases in the ratio, whereas recovery is accompanied by a decrease in this value, and c) the abnormal T A N / A N ratios are not due to febrile

states Comparable T A N /A N ratios derived from urinary tryptophane figures obtained by the microbiological or Shaw-McFarlane techniques did not yield differentiating values for the tuberculosis patient as found by the Albanese-Frankston method. Moreover the high ratios do not arise from an increased output by the tubercular subject of a non-diffusible bound N component. This however does not exclude a possible increase in the output of diffusible bound N. The metabolic and nutritional implications of these observations will be discussed.

Biological value of wheat and corn proteins in the male infant. ANTHONY A. ALBANESE, L. EMMETT HOLT, JR., SELMA E. SNYDERMAN (by invitation), MARILYN LEIN (by invitation), EMILIE M. SMETAK (by invitation) and BETTY VESTAL (by invitation) *Dept. of Pediatrics, New York Univ. College of Medicine, New York City.* During the past year we have made measurements on the food value of some vegetable proteins in the infant. We felt this worthwhile for two reasons, a) vegetable protein containing diets might prove useful in feeding infants allergic to animal protein diets and, b) the use of vegetable protein diets might prove necessary if predictions regarding the failure of future food production to keep pace with population growth are realized. A synthetic diet containing lysine reinforced granulated wheat gluten (4% lysine content) as the principal N component was found to support N retention, body weight gains and blood protein concentration in 3 male infants equal to those achieved with an evaporated milk formula fed at comparable N, caloric and fluid levels. On the other hand a similar diet fed under similar conditions in which corn gluten reinforced with 6% L-lysine and 1% L-tryptophane constituted the principal protein component was found not to support N retention and weight gains at levels comparable to those attained with the evaporated milk formula. The inferior biological value of the corn gluten diet was not due to poor digestibility which we had previously found to be cause of the poor nutritional quality of commercial zein. In the light of recent investigations on the effects of amino acid imbalance on the growth of experimental animals reported from various laboratories we venture to suggest that the inferior nutritional quality of reinforced corn gluten in the infant may arise in part from its failure to provide an amino acid pattern compatible with growth.

Isolation of adenosine triphosphate from plant tissue. H. G. ALBAUM, M. OGUR (by invitation), and A. HIRSHFELD (by invitation) *Depts. of Biology and Chemistry of Brooklyn College, Brooklyn, N. Y.* We have previously reported (*Fed. Proc.* 7:141, 1948) an adenine-pentose-pyrophosphate in trichloroacetic acid extracts of the mung bean. The isolation of adenosine triphosphate (ATP) from a

plant source, seemed a necessary experimental step in the extension of common pathways of metabolism to plant tissues. The present work deals with the isolation of the plant ATP with a minimum purity of 70% when calculated as $\text{Ba}_2\text{ATP} \cdot 4\text{H}_2\text{O}$. A trichloroacetic acid extract of mung bean sprouts was fractionated with barium into two soluble fractions (pH 4.5-8, and pH 1-4.5), and a residue insoluble at pH 1. Approximately 80% of the material showing ultraviolet absorption at 260 $m\mu$ was found in the fraction insoluble at pH 1. This material, which may be dissolved and freed of barium by sodium sulfate exchange, represents an ATP extract of approximately 10% purity. Precipitation of the silver salt, decomposition with HCl, and reprecipitation of the barium salt increased the purity to approximately 50%. This barium salt is pigmented and shows absorption maxima at 320 $m\mu$ as well as at 260 $m\mu$. Removal of the barium by ion exchange, using Amberlite IR 100, and treatment with four volumes of acetone yielded a white barium salt with no absorption at 320 $m\mu$. This preparation has a ratio of pentose:adenine:labile P:total P of 1:1:2:3 and a minimum purity of 70%. It is active in the transphosphorylation of glucose by yeast hexokinase. After treatment with myokinase and hexokinase, it is deaminated by a muscle deaminase at a slow rate compared with animal ATP.

In vitro inhibition of hyaluronidase activity. HARVEY E. ALBURN, ROBERT W. WHITLEY and LOUISE C. HALL (introduced by SAM SEIFTER) *Wyeth Institute of Applied Biochemistry, Philadelphia, Pa.* In order to study the nature and amount of hyaluronidase inhibitor present in hyaluronic acid preparations, the inhibition by heparin and other sulfated polyuronides and the neutralization of this inhibition by salts and protamine were investigated. The hyaluronidase activity was determined by a modification of the Kass and Seastone turbidimetric method and a highly purified hyaluronic acid from human umbilical cord was used as the substrate. With 0.1 mg of hyaluronate and sufficient hyaluronidase to hydrolyze it almost completely in 1 ml of pH 6.0, 0.1 M potassium acetate buffer in 30 min, 0.0033 gamma of heparin gave detectable inhibition and 0.33 gamma gave maximal inhibition in the absence of sodium chloride. In the presence of 0.075M sodium chloride, the optimal concentration, 10 gamma of heparin was required to give detectable inhibition. Similar data were obtained for other salts and other polyuronides. These data were used to calculate the amounts of inhibitor in various hyaluronate preparations. Protamine was found to neutralize the inhibition of hyaluronidase by heparin in very low concentrations, probably due to combination with the heparin. In higher concentrations protamine was found to inhibit hyaluronidase activity.

Effects of 2-acetylaminofluorene on liver func-

tion JAMES B ALLISON, ARTHUR W WASE (by invitation) and WALTER W WAINIO (by invitation) *Bureau of Biological Research, Rutgers Univ, New Brunswick, N J* Studies are underway on the effects of the carcinogenic agent, 2-acetylaminofluorene, on liver function in dogs and rats. The animals are being fed a synthetic diet (*J Nutrition* 29 413, 1945) consisting of casein 12%, lard, carbohydrate, agar, minerals, synthetic vitamins and 0.03 to 0.05% 2-acetylaminofluorene. Preliminary results on the dogs demonstrate that after 4 months subsistence on this diet the excretion of uric acid increased markedly while the excretion of allantoin decreased, changes which have been associated with decreased uricase activity in damaged livers. Decreased liver function was indicated also by an increased retention of bromsulfalein, and by jaundice. At the end of 6 months a hepatoma had developed which was sufficiently advanced in 1 dog to cause death. The neoplasm had spread also to the spleen in this animal. The correlation between these and other metabolic changes associated with the development of hepatomas are being followed together with the retention of nitrogen in the body of the animal.

Administration of parenteral and oral casein hydrolysates to depleted dogs. CARL ALPER (by invitation), BACON F CHOW and SHIRLEY Q DELBRASE (by invitation) *Nutrition Dept, Div of Development, and the Div of Protein Chemistry, The Squibb Institute for Medical Research, New Brunswick, N J* Mongrel dogs, previously depleted by feeding a protein-free diet at normal caloric intake, with or without the accompaniment of plasmapheresis, were fed parenterally for a period of four weeks, and then orally for a similar period, a tryptic digest of casein as the sole source of nitrogen at a level sufficient to maintain positive nitrogen balance. The object of this experiment was to study the effectiveness of parenterally and orally administered casein hydrolysates in the repletion of plasma protein, and the maintenance of positive N balance. Maintenance of N balance, and the repletion of plasma protein were measured by urine, fecal, and blood protein N determinations and blood-volume determinations. Results of these experiments, which were carried out in 10 dogs, showed 1) no striking increments in weight during repletion, and 2) dogs retained 2 to 3-fold more N via the oral route than via the intravenous route, even though partial repletion had already been effected by the intravenous route. Also it was observed that, contrary to the current belief, the degree of hydrolysis varying from 18 to 40% did not appear to influence the utilization of N. Between 5-9% of the N retained by parenteral feeding was utilized for plasma protein synthesis. About 15-25% of the N retained by oral feeding was utilized for plasma protein synthesis.

Microbially synthesized APF (animal protein factor) STEFAN ANSBACHER, HARLEY H HILL, JR (by invitation), JESSE W TIEMAN (by invitation), JEAN F DOWNING (by invitation) and JOHN H CALDWELL, JR (by invitation) *Research Laboratory, Vi-D-Co, Marion, Ind* The need of single-stomached animals for APF appears to increase when increasing amounts of soybean oil meal (SBOM) are fed. It would seem logical, therefore, to impart APF potency to SBOM. This has been accomplished by growing certain micro-organisms on media, the principal constituent of which was SBOM. Yeast, although an excellent source of the vitamin B-complex, has normally no APF activity, but it too may be endowed with high APF potency by certain fermentation processes. The micro-organisms that selectively synthesize APF have been isolated for use in these processes. The physiological activity is expressed in APF units. Fish meal, fed to chicks at a level of 5% of the basal (all-plant) ration, has been assigned a potency of one unit. If a product fed at the same level causes chicks to grow approximately as well as those receiving the fish meal ration, it has a potency of one unit. The potency is two units, if the oral administration of a product at 2½% results in growth about equal to that obtained from the fish meal ration. Products containing at least 20 APF units have been obtained by fermentation processes without resorting to chemical purification procedures.

Chromatography of urinary steroids and products of acid hydrolysis of pure steroids. R M ARCHIBALD *Hospital of the Rockefeller Institute for Medical Research, New York City* CCl_4 extracts of hydrolysates of 10 to 50 cc urine have been chromatographed on columns in a Technicon fraction cutter. Eluting agents used were benzene, benzene plus 0.1%, 0.5%, and 2.0% ethyl alcohol, 95% ethyl alcohol, water, and 50% pyridine in water. Ultraviolet absorptions at 215, 240, 260 $\text{m}\mu$ were determined on each of ninety 3 cc fractions, then aliquots of each were used for Zimmermann, Liebermann-Burchard and modified Kober (color and fluorescence) reactions, and the colorimetric method of Munson, *et al* for dehydroisoandrosterone. By these methods comparisons were made of chromatograph fractions of extracts obtained after hydrolysis of pure steroids and urines with HCl , H_3PO_4 , H_2SO_4 , or NaOH a) at room temperature b) after reflux boiling 30 minutes. Application of these techniques to urine of a woman before and after surgical removal of a hirsutizing adrenal tumor, and to an extract of the tumor, indicated the presence of a large amount of material easily eluted with benzene in the tumor and in the urine prior to operation, but absent from urine after operation.

Determination of dimethylethanolamine in biological materials. CAMILLO ARTOM and MARIETTA CROWDER (by invitation) *Dept of Biochemistry,*

Bowman Gray School of Medicine, Winston-Salem, N C The method is based on the observation that dimethylethanolamine (DME), a likely intermediate in choline metabolism, is rapidly and quantitatively displaced from its alkaline solutions by steam distillation. The protein-free material is treated with HNO_2 to destroy primary and secondary amines, transferred to the distilling flask of the Parnas-Wagner apparatus, brought to pH 10, and aerated at room temperature to remove volatile tertiary amines. DME is then steam distilled for 15 minutes into an excess of standard acid and the excess acid titrated iodometrically with 0.005 N $\text{Na}_2\text{S}_2\text{O}_3$. 95 to 100% of the substance in pure solutions or added to tissue extracts could be recovered. Choline, ethanolamine, methylethanolamine, serine, urea, glycine, betaine or trimethylamine do not interfere appreciably. The method has been applied to aqueous extracts of liver and muscle, deproteinized with colloidal $\text{Fe}(\text{OH})_3$, and to acid hydrolysates of the lipides, extracted from the tissues of rats under various dietary conditions. Traces of DME were found only occasionally, the results being negative in most determinations. However, quite definite amounts of DME were detected in the liver lipides of rats, killed 3 or 6 hours after the administration of a single large dose of the compound. This finding indicates that DME can be incorporated in the phospholipide molecule even before further methylation to choline. Determinations of DME in the tissues of animals receiving large doses of choline or ethanolamine are in progress.

Response of pteroylglutamic acid (PGA) depleted rats to supplementation with natural products CONRADO F. ASENJO *Dept. of Chemistry and Nutrition, School of Tropical Medicine, San Juan, Puerto Rico* PGA-depleted rats, 7 weeks old, were continued on the highly purified PGA-free diet, containing 2% succinylsulfathiazole, but, in addition, were given daily weight amounts of natural products for a period of 5 consecutive weeks. The amount of the supplement was never more than 2 gm, so as not to interfere to any great extent with the basal diet intake. Two % by weight of SST was mixed with the daily supplement. Growth response, white cell regeneration, degree of alopecia, and spleen condition were recorded at the end of the supplementation period. As the depleted rat requirement of PGA to regain normalcy is approximately known (*J. Nutrition*, 38: 601, 1948), the response exhibited to the different supplements by the depleted animals can be evaluated roughly in terms of PGA. Of 10 fresh tropical fruits so far assayed, the only one to exhibit appreciable curative action on the rat was the avocado. Boiled and dried pigeon peas, red kidney beans, and breadfruit seed proved to be rich sources of PGA, inducing very rapid and pronounced recovery. In the present

report the results obtained with 20 natural products commonly used in the West Indian dietary, especially tropical products, are presented.

Action of ribonuclease on ribonucleic acid JAMES E. BACHER (by invitation) and FRANK WORTHINGTON ALLEN *Div. of Biochemistry, Univ. of California Medical School, Berkeley, Calif.* Ribonucleic acid from yeast was purified and dialyzed, neutralized to pH 7.0 and subjected to ribonuclease action. Dialysis was continued and the dialyzable and non-dialyzable fractions were isolated by lyophilization. Control experiments without enzyme were treated similarly. Application of rate studies to fractions that were isolated gave the following data:

	Ribonucleic Acid	Control Na salt	Dialyzable Fraction	Non-dialyzable Fraction
	%	%	%	%
Total N	15.4	13.7	12.3	13.7
Guanine N				
Total N	34.3	34.9	24.6	39.2
Purine N				
Total N	67.9	67.4	53.8	73.0
Total P	9.0	8.2	8.3	7.8
Labile P				
Total P	50.6	51.2	34.6	57.3
Stable P				
Total P	49.4	48.8	65.4	42.7
	atoms	atoms	atoms	atoms
Purine N	5.06	4.89	5.11	4.98
Labile P				
Pyrimidine N				
Stable P	2.44	2.48	2.32	2.47

Ribonucleic acid contains approximately equivalent quantities of each nitrogenous constituent. After ribonuclease action the dialyzable fraction, which comprises 25%, contains 2 pyrimidines per purine, more adenine than guanine and approximately twice as much uracil as cytosine. The non-dialyzable fraction, which comprises 70%, reflects the contrary.

Transaminase activity in the skeletal muscles of normal and vitamin E-deficient animals MARY ALICE BARBER (by invitation), DANIEL H. BASINSKI (by invitation) and H. A. MATILL *Dept. of Biochemistry, State Univ. of Iowa, Iowa City, Iowa* To examine further into the altered energy metabolism accompanying the dystrophy of vitamin E deficiency, aspartic α -ketoglutaric transaminase activity was investigated in skeletal muscle homogenates from normal and E-deficient guinea pigs and rabbits. In dystrophy, this trans-

amination reaction was decreased by $\frac{1}{2}$ in guinea pigs and by $\frac{1}{3}$ in rabbits. The diminution of activity was consistent whether the results were calculated on the basis of wet weight of the tissue or on the dry weight or N content of the homogenate. The concentration of the coenzyme, pyridoxal phosphate, appeared not to be the limiting factor. The reduced activity was not an artifact produced by altered removal of the oxalacetic acid formed. On the contrary, normal muscle seemed to possess a better alternative mechanism for disposing of excess oxalacetate than did dystrophic muscle. The significance of these results in the interpretation of the abnormal energy metabolism of these paralyzed muscles is briefly discussed.

Intracellular heterogeneity of liver ribonucleic acid as evidenced by P^{32} uptake. CYRUS P. BARNUM and ROBERT A. HUSEBY (by invitation), *Depts. of Physiological Chemistry and Physiology, Univ. of Minnesota Medical School, Minneapolis, Minn.* From fasted, homogenized mouse livers ribonucleic acid (RNA) has been isolated from the nuclei (N-RNA), the cytoplasmic particulate fractions (M-RNA), and the cytoplasmic fraction that does not sediment in 90 minutes at 23,000 *g* (MS-RNA). The mice had been injected intraperitoneally with inorganic P^{32} at times varying from 15 minutes to 24 hours prior to sacrifice. The N-RNA shows a specific activity (S.A. -disintegrations/min./ γ total P) which rises rapidly so that at 1 hour it is 14% of the S.A. of tissue inorganic P (I.P.) as compared to cytoplasmic phospholipid P for which the S.A. is 10.5% that of I.P. The S.A. of N-RNA passes through a maximum around 3 hours and is below that of phospholipid at 6 hours. The M-RNA takes up P^{32} very slowly and at 1 hour its S.A. is only about 0.6% that of N-RNA but rises to 3.1% at 3 hours and 21.5% at 20 hours. No significant differences have been observed in the S.A. of M-RNA samples obtained from mitochondria (sedimented in 5 minutes at 23,000 *g*) or microsomes (sedimented in 90 minutes at 23,000 *g*). The MS-RNA follows a S.A. time curve intermediate between those of N-RNA and M-RNA. At 1 hour its S.A. is about 6.8% that of N-RNA while at 3 hours it is 9.1% and at 20 hours 30.5%. These data point up the relationship between intracellular location of RNA and its rate of incorporation of newly administered inorganic phosphate.

Anterior pituitary growth hormone preparations and the glutamine-glutaminase system. PAUL D. BARTLETT (introduced by OLIVER H. GAEBLER), *Edsel B. Ford Institute for Medical Research, Henry Ford Hospital, Detroit, Mich.* Van Slyke *et al.* (*J. Biol. Chem.* 150: 481, 1943) have shown that transport and storage of ammonia are physiological functions of glutamine. Storage of amino nitrogen might be another function, and, in this respect,

glutamine might be considered a major constituent of Schoenheimer's 'metabolic pool' of nitrogen. Since in this and other laboratories weight gain and nitrogen storage have been produced in rats and dogs with growth hormone preparations it seemed of interest to study the *in vivo* effect of the recent Fishman, Wilhelm, Russell preparation on the glutamine-glutaminase system. In 19, 20, and 28 day experiments on dogs 3 different lots of this preparation were tested. Increases in plasma glutamine of 19, 30, and 33% above the average control level were obtained. Nitrogen storage and weight gain were produced and excretion of urinary ammonia was found to parallel changes in plasma glutamine. Experiments now in progress will determine whether or not the increase in plasma glutamine occurs independently of total free amino acids. In *ad libitum* feeding experiments on hypophysectomized rats and on hypophysectomized rats treated with growth hormone glutaminase assays of 21 and 31 μ /ml of 25% kidney emulsion respectively were obtained. Food consumption of treated rats did not increase during the 7-day experimental period. Kidneys from normal rats maintained on a food intake based on that of the hypophysectomized-treated rats and from normal rats fed *ad libitum* assayed respectively 35 and 56 μ /ml of 25% emulsion.

Behavior of the thyroid toward elements of the seventh periodic group II Rhenium. EMIL J. BAUMANN, N. ZIZMER (by invitation), ELEANOR OSHRY (by invitation) and S. M. SEIDLIN (by invitation), *Laboratory Div. and Laboratory of Medical Physics, Montefiore Hospital, New York City.* Two years ago we presented experiments to the Society which showed that the thyroid filters not only I from the circulation but other halogens as well. We expressed the view that the thyroid distinguishes between the halogens imperfectly if at all in the filtration process. Further, it was suggested that if a very large amount of one of the halogens were administered, compared to the amount of any of the other halogens ingested, the one given in excess will tend to wash the others out of the thyroid in a mass action like effect. On the basis of other work, we postulated then that other elements of group VII of the Periodic Table would behave as the halogens do and we now report our experiments with Rhenium (element 75). Perrhenic acid given parenterally to rats and rabbits was found to accumulate in the thyroid in greater concentration than in any other tissue—25 to over 100 times more than in the tissue having the next highest concentration. The maximum concentration occurs 1 to 4 hours after injection. Most of the Re is excreted in the urine during the first day and the remainder is practically all eliminated in the next 24-hour period.

Effects of glucose, bile salts, and protein on re-

covery of neutral 17-ketosteroids WILLIAM T BEHER (by invitation) and O H GAEBLER *Edsel B Ford Institute for Medical Research, Henry Ford Hospital, Detroit, Mich* Rates of color development of individual 17-ketosteroids, and effects of varying other conditions in the Zimmermann reaction, have been studied extensively (*Endocrinology* 33 189) Little attention has been given to effects of normal and pathological constituents of bile and urine on recovery of 17-ketosteroids In studying this problem, we have used a method involving the usual hydrolysis and continuous extraction The Zimmermann reaction was then applied after removing the estrogens, or after separating the neutral 17-ketosteroid fraction with Girard's reagent, according to the general scheme outlined by Pincus (*Clinical Endocrinology* 5 291) In either case, 2 color spectrophotometry was the final step Additions of glucose to solutions of free 17-ketosteroids (androsterone and dehydroisandrosterone), or to urine, did not influence the results Addition of bile salts or purified human serum albumin to solutions of free 17-ketosteroids did not affect the results when solutions were analyzed without preliminary hydrolysis, but lowered them when hydrolysis was included Low values due to addition of protein could be overcome by doubling the extraction period, but bile acids apparently increased the destruction of ketosteroids during hydrolysis, so that longer extraction did not influence the result Analysis of urines with and without addition of bile salts or purified human serum albumin showed that the same findings apply when one deals with conjugated 17-ketosteroids Effects of ultrafiltering to remove protein, and of varying the acid used in hydrolysis, were also studied

Intravenous administration of commercial cytochrome C to rats previously treated with radioactive iron HELMUT BEINERT and KURT R REISSMANN (introduced by FRED W OBERST) *USAF School of Aviation Medicine, Randolph Air Force Base, Texas* The biosynthesis of iron-labeled cytochrome c showing a radioactivity suitable for tracer studies has been reported previously (*Science* 108 634, 1948) Experiments were made to obtain knowledge about the penetration of injected cytochrome c into tissue cells Two pairs of litter-mate rats previously treated with radioiron were nephrectomized unilaterally One animal of each pair was then injected intravenously with commercial cytochrome c (8 mg) Immediately thereafter, both pairs were taken to a simulated altitude of 20,000 ft to favor utilization of injected cytochrome, if such actually does occur All animals were sacrificed 24 hours after injection, to assure return of tissue cytochrome concentration to pre-injection level Kidneys, hearts, and skeletal muscle were assayed for cytochrome concentration

and the isolated cytochrome was checked for radioactivity Comparative tests showed that the cytochrome content per unit weight of the left and right kidney removed under the conditions of the experiment generally does not differ by more than $\pm 5\%$. Unilateral nephrectomy, therefore, represents a useful means of internal control By this technique and comparison with the control litter-mates no significant elevation of the cytochrome values in the organs of the injected animals was found, indicating that no appreciable quantity of cytochrome was left in intercellular spaces No significant dilution of the originally occurring radioactive cytochrome could be demonstrated in the cytochrome-injected animals Iron analysis indicates that at least 94% of the measured radioactivity is due to cytochrome iron This experiment does not give evidence of any significant incorporation of injected cytochrome c into tissue cells

The fate of ingested uric acid in man AARON BENDICH (by invitation), WILLIAM D GEREN (by invitation), OSCAR BODANSKY and GEORGE BOWORTH BROWN *The Sloan-Kettering Institute for Cancer Research, New York, N Y* A normal male ingested 150 mg of uric acid labeled with an excess of N^{15} in positions 1 and 3 Total urine collections were made over specific periods for 7 days and were analysed for total nitrogen, uric acid, urea and ammonia For isotopic nitrogen analyses, uric acid was isolated by adsorption on Norite, elution with dilute alkali and purified by repeated crystallization Urea was obtained as the dixanthidol derivative and urinary ammonia was isolated The course of the urinary excretion of each of the stated compounds, as well as their isotope content, was determined During a period of 4 days, 80% of the administered isotope appeared in the urine Of this amount, about $\frac{1}{4}$ was present as urea N and about $\frac{1}{4}$ as uric acid N The results are discussed with respect to the question of uricase in human tissues, to bacterial action in the intestine, and to the relative contribution of purine nitrogen to urinary urea and uric acid nitrogen

Human uric acid metabolism JEAN D BENEDICT (by invitation), PETER H FORSHAM (by invitation) and DEWITT STETTIN, JR *Dept of Biological Chemistry, Harvard Medical School, Dept of Medicine, Peter Bent Brigham Hospital, Boston, Mass, and the Div of Nutrition and Physiology, Public Health Research Institute of The City of New York, New York City* Uric acid has been synthesized in such a fashion as to include a high concentration of N^{15} in the pyrimidine ring and this material has been administered intravenously in tracer dose to human subjects while maintained in balance on a purine-poor diet From the concentrations of N^{15} in the urinary uric acid samples in the succeeding period it has been possible to estimate 1) the quan-

tity of uric acid which mixed with and diluted the injected isotopic uric acid, 2) the rate of *in vivo* formation of uric acid in man under these circumstances. These quantities have been compared with the prevailing level of plasma uric acid during the experiment and with the independently determined rate of urinary uric acid excretion. Experiments have been conducted on both normal subjects and patients suffering from gout.

Partial purification and properties of hydantoinase FREDERICK BERNHEIM, MARY L C BERNHEIM (by invitation) and G S EADIE (by invitation) *Depts of Physiology, Pharmacology and Biochemistry, Duke University Medical School, Durham, N C*. The enzyme in rat liver which hydrolyzes hydantoin to hydantoic acid has been partially purified by removing proteins insoluble in slightly alkaline and acid solutions and by dialysis. The enzyme remains in a clear solution 0.25 ml of which hydrolyzes under optimal conditions 4.0 mg of hydantoin in 15 minutes. The solution also contains an active esterase for simple esters but only traces of choline, demerol and procaine esterases. If the activity is measured by the liberation of CO_2 , the end point depends on the pH probably because of the simultaneous formation of an NH_2 group. That the end point at any pH corresponds to the quantitative formation of hydantoic acid was proved by the determination of the latter with the diacetyl monoxime reagent. The enzyme does not hydrolyze parabanic acid, dimethyl- or methyl ethyl hydantoin but they inhibit its action on hydantoin. Its pH optimum is about 8.6. The reaction is monomolecular throughout the greater part of the hydrolysis. The relation between initial velocity and substrate concentration is of the Michaelis-Menten type. Preliminary experiments indicate a dissociation constant not far from 0.01, showing relatively small affinity between enzyme and substrate.

Conversion of carotene to vitamin A by the rat J G BIERI (introduced by M O SCHULTZE) *Div of Agricultural Biochemistry, Univ of Minnesota, Minneapolis, Minn*. Further evidence for the conversion of carotene to vitamin A by hypothyroid rats is presented from studies of the blood serum vitamin A content of hypothyroid rats given carotene. Two of four groups of rats on a vitamin A-deficient ration were made hypothyroid by feeding 0.2% thiouracil. When the animals showed weight plateaus, definite xerophthalmia and no measurable serum vitamin A, 200 γ of B-carotene in Tween 80-water (1:1) was given weekly for 5 weeks orally or intramuscularly. Xerophthalmia disappeared. The hypothyroid rats gained weight for 15-19 days only, the others continued to gain. Vitamin A in blood serum determined by the method of Bessey before, and 12 hours after, the last administration of carotene showed the following:

Thyroid	Carotene	Serum Vitamin A, $\gamma\%$	
		Before	After
Normal	Oral	7.2	13.7
Hypothyroid	Oral	8.3	21.6
Normal	Intramuscul	7.0	10.2
Hypothyroid	Intramuscul	5.8	26.3

Several laboratories have reported that the small intestine is involved in the conversion of carotene to vitamin A by the rat. Preliminary studies show that vitamin A-deficient rats given 400 γ of B-carotene in Tween intramuscularly after surgical removal of the small intestine have in the blood serum, after 5 hours, appreciable amounts (equivalent to 12-50 $\gamma\%$ of vitamin A) of a fat soluble light-sensitive material absorbing at 328 m μ . Operated control rats given Tween show no appreciable change in serum fat-soluble components absorbing at 320-350 m μ .

Cystathionase FRANCIS BINKLEY *Laboratory for the Study of Hereditary and Metabolic Disorders, Univ of Utah School of Medicine, Salt Lake City, Utah*. The enzyme responsible for the conversion of cystathionine to a compound reacting in the test of Sullivan for cysteine has been obtained in a condition of high purification from rat and pig liver. The purified enzyme was obtained in crystalline form and was dissociated, under acid conditions, into an inactive crystalline protein and an alcohol-soluble coenzyme. The inactive protein was reactivated by incubation with the coenzyme or a boiled extract of liver at values of pH near 8. Magnesium ions and inorganic phosphate were required for the activity of the enzyme but it was not possible to demonstrate an uptake of phosphate in the reaction. The rate of reaction was dependent upon the concentration of inorganic phosphate as well as upon the concentration of cystathionine or enzyme. The enzyme was active in the desulfhydration of cysteine, inorganic phosphate was required for the desulfhydration of cysteine. Cysteine was not a primary product of the action of the enzyme on cystathionine, the product responded to the Sullivan reaction but not to the reaction of Nakamura and Binkley. If, however, the reaction product were incubated with crude extracts of liver or kidney, cysteine was produced. Whereas iodometric titrations indicated a complete cleavage with the purified enzyme, the Sullivan reaction indicated less than 40% cleavage.

Hydrolysis of conjugated 17-ketosteroids in urine concentrates by acetate buffers JOEL BITMAN (by invitation) and SAUL L COHEN *Dept of Physiological Chemistry, Univ of Minnesota Medical School, Minneapolis, Minn*. Butanol extracts of male urine prepared for studies on the hydrolysis of concentrates of the conjugated ketosteroids

showed a considerable loss (up to 45%) of these conjugates from the butanol extracts if the extraction was carried out at pH above 3.0. Similar losses occurred if the butanol concentrates were washed with buffers of pH above 3.0. This indicates the presence in urine of some conjugated ketosteroids whose salts are more soluble in water than in butanol. The BaCl_2 -acetate buffer hydrolytic technique of Talbot *et al* (1943) when applied to butanol extracts of normal male urine liberated 20–40% of the total ketosteroids, of which 40–60% was of the beta type. These amounts were independent of the pH of the urine existent when the butanol extracts were prepared. The same degree of hydrolysis occurred even when the BaCl_2 was omitted from the hydrolytic mixture and the butanol residues were heated with buffer alone. Hydrolysis with pH 5.8 acetate buffer was found to be 85% complete in 4 hours. No marked increase in the proportion of ketosteroids liberated by buffers occurred when the buffer pH was reduced until a pH of 3.0 or less was achieved. These results tend to indicate the presence in normal male urine of a specific group of conjugates whose salts are much more soluble in butanol than in water and which are sufficiently labile to be hydrolyzed by relatively mild conditions.

Influence of magnesium and cobalt on the inhibition of phosphatases by α -amino acids OSCAR BODANSKY *Memorial Hospital for The treatment of Cancer and Allied Diseases and the Sloan-Kettering Institute for Cancer Research, New York City*. In the presence of inhibiting concentrations of α -amino acids or cyanide the usual activating effect of magnesium on bone phosphatase was decreased and in some instances was transformed into a retardant effect. The extent of the activating effect of cobalt on bone phosphatase was decreased slightly as the concentration of amino acid was increased. In the conjoint presence of cobalt and magnesium, accomplished either before or after the start of the reaction, the degree of activation of bone phosphatase which had been inhibited by amino acids was much greater than with either cobalt or magnesium alone and counteracted to varying degrees the inhibition by amino acid. The activating effect of magnesium on intestinal phosphatase was unaffected as the concentration of amino acid was increased, the activating effect of cobalt was decreased. The extent of activation of intestinal phosphatase which had been inhibited by amino acids was about the same in the conjoint presence of cobalt and magnesium as in the presence of magnesium alone. Human osteogenic sarcoma phosphatase resembled normal rat bone phosphatase with respect to the extent of inhibitions by various amino acids and the influence of cobalt and magnesium on these inhibitions. The present findings are interpreted to indicate that α -amino acids inhibit bone, intestinal and osteo-

genic sarcoma phosphatases by combining with an essential metal component of these enzymes and that this component normally mediates the activation of bone and osteogenic sarcoma phosphatases, but not of intestinal phosphatase, by magnesium.

Quantitative separation and determination of small amounts of histidine and tyrosine employing paper chromatography DIANA BOLLING (by invitation), HERBERT A. SOBER (by invitation) and RICHARD J. BLOCK *Dept of Biochemistry and Physiology, New York Medical College, Flower and Fifth Ave Hospitals, New York City*. Pauly (1904) reported that histidine and tyrosine gave a red color when treated in alkaline solution with freshly diazotized sulfanilic acid. The use of this reaction for quantitative determinations has been limited by the difficulties in obtaining complete separation of these 2 amino acids. The excellent technique for the separation of amino acids by paper chromatography, introduced by Consden *et al* (*Biochem J* 36: 224, 1944), and the adaptation of this method for their estimation, based on the observation that the concentration of an amino acid on the paper was proportional to the area of the spot multiplied by the color density (Block, *Science* 108: 608, 1948), suggested that the Pauly reaction could be readily adapted to the determination of histidine, tyrosine, etc. Method: Histidine and tyrosine are separated by chromatography using a mixture of n-butanol, 100 parts, glacial acetic acid, 10 parts, saturated with water. Ascending chromatograms on S&S 598 or 596 paper are run for 3 hours. The paper is removed, dried, and sprayed with freshly diazotized sulfanilamide dissolved in n-butanol. The paper is allowed to air-dry for exactly 5 minutes. It is then sprayed with an emulsion of 5% sodium carbonate in 2 volumes of butanol. The chromatograph is air dried and the quantities of histidine and tyrosine are determined from the product of the color area times density. The results obtained on casein, zein and hemoglobin agree with the better values in the literature.

Influence of pituitary and adrenal glands on ketone body production by rat liver slices PHILIP K. BONDY (by invitation) and ALFRED E. WILHELM *Dept of Physiological Chemistry, Yale Medical School, New Haven, Conn*. Slices of rat liver were incubated in Krebs Ringer medium, with phosphate buffer, pH 7.4, in oxygen at 38°C. Ketone bodies were determined by the method of Greenberg and Lester on the total contents of the flask. All animals were fasted 18 hours before the experiment. Livers from normal rats produce $2.4 \pm 1.6 \mu\text{M}/100 \text{ mg}$ (dry weight) in 15 minutes, and $8.8 \pm 4.6 \mu\text{M}/100 \text{ mg}$ in 120 minutes, an increment of $3.5 \pm 4.3 \mu\text{M}/100 \text{ mg}/\text{hour}$. The rate of appearance of ketone bodies was constant from 5 minutes to 120 minutes. Livers from adrenalectomized rats formed $2.5 \pm 1.9 \mu\text{M}/100 \text{ mg}$ in 15 min-

utes, and $10.2 \pm 4.3 \mu\text{M}$ in 120 minutes, an increase of $4.4 \pm 2.9 \mu\text{M}/100 \text{ mg}/\text{hour}$. These results are not significantly different from the data obtained from normal rat livers. The livers of rats hypophysectomized several months previously produced $1.5 \pm 1.0 \mu\text{M}/100 \text{ mg}$ in 15 minutes, and $3.7 \pm 2.0 \mu\text{M}/100 \text{ mg}$ in 120 minutes, a rate of $1.3 \pm 2.0 \mu\text{M}/100 \text{ mg}/\text{hour}$. These values are significantly lower than those obtained with either normal or adrenalectomized animals. In normal rats, the body weight was found to be a significant factor, since liver slices from heavier rats produced more ketone bodies than did slices from lighter animals ($r = 0.8428$, p less than 0.05). This correlation was observed only in the 120-minute period. No such relationship between body weight and ketone body formation could be demonstrated for hypophysectomized or adrenalectomized animals.

A microbiological determination of D-phenylalanine ERNEST BOREK and HEINRICH WAELSCH *Depts. of Biochemistry, New York State Psychiatric Institute and Columbia Univ., New York City*. The microbiological determination of D-amino acids has been previously achieved by the use of two different varieties of microorganisms one of which utilizes only the L-amino acid, and the other, either the D or the D-L form. During studies of the metabolism of phenylalanine and its derivatives in *Lactobacillus Arabinosus* we found that, while these organisms can utilize only L-phenylalanine, they readily metabolize both optical antipodes of phenyllactic acid in lieu of the amino acid. Since the growth response of the microorganisms is nil to D-phenylalanine but complete to D-phenyllactic acid, and, since the chemical transformation of the amino acid to its hydroxy analogue is almost quantitative, a convenient determination of the D-amino acid is possible. Samples of D-L phenylalanine in microgram quantities, produce, after reaction with nitrous acid, almost twice the growth response of an identical, untreated aliquot. The blood of patients with oligophrenia phenylpyruvica has been subjected to a search for D-phenylalanine which has been claimed to accumulate in these subjects. Within the limits of error of the method the phenylalanine, which circulates in concentrations as high as 30 mg % the blood of these subjects is the L-isomer.

Incorporation of labeled lysine into the proteins of guinea pig liver homogenate HENRY BORSOOK, CLARA L. DEASY (by invitation), ARIE J. HAAGENS-MIT (by invitation), GEOFFREY KEIGHLEY (by invitation) and PETER H. LOWY (by invitation) *Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena, Calif.* The incorporation of L-lysine (labeled with C^{14} in the ϵ -position) into the proteins of guinea pig liver homogenate was studied using either the whole

homogenate or the sedimented fraction obtained by centrifuging the 15-fold diluted homogenate at 2500 g. Characteristics of the reaction with the whole homogenate are: its optimum pH is at 6.1, the presence of calcium is obligatory, the reaction proceeds as well in nitrogen as in oxygen, it is inhibited nearly completely by fluoride, and less completely by arsenate, arsenite, azide, cyanide and dinitrophenol, the concentration of L-lysine incorporated is approximately a linear function of the lysine concentration and is independent of the homogenate concentration. Characteristics of the reaction with the sedimented fraction are: its optimum pH is approximately 7.3, calcium accelerated the reaction only slightly and its presence is not obligatory, it proceeds under nitrogen but more slowly than under oxygen, it is inhibited slightly by fluoride and practically uninhibited by arsenate, arsenite, azide, or dinitrophenol, the concentration of lysine incorporated is a function of the total amount of lysine in the reaction mixture. The rate of incorporation of lysine into the proteins of the whole homogenate is approximately the same as that *in vivo*, the rate with the sedimented fraction is several times faster.

Passage of hemoglobin through the glomerular membranes and its excretion by the amphibian kidney PHYLLIS A. BOTT *Dept. of Physiological Chemistry, Woman's Medical College of Pennsylvania, Philadelphia, Penna.* Kidneys of amphibia were perfused with Ringer's solution containing human hemoglobin prepared by Drabkin's method (*J. Biol. Chem.* 164: 703, 1946). 'Direct' glomerular puncture experiments and 'indirect' (hemoglobin-inulin) experiments were made with methods similar to those used previously with other proteins (Bott and Richards, *J. Biol. Chem.* 141: 291, 1941). Indirect experiments. Hemoglobin excretion in ureteral urine was influenced by pH as in the experiments of Webster, Engel, Laug and Amberson (*J. Cell and Comp. Physiol.* 5: 399, 1934) with laked cells, and the percentage filtration calculated from these by the indirect method also seemed to be so influenced. In a number of cases the calculated percentage filtration of hemoglobin did not show an increase when the concentration of hemoglobin in the perfusion fluid was increased. Direct experiments. From glomerular collection analysis there appears to be considerable variation in the degree of completeness of hemoglobin filtration by the individual glomeruli, but possibly a trend toward more complete filtration with increasing concentration of hemoglobin in the perfusion fluid. Glomerular fluid and ureteral urine were collected simultaneously in a number of experiments.

Antithromboplastic activity of navy bean trypsin inhibitor *in vivo* DONALD E. BOWMAN *Dept. of Biochemistry and Pharmacology, Indiana Univ.*

School of Medicine, Indianapolis, Ind A highly active and abundant navy bean trypsin inhibitor has been used to retard the *in vivo* coagulation which results from the intravenous injection of trypsin in rabbits. This inhibitor was prepared by a modification of an earlier procedure employing isoelectric precipitation of inert proteins, fractional salting out of the active material and fractionation with alcohol. In rabbits the navy bean trypsin inhibitor confers a considerable degree of protection against the lethal effects of single large doses of intravenously injected trypsin or repeated small doses of the enzyme. Extensive pulmonary embolism which is almost invariably observed upon immediately examining rabbits killed with trypsin alone is not found upon sacrificing those receiving the enzyme plus its inhibitor. In rabbits receiving repeated small injections of trypsin the coagulation time of carotid blood is first observed to increase markedly and then gradually decrease, finally reaching a point considerably below the control level at the time of fatal termination. The initial rise is also observed with the enzyme plus its inhibitor, however, the subsequent fall is not as severe. Iodinated trypsin which retains relatively more of the proteolytic activity as compared with the hypotensive effect of the original enzyme also gives rise to pulmonary emboli particularly with repeated small doses. Single large doses have been less toxic than the untreated enzyme given in amounts sufficient to provide equivalent proteolytic capacity. Evidence of anaphylactic reactions was not observed upon intravenous injection of the inhibitor at 14-day intervals.

Tuberculostatic properties of autoclaved human plasma DONALD E. BOWMAN *Dept of Biochemistry and Pharmacology, Indiana Univ School of Medicine, Indianapolis, Ind* During an investigation of enzymatic factors in tuberculosis it has been observed that undiluted autoclaved human plasma containing glycerol is less able to support growth of the H37 strain of tubercle bacilli than is sterile plasma which has been coagulated at 80° for 10 min. On the latter surface growth of the organisms having typical staining properties can be observed in 2 to 4 days after inoculation while growth on plasma which has been autoclaved for 15 min at 15 lb pressure requires a week or more to reach a comparable point and subsequently shows much less growth. With non-autoclaved dog plasma or restored dessicated human plasma growth is comparable to that on autoclaved fresh human plasma. That the addition of a concentrate of the fluid derived from autoclaved human plasma to synthetic media consistently inhibits growth is readily observed and can be estimated by determining the nitrogen content of the washed organisms by the procedure described by Youmans.

Fluid derived from plasma coagulated at 80° does not inhibit growth. In fact in less favorable media such as that in which the yeast extract and surface active accelerating agents have been omitted from Dubos' synthetic media, fluid pressed from 80° coagulated plasma actually accelerates growth. Uric acid shows an inhibiting effect which it partially loses upon being autoclaved.

A biologically-active, reversible oxidation product of α -tocopherol PAUL D. BOYER *Div of Biochemistry, Univ of Minnesota, St Paul, Minn* A biologically-active oxidation product of α -tocopherol has been formed by reaction with two equivalents of Fe^{+++}/M and isolated as an unstable, colorless oil by extraction and chromatographic procedures. It has an absorption maximum at 237 $\text{m}\mu$ in isooctane ($\epsilon_M = 1.2 \times 10^4$), and is readily reduced to tocopherol by ascorbic acid or hydro-sulfite. The product is a structural isomer of α -tocopherylquinone, $\text{C}_{29}\text{H}_{50}\text{O}_3$, into which it is readily converted by a slight excess of Fe^{+++} and by acids and alkalis. Its antisterility activity is 1/10 parenterally and 1/30 orally of that of α -tocopherol. This suggests a relation of the oxidation product to the biochemical function of tocopherol. Tentatively the product is considered to be 2,5,7,8-tetramethyl-2-(4',8',12'-trimethyltridecyl)-6-keto-7-hydro-8,9-epoxychroman. The absence of an OH group is demonstrated by failure to react with reagents for OH groups and by the infra red absorption spectrum. The hetero ring is thus intact and the extra oxygen must be associated with the carbon ring in a cyclic or peroxide structure. The product liberates I_2 from NaI in Ac_2O , and this property, together with the ease of conversion to the quinone, has prevented direct reaction with carbonyl reagents. The ultraviolet absorption spectra and other properties of the molecule are best explained by an α - β unsaturated keto structure. Similar products from γ - and δ -tocopherols show maxima about 10 $\text{m}\mu$ shorter wave length, consonant with the postulated structure. Properties of the molecule and structural considerations suggest the location of the extra oxygen in a three-membered ring in the 8-9 position.

Evaluation of some lactic acid producing bacteria in the microbiological assay of amino acids MYRON BRIN (by invitation) and HAROLD H. WILLIAMS *Dept of Biochemistry and Nutrition and The School of Nutrition, Cornell Univ, Ithaca, N Y* The amino acid assay of complex feedstuffs containing relatively large amounts of lipids, complex carbohydrates, or both, offers difficulties not apparent in the assay of purified protein materials. This report deals with the comparative analysis of complex feeds by 5 bacteria previously proposed for amino acid assay. Whole egg, soybean oil meal, and clover hay were chosen as representative of

feedstuffs, and casein was included as a standard of comparison with literature values. Depending upon the amino acid requirements for the respective organisms, comparative assays for the essential amino acids were run on the above materials with *Lactobacillus arabinosus* 17-5, *Lactobacillus casei*, *Lactobacillus delbrueckii* LD5, *Leuconostoc mesenteroides* P-60, and *Streptococcus faecalis*. In order to measure differences of response between organisms to the test materials, a uniform basal medium was developed which is applicable to the 5 bacteria used. Comparative results indicate that all of the organisms do not yield equivalent amino acid values under similar conditions. It is concluded that under the conditions of this study 3 bacteria yielded the most accurate values, namely *Streptococcus faecalis*—arginine, histidine, lysine, methionine, threonine, tryptophan and valine, *Leuconostoc mesenteroides* P-60—histidine, lysine, methionine and phenylalanine, *Lactobacillus arabinosus* 17-5—leucine, leucine, and tryptophan.

Mechanism of histidine synthesis in lactic acid bacteria HARRY P. BROQUIST (by invitation) and ESMOND E. SNELL *Dept of Biochemistry, Univ of Wisconsin, Madison, Wis*. If *Lactobacillus arabinosus* 17-5 is cultured in a medium deficient in histidine and purine bases, the organism grows very slowly. Much more rapid growth is obtained on the addition of either histidine or individual purine bases. This suggests a relationship between histidine and purine bases in metabolism. The possibilities (reaction *a*) that histidine may serve as a precursor of purines, or (reaction *b*) that the purines may serve as a precursor of histidine are considered. Reaction *a* has been frequently proposed, and appears unlikely in view of present knowledge. Furthermore, the histidine requirement of *Streptococcus faecalis* R is not increased by culturing in the absence of the proposed product, purine bases. Data to be presented indicate rather that the purine bases serve as a precursor for the imidazole ring of histidine (reaction *b*) and that xanthine is particularly effective in this relationship. In the absence of vitamin B₆, histidine becomes essential for growth of *L. arabinosus* and cannot be replaced by purine bases, implying that vitamin B₆ is involved somewhere in reaction *b*. Imidazole pyruvic acid replaces histidine for both *L. arabinosus* and *S. faecalis*, but only when vitamin B₆ is present in the medium. Thus B₆ appears to function in conversion of this keto acid to the amino acid. If imidazole pyruvic acid is the immediate precursor of histidine this implies a wider significance for the transamination reaction than has recently been indicated.

Changes in sodium sulfate solutions in the upper and lower intestines of dogs GLADYS R. BUCHER (by invitation), CARL E. ANDERSON (by invita-

tion) and C. S. ROBINSON *Dept of Biochemistry, Vanderbilt University, Nashville, Tenn*. The ability of the small intestine to alter sodium sulfate has been studied in a double period test in a series of acute dog experiments using 2 methods of isolating the gut segments, and also in chronic dogs with Thury-Villa loops. In the acute experiments, segments were isolated using the terminal end of the Miller-Abbott apparatus in the jejunum and 60 cm from the ileocecal valve, or by mechanical obstruction with flat clamps in these regions. After a sulfate wash, each loop was filled with Na₂SO₄ (35–40 cc, 204 mEq/l) and drained for analysis after 20 minutes. Residual volume was obtained by determining the conductivity of an equal volume of 10% sucrose solution used as a rinse. Following a rinse with sulfate, a second 20-minute test was made. The analysis consisted of determining volume, pH, total base, ammonia, sulfate, chloride and total CO₂. The data from the 3 types of preparation were statistically homogenous. The first period samples in both upper and lower loops showed some significant differences from the second period samples. In upper loops, the second period samples were significantly different in possessing a more acid pH, higher sulfate and a lower chloride concentration. In the lower loops, the second period samples were significantly different in possessing a more acid pH, increase in total base concentration and a decrease in the concentration of ammonia. These ionic shifts occurred when no consistent differences in volume were detected. The sample differences bespeak a reduction in the rate of ionic shifts in the second period, due possibly to a fatigue or saturation phenomenon.

Anaerobic and aerobic glycolysis of parasitic tissue helminths ERNEST BUEDEL *Dept of Pharmacology, School of Medicine, Western Reserve Univ, Cleveland, Ohio*. In contrast to tissues from vertebrates and to many bacteria, lactic acid is not the major end product of the anaerobic carbohydrate metabolism of parasitic helminths living in the intestinal tract or the bile ducts. However, lactic acid production accounted for at least 80% of the total amount of carbohydrate utilized anaerobically by 2 tissue parasites, the filarial nematode, *Litomosoides carinii*, and the trematode, *Schistosoma mansoni*. This was established by isolation and identification of the benzimidazole and the p-bromphenacyl derivatives from media in which the parasites had been incubated in the presence of glucose. Glycolysis of *Litomosoides carinii* was greatly reduced by aerobiosis and inhibition of its respiration resulted in a compensatory increase of glycolysis. In schistosomes and in another tissue parasite, the filarial nematode, *Dracunculus insignis*, the rates of glucose utilization and of lactic acid production remained

constant under anaerobic and aerobic (1-100% oxygen) conditions, and were not affected by inhibitors of respiration. Evidence will be discussed indicating that a large amount of energy derived from oxidative reactions is essential for survival of *Leishmaniasis carinii*, but not for that of schistosomes, in which respiration and glycolysis occur independently of each other. Glycolysis of the parasites was reduced markedly by low concentrations of -SH inhibitors. This effect could not be reversed by glutathione, dithiothreitol or H₂S. Dithiothreitol (but not glutathione) greatly enhanced the inhibitory action of p-chloromercuric benzoate on glycolysis of the schistosomes.

Bacterial glucuronidase HENRY J. BUEHLER (by invitation), PHILIP A. KATZMAN and EDWARD A. DOIST, *Laboratory of Biological Chemistry, Saint Louis Univ. School of Medicine, Saint Louis, Mo.* The estimation of several important substances excreted in the urine as glucuronides depends upon an initial hydrolysis. Since the prevailing methods for hydrolyzing such substances result in considerable destruction, we have directed our efforts toward the preparation of potent glucuronidase extracts of bacterial origin. Of fourteen organisms grown in a 1% peptone medium only *C. rosea*, *C. hoffmanni* and *E. coli* produced glucuronidase as indicated by the hydrolysis of phenolphthalein glucuronide. *E. coli* was selected for further study because it showed the greatest glucuronidase production under these conditions. The optimum pH for enzyme production by this organism at 25°C in a peptone-beef extract medium containing 0.05M phosphate buffer was found to be about 7.3. The optimum pH for enzyme activity using phenolphthalein glucuronide as the substrate and 0.033M KH₂PO₄ as buffer was determined to be approximately 6.2. The production of glucuronidase by *E. coli* can be markedly increased by the addition of menthol glucuronide to the growth medium. The addition of 0.5% menthol glucuronide in this manner has effected as high as a 70-fold increase in the yield of the enzyme. Employing as the enzyme preparation the culture fluid after dialysis and filtration, phenolphthalein glucuronide was quantitatively hydrolyzed in 2 hours. The extent of hydrolysis for shorter periods was not measured.

L-Alanine oxidase of rabbit kidney and liver M. V. BUELL and J. L. STILL (introduced by D. E. GREEN), *Institute for Enzyme Research, Univ. of Wisconsin, Madison, Wis.* Liver and kidney cytochrome preparations can catalyze the complete oxidation of L-alanine to CO₂, NH₃ and H₂O. This process requires the presence of some member of the citric acid cycle in sparking concentration. It appears that the first step in the complete oxidation is a transamination between alanine and α-ketoglutarate with formation of pyruvate and

glutamate. Once the process is properly started the oxidation of pyruvate and glutamate both of which pass through α-ketoglutarate as an intermediary step provide a continuous supply of α-ketoglutarate for the initial transamination reaction. The alanine-glutamic transaminase is capable of working at catalytic concentrations of α-ketoglutarate. Under appropriate conditions the cytochrome system can synthesize L-alanine from pyruvic acid and NH₃. This synthesis involves both the synthesis of glutamate from α-ketoglutarate and NH₃ and the reversal of the transamination reaction. $2 \alpha\text{-ketoglutarate} + \text{NH}_3 \rightarrow \text{glutamate} + \text{succinate}$ $\text{glutamate} + \text{pyruvate} \rightarrow \alpha\text{-ketoglutarate} + \text{alanine}$. The synthesis of glutamate from α-ketoglutarate and NH₃ proceeds very rapidly under aerobic conditions. α-Ketoglutarate in excess suppresses almost completely ammonia production from either glutamate or alanine in accordance with the dictates of the above equations.

Effect of amino acid administration on amino acid oxidase activity R. N. CAGAN (by invitation), J. L. GRAY (by invitation) and H. JENSEN, *Medical Dept. Field Research Laboratory, Fort Knox, Ky.* The effect of intraperitoneal injection of an amino acid mixture on the amino acid oxidase activity of the liver and kidney was studied in normal, hypophysectomized, adrenalectomized and thyroidectomized rats. The enzyme activity of a buffered extract of homogenized tissue was measured. Simultaneous determinations of blood amino acids, urea and glucose were made. An increase in the amino acid oxidase activity of the liver of normal animals was found after amino acid administration while no such increase was observed in the operated animals. The amino acid oxidase activity of the kidney was found to be dependent principally upon the amino acid level of the blood and not upon hormonal control. Comparison of the amino acid oxidase activity of normal and operated animals was made.

Direct utilization of sucrose by *Leuconostoc* WARNER W. CARLSON and VIRGINIA WHITESIDE CARLSON (introduced by EMMETT B. CARMICHAEL), *Biochemistry Dept., Medical College of Alabama, Birmingham, Ala.* Several instances of direct utilization of disaccharides by microorganisms have been reported in the literature. With the disaccharide as the carbohydrate source, earlier growth and acid production were observed than resulted from the use in the media of the constituent monosaccharides. In the case of *Leuconostoc mesenteroides* and *L. dextranicum* direct utilization of sucrose has been indicated as a result of the fact that these organisms synthesize dextran only from the intact disaccharide, no polysaccharide being formed when glucose, fructose, or a mixture of the monosaccharides are employed. We have

observed earlier growth and more rapid acid production from sucrose by various strains of *Leuconostoc* regardless of whether a particular strain formed large amounts or only a trace of the polysaccharide. Investigation of the growth requirement of certain strains of *Leuconostoc* in a chemically defined sucrose medium, and in media containing the constituent monosaccharides revealed several differences, the most important of which was an apparent absence of a biotin requirement when the disaccharide was employed, as compared with a need for this vitamin when glucose or fructose were employed. Since sucrose often is known to contain significant amounts of biotin, the studies were also made with media containing different amounts of raw egg white.

Toxicity studies with demerol hydrochloride (isonipECAINE) on adult male guinea pigs EM-METT B. CARMICHAEL, WALTER H. JOHNSON (by invitation) and FRANK A. KAY (by invitation) *Biochemistry Dept., Medical College of Alabama, Birmingham, Ala.* Toxicity studies have been made with Demerol hydrochloride on adult male guinea pigs. A fresh aqueous solution was injected intraperitoneally. The doses varied by 10 mg increments from 80 to 170 mg/kg. The smallest dose to cause death was 110 mg/kg while a dose of 170 mg/kg killed 100% of the animals. With the large doses, animals usually became nervous and excitable within 5 to 10 minutes and as the symptoms became more severe, convulsions developed. Then an animal would fall on its side during a convulsion and if the dose was lethal, the animal usually would not stand up before expiring. The survival time for those animals that died varied from 12 to 323 minutes with a majority of the animals dying in less than 2 hours. The LD₅₀ of Demerol for adult male guinea pigs seems to be in the range 130 to 150 mg/kg.

Separation of three naturally occurring adenine ribonucleotides by paper chromatography and ion-exchange C. E. CARTER (by invitation) and WALDO E. COHN *Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.* Adenylic acid prepared from yeast nucleic acid was assigned the structure of adenosine-3-phosphoric acid by Levene. It has been found by techniques of paper chromatography that yeast adenylic acid may be resolved into two components and by an anion exchange procedure these two components may be separated from each other and from muscle adenylic acid. The two adenylic acids in yeast nucleic acid are degraded to adenine by acid hydrolysis and to adenosine by weak alkaline hydrolysis and kidney nucleotidase. Neither component is desaminated by Schmidt's muscle adenylic acid desaminase and the phosphorous of both is acid labile. These findings suggest that nucleic acid contains adenosine-2-phosphoric acid and adenosine-3-

phosphoric acid (the 1 and 4 positions on the ribose carbon chain being excluded by the furanose ring and the 5 position by the acid labile nature of the phosphorous of yeast adenylic acid) although other possibilities for isomerism are not excluded. The ion-exchange separation of adenylic acids was achieved by elution with dilute formic, acetic or hydrochloric acid solutions from strong-base anion exchangers. Separation of nucleotides by paper chromatography was obtained in a two phase system consisting of isoamyl alcohol and 5% Na₂HPO₄ in water.

Inositol lipids of soybean HERBERT E. CARTER, WALTER D. CELMER (by invitation), MACK H. McCORMICK (by invitation), CLIFFORD O. NYMAN (by invitation) and FRANCES W. SAUNDERS (by invitation) *Div. of Biochemistry, Noyes Laboratory of Chemistry, Urbana, Ill.* Attempts to prepare inositol lipid from soybean by the procedure of Woolley (*J. Biol. Chem.*, 147: 581, 1943) yielded non-homogeneous products containing a non-reducing carbohydrate which gave positive Selivanoff and diazouracil tests. Therefore, other fractionation procedures were studied, using crude-soybean phosphatide from several commercial sources. The crude material was freed of oil and the product (3-4% inositol) was extracted with glacial acetic acid. The residue was a crude inositol lipid (10-13% inositol) which contained about 80% of the original inositol. Addition of hexane to the glacial acetic acid extract gave a white precipitate (2-5% inositol) containing a large amount of the non-reducing carbohydrate. This material when freed of inositol gave a positive test for galactose and sucrose. The optical rotation and the properties of the acetyl derivative were similar to those of raffinose. The acetic acid insoluble fraction was further purified by distribution between aqueous acetic acid and cyclohexane giving a white powder containing 11-14% of inositol (Selivanoff test negative). Distribution of this material between hexane and methanol gave two inositol-containing fractions.

Variability in the Beckman spectrophotometer W. O. CASTER (by invitation) and OLAF MICKELSEN *Nutrition Section, U. S. Public Health Service, Bethesda, Md.* Different Beckman Spectrophotometers may yield E_{1%¹cm} values differing by as much as 1-5% (Ewing and Parsons, *Anal. Chem.* 20: 423, 1948). One instrument was subjected to a series of control studies in order to determine the cause of this variation. Analysis of variance revealed several distinct and statistically significant sources of variability. Under constant conditions duplicate readings can be repeated within $\sigma = 0.2\%$. This is only slightly above the error in reading the instrument scale. This has often been considered the 'true error' of this instrument. Quartz cell correction factors, (i.e. to compensate

for the lack of agreement between readings obtained from different cells when all are filled with identical solutions) change with changes in wave length (and simultaneous changes in slit width) to give an additional variation of 0.2%. From the circuit the phototube appeared to be by far the greatest source of inconsistency. The variation between a series of ultraviolet-sensitive phototubes was $\sigma = 1.1\%$ and between red-sensitive and ultraviolet-sensitive phototubes in their overlapping range, 600–625 $m\mu$, was $\sigma = 3.2\%$. Changes in slit width over an 0.08 mm range resulted in a 0.9% variation in $E_{1\text{cm}}^{1\%}$ values. Changes in wave length over a 23 $m\mu$ range likewise resulted in a 0.5% variation. Together these error factors are considerably greater than the 0.2% so often reported as the variability of this instrument. In the exact interpretation of $E_{1\text{cm}}^{1\%}$ values these factors should be considered.

Fractionation and chemical studies of proteins of rat liver. ALFRED CHAUTIN, E. C. GJESSING and STEPHAN LUDEWIG, *Biochemical Laboratory, Univ. of Virginia, Charlottesville, Va.* The proteins of saline extracts of rat liver have been fractionated by isoelectric precipitation and ethanol. A relatively large fraction containing a protein-lipide-nucleic acid complex was separated by isoelectric precipitation. A series of proteins were obtained by adjusting the ethanol and hydrogen-ion concentrations. These fractions were analyzed for their nitrogen, lipid and nucleic acid contents. Data for the distribution of arginase, phosphatase, catalase, esterase, succinic dehydrogenase and fluorophosphatase in these fractions will be presented.

Concentration of amino acids by the rat diaphragm suspended in artificial media. HALVOR N. CHRISTENSEN, *Children's Hospital and Dept. of Biological Chemistry, Harvard Medical School, Boston, Mass.* A study has been made of the activity of excised rat diaphragm in maintaining cellular amino acids at much higher concentrations than those of the extracellular fluid. Two procedures were used. 1) One hemidiaphragm was analyzed immediately, the other after shaking in a given medium. The amino acid concentration of each was referred to the appropriate extracellular fluid. 2) The two hemidiaphragms were shaken simultaneously in media identical except for one component. A solution containing 103 mM NaCl, 25 mM NaHCO_3 , 2.5 mM CaCl_2 and 20 mM sodium pyruvate in an O_2 - CO_2 atmosphere maintained the concentrating activity for glycine unchanged for 3 hours. α -Ketoglutarate served in place of pyruvate. When supplemented with pyruvate, rat plasma was equally satisfactory. The diaphragm glycine was responsive to the fluid glycine, falling at one fluid level and rising at a slightly higher one. In the absence of oxygen, or the presence of cyanide, arsenite, 80 mM potassium, or 2,4-dinitro-

phenol at 10^{-3} to 5×10^{-6} M, glycine was rapidly lost from the diaphragm. Pyruvate and dinitrophenol added to rat plasma stimulated and inhibited respectively the concentration of α -amino acids (measured collectively by ninhydrin). These observations relate to the energy source for amino acid concentration. All α -amino acids added to the medium at 21 mM concentration diminished by about $\frac{1}{3}$ the glycine retained by the suspended diaphragm. Only α -amino acids had this effect, and pairs of optical antipodes were equally inhibitory. This inhibition, earlier shown in the intact animal, is interpreted as a competitive phenomenon.

Nitrogenous metabolism of the earthworm (*Lumbricus terrestris*). STANLEY COHEN (by invitation) and HOWARD B. LEWIS, *Dept. of Biological Chemistry, Medical School, Univ. of Michigan, Ann Arbor, Mich.* In the normal earthworm, the chief nitrogenous component of the excreta was ammonia (66–91%), with traces only of urea (<10%). During inanition, the urea content was greatly increased so that urea was the most important nitrogenous constituent of the excreta. Thus, after 18 days' fasting, from 82 to 86% of the nitrogen was present as urea and less than 10% as ammonia. When arginine was fed by tube to normal earthworms, the excretion of urea was greatly increased. Small increases in the amount of urea excreted were noted after the oral administration of citrulline, but not after ingestion of ornithine, glutamic acid, glutamine, glycine, alanine, histidine, or ammonium chloride. When citrulline was fed simultaneously with glycine, alanine, or glutamic acid, the urea excretion exceeded that observed when citrulline or the other amino acids were fed alone. The administration of ornithine with other amino acids did not alter the excretion of urea.

Detection of 7-keto-cholesterol in blood plasma. H. B. COLLIER and R. H. COX (by invitation), *Dept. of Biochemistry and College of Pharmacy, University of Saskatchewan, Saskatoon, Sask., Canada.* In connection with the administration of pure 7-keto-cholesterol to rabbits a method was sought for its detection in plasma filtrates. The Liebermann-Burchard reaction is negative, but with the Tschugaeff reagent (Trappe, *Z. Physiol. Chem.* 273:177, 1942) the compound gives an intense yellow color with an absorption maximum at 432 $m\mu$ (Coleman model 11 spectrophotometer) and a green fluorescence. Cholesterol gives a pink color, with absorption maximum at 503 $m\mu$, and a yellowish fluorescence. (A similar color, with maxima at 515–520 $m\mu$, is also given by cholestadiene (3,4,5,6), coprostene-(4,5)-one-3, and dicholesteryl ether.) When the reaction is applied to dried acetone-alcohol filtrates of plasma, 7-keto-cholesterol may be detected by a peak in the spectrophotometric absorption curve at 432 $m\mu$. However, with mixtures of cholesterol and 7-keto-cholesterol, nei-

then the optical densities in the visible spectrum nor the fluorescence intensities are additive. Semi-quantitative separation and estimation of cholesterol and 7-keto-cholesterol may be achieved by adsorption on alumina from petroleum ether, elution of cholesterol by chloroform and of 7-keto-cholesterol by acetone, followed by color development.

Demonstration of TPN dehydrogenases in the tissues of higher plants ERIC E. CONN (by invitation), BIRGIT VENNESLAND and E. A. EVANS, JR. *Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.* Ammonium sulfate fractions of seven different higher plants which contain oxaloacetic carboxylase activated by manganous ions have been shown to contain a malic dehydrogenase requiring triphosphopyridine nucleotide (TPN) as a coenzyme. The overall reaction catalyzed by these preparations may be written

malate + TPN_{oxidized} \rightleftharpoons

pyruvate + CO₂ + TPN_{reduced}

and is analogous to the reaction catalyzed by the 'malic enzyme' of pigeon liver as described by Ochoa. The main difficulty in demonstrating the occurrence of the reaction in plant preparations is associated with the widespread distribution of enzymes which destroy TPN. This destruction can be prevented by muscle adenylic acid, adenosine triphosphate, diphosphopyridine nucleotide, and to a lesser extent, yeast adenylic acid, but not by nicotinamide or adenosine. The destruction of reduced TPN is accompanied by the disappearance of the absorption band at 340 mμ and therefore simulates a reoxidation of the nucleotide. The practical significance of these facts in connection with the procedures used to demonstrate the reaction will be discussed. A detailed study of the dehydrogenase from wheat germ has been made. Observations with parsley root, parsnips, beets, carrots, spinach and peas indicate a general similarity in the properties of the dehydrogenases and of the factors which destroy TPN in crude protein preparations from all these different plant sources.

Formation of acetoacetic acid from radioactive leucine MINOR J. COON (by invitation), SAMUEL GURIN and D. WRIGHT WILSON. *Dept. of Physiological Chemistry, School of Medicine, Univ. of Pennsylvania, Philadelphia, Penna.* A fasted phlorhizinized rat was injected subcutaneously with a solution of 100 mg. of DL-leucine labeled in the β position with C¹⁴. The acetoacetate which appeared in the urine was degraded by standard procedures. The acetone fraction was found to be radioactive, and iodoform prepared from it accounted for all of the isotope present. These findings are in accord with the view that the carboxyl group of leucine is lost early in the course of metabolism. In other experiments, the radioactive amino acid was incubated with rat liver slices. The aceto-

acetate formed was oxidized with permanganate to produce formic and acetic acids, both of which proved to be radioactive. It was calculated that the methyl and methylene carbons of the acetoacetate had approximately the same concentration of isotope. Accordingly, at some stage in the metabolism of leucine, its α and β carbons must split off as a two-carbon intermediate which is capable of condensing to form acetoacetate.

Effects of feeding an ethanolamine analogue (diethanolamine) on the formation of liver phospholipides W. E. CORNATZER (by invitation), CAMILLO ARTOM and MARIETTA CROWDER (by invitation). *Dept. of Biochemistry, Bowman Gray School of Medicine, Winston-Salem, N. C.* With the aid of radioactive P as an indicator it was previously shown that the administration of a single large dose of diethanolamine to rats on low protein diets stimulates the lipid phosphorylation in the liver to a degree similar to, or greater than that observed after ethanolamine was given under the same conditions. With either substance, the stimulation involves both lecithin and cephalin fractions. The results are quite different when diethanolamine is added to the diets and fed over a period of 7 to 12 days. Under this condition the rate of lipid phosphorylation in the liver becomes markedly lower than in the controls on the same diets, unsupplemented or supplemented with ethanolamine. The decrease is mainly due to a diminished rate of formation of lecithins. Changes in the amount and distribution of liver phospholipides (increases in the total and non-choline containing phospholipides with a considerable decrease in the ratio lecithins to total phospholipides) were also noted. Indirect evidence was obtained for the presence of diethanolamine in the liver phospholipides of rats on diets supplemented with this compound. It is suggested that in these animals considerable amounts of diethanolamine-containing phospholipides are formed, and that these atypical phospholipides, being less easily metabolized than the natural cephalins, accumulate in the liver. This effect, in turn, would lead to a marked decrease in the formation of lecithins through a mechanism which remains obscure.

Electrophoretic patterns of plasma proteins during pregnancy and following delivery MARGARET N. CORYEIL (by invitation), ELIOT F. BEACH, and ABNER ROBINSON (by invitation). *Research Laboratory, Children's Fund of Michigan, Detroit, Mich.* The changes which occur in the plasma proteins of women at various intervals in pregnancy and postpartum are demonstrated by data obtained by use of the Tiselius electrophoresis technique with 89 plasma samples from the blood of 11 non-pregnant women, 23 women during pregnancy and postpartum, and the cords of 12 infants. Determinations were made with 3, 7, and 17 samples ob-

tained during the first, second, and third trimesters of pregnancy respectively, 16 maternal samples obtained at delivery, 10 samples within 5 days, and 13 samples 5 or more weeks postpartum. The data reveal a smooth progression of changes in all plasma protein fractions during the 3 trimesters of pregnancy. Comparison of the averages for non-pregnant patterns and those at delivery shows decreases in the absolute levels of gamma globulin of from 28 to 35%, respectively, while α_1 , α_2 , and beta globulins, and fibrinogen, increased 29, 41, 36, and 29%, respectively. During the 5 days following delivery there was some further fall in albumin and gamma globulins and a rise in α_1 globulin. By 5 to 9-weeks postpartum all subjects had plasma protein patterns similar to those of non-pregnant women, with complete restoration of the albumin fraction although gamma globulin was still reduced and α_1 and beta globulins were slightly elevated. Effects of factors such as milk flow, lactation failure, or renewed menstruation, upon the plasma protein patterns were not apparent.

Oral ingestion of histidine and the glutamic acid blood level in rats. H. R. CROOKSHANK and BERNARD F. CLOWDUS (introduced by EMMETT B. CARMICHAEL) *Biochemistry Dept., Medical College of Alabama, Birmingham, Ala.* The course of histidine metabolism and the conversion of histidine to glutamic acid was studied in previously fasted rats by assaying the blood for histidine, total imidazoles, glutamic acid, and amino nitrogen both before and at intervals after feeding single doses of L-Histidine. The histidine, total imidazoles, and amino nitrogen reached maximal concentrations in 1 hour after feeding and returned to the fasting level in 6 to 10 hours. No significant change in the glutamic acid level was observed, either before or at any interval after ingestion of L-Histidine. The data seem to indicate either little conversion of histidine to glutamic acid or that the glutamic acid thus formed is produced too slowly to be detected by our method or is converted to another metabolite which retains the alpha-amino nitrogen.

Incorporation of P^{32} during the operation of the cyclophorase system. R. CROSS, W. ATCHLEY and H. ALBAUM (introduced by D. E. GREEN) *Enzyme Laboratory, College of Physicians and Surgeons, Columbia University, New York City.* When the cyclophorase system is allowed to carry out the oxidation of any member of the citric acid cycle, of proline, glutamate and β -hydroxybutyrate in presence of radioactive inorganic phosphate it is found that the enzyme gel after thorough washing with 0.9% KCl is radioactive and the radioactivity is from 5-10 times as high as that of the blank treated in the same way except without added substrate. Under optimum conditions the radioactivity μ mol of total phosphate extractable from the gel by treatment with 5% trichloroacetic acid

approaches the value for the radioactivity/ μ mol of inorganic phosphate in the original digest. Arsenite and capryl alcohol abolish this incorporation of radioactive phosphate. Gramicidin and dinitrophenol only partially inhibit the incorporation though both together reduce the level of radioactivity to the level of the blank without substrate. Analysis of the radioactive gel has shown that either inorganic phosphate or one or more substances which estimate as inorganic phosphate are largely responsible for the radioactivity. The fraction corresponding to the adenosine pyrophosphate is also highly radioactive and it has been shown by enzymatic procedures that the radioactivity actually resided in the pyrophosphate moieties of ATP and ADP. Incorporation of P^{32} accompanies the oxidation of succinic acid in the cyclophorase system but not oxidation in the classical succinic oxidase system.

Influences of dietary protein transmittal of growth factor. FRANK A. CSONKA and M. W. OLSEN (by invitation) *Bureau of Human Nutrition and Home Economics and Bureau of Animal Industry, Agricultural Research Admin., U. S. D. A., Washington, D. C.* Evidence has been presented previously that the cystine and methionine content of a hen's egg may be increased by feeding a high-protein casein diet (Csonka, Denton, and Ringel, *J. Biol. Chem.*, 169: 259, 1947). In the present work, two pens of chickens were used to furnish eggs for incubation, one group was fed a high-protein diet of which 20% was casein and the other group was fed a low-protein diet containing no casein. Eggs were incubated from both pens at the same time and the chicks were reared together to 4 weeks of age on the same growing mash ration which had no casein. The chicks from the hens fed the casein diet grew faster than those from the hens fed the low protein diet, as shown by their greater body weight at 4 weeks of age. This difference in chick growth is related to the maternal dietary protein as the chief variable component in our experimental arrangement, the growth effect being transmitted through the egg to the chick. When the casein was replaced in the hen's ration with an equivalent quantity of soybean meal, the same accelerated growth effect was not observed. Addition of dried cow manure or Wilson's liver fraction 'L' to the chick mash inhibited the growth of the chicks that originated from the hens fed the high protein casein ration. The same supplements, however, slightly accelerated the growth of the chicks that had descended from parents fed the low protein ration. The growth of the chicks on the supplemented diet whose parents received a high protein soybean ration was not affected.

An ultraviolet spectrophotometric method for the determination of benzene hexachloride. BERNARD DAVIDOW and GEOFFREY WOODARD (introduced by R. L. GRANT) *Div. of Pharmacology, Food and*

Drug Administration, Federal Security Agency, Washington 25, D C A method has been developed for the estimation of small quantities of benzene hexachloride. It is based upon the conversion of the benzene hexachloride to 1,2,4-, 1,2,3- and 1,3,5-trichlorobenzene by alkaline hydrolysis. Of the mixture of trichlorobenzenes formed approximately 82% is the 1,2,4-trichlorobenzene which has a characteristic spectral absorption curve in the ultraviolet region. Advantage was taken of these facts in developing a quantitative method. The method consists of the initial extraction of the benzene hexachloride with ether from the material to be examined, and the conversion of the benzene hexachloride to the 1,2,4-trichlorobenzene by refluxing with 1N methanolic potassium hydroxide for one hour. Purification of the trichlorobenzene is accomplished by extracting with hexane and washing with water, and when necessary by the use of magnesium oxide or alumina chromatographic columns. The quantity of the trichlorobenzene is estimated by measurement of its optical density at $286\text{ }\mu$. Spectral absorption due to other substances is eliminated by applying a formula for the resolution of a two component color system. In this manner as little as $100\text{ }\mu\text{g}$ /total sample may be determined in biological tissues, in spray residues on spinach and cabbage, and in animal laboratory diets.

Isolation of biochemically deficient mutants of bacteria by means of penicillin BERNARD D. DAVIS, U S P H S, *Tuberculosis Research Laboratory, Cornell Univ. Medical College, New York City*. Mutants with increased nutritional requirements have been difficult to isolate efficiently because any medium enriched to permit growth of the mutants will also foster growth of non-mutant organisms. A method of selectively isolating such mutants from large populations suggested itself on the basis of the reports that penicillin sterilizes bacteria only under conditions which permit growth. Mutants were therefore exposed to penicillin in minimal medium in which they were unable to grow, and were found to survive while the predominant non-mutant population was eliminated. This screening technique proved to be very efficient when applied to ultraviolet irradiated *E. coli*, and made it possible in a short time to isolate mutants with individual requirements for almost all the vitamins and naturally occurring amino acids, and less specific requirements for purines or pyrimidines, as well as requirements for unknown factors in yeast extract. By testing the syntrophic effect of certain mutants on others grown adjacent to them on solid media, it has been possible to demonstrate several instances in which mutants with a common requirement are blocked at different stages in the synthesis of the nutritive (arginine, proline, histidine, phenylalanine plus tyrosine, thiamine).

Those mutants which visibly feed others must secrete into the medium considerable amounts of a stable compound which is used in biosynthesis.

Peptides: metabolic and structural intermediates in protein metabolism CLARA L. DEASY (by invitation), HENRY BORSOOK, ARIE J. HAAGEN-SMIT (by invitation), GEOFFREY KEIGHLEY (by invitation), and PETER H. LOWY (by invitation) *Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena, Calif.* A large peptide (designated Peptide A) was isolated by starch chromatography from the liver of albacore, beef, guinea pig, hog, horse, lamb and rat and from Witte's peptone. The peptide isolated from different sources had (within the limits of analytical accuracy) the same amino acid composition. The following amino acids have been demonstrated in it: alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, proline, serine, threonine, tryptophan and tyrosine. Guinea pig liver was found to contain a number of other peptides. After incubating the liver homogenate with C^{14} -labeled glycine, leucine or lysine the labeled amino acids were extensively incorporated into Peptide A. In addition leucine is rapidly incorporated into one other peptide and more slowly into another. Labeled glycine (or an amino acid derived from it) was rapidly incorporated into two other peptides. The peptides into which labeled glycine, leucine and lysine are incorporated are normally present in guinea pig liver. They and free amino acids increase during incubation of liver homogenate, more at pH 6.0-6.5 than at 7.0-7.5. The incorporation of glycine and leucine is much faster into the peptides than into the proteins. This fact and the finding of the same peptide (Peptide A) in liver and as a major product of the peptic digestion of fibrin (Witte's peptone), are evidence that peptides are metabolic as well as structural intermediates in protein metabolism.

Studies of nuclei isolated from normal rat and rabbit liver by a modified Behrens technique ALEXANDER L. DOUNCE, GARSON H. TISHKOFF (by invitation), SHIRLEY R. BARNETT (by invitation), and RICHARD M. FREER (by invitation) *Dept. of Pathology and Biochemistry, School of Medicine and Dentistry, Univ. of Rochester, Rochester, N. Y.* Nuclei were prepared from normal rat and rabbit liver by a modification of the method of Behrens in order to avoid the use of aqueous solvents. The desoxyribonucleic acid of the Behrens nuclei was difficultly extractable, and was at least 50% lower in concentration than the desoxyribonucleic acid of nuclei prepared by aqueous solvents at pH 6.0. This finding apparently is due to the loss of much material other than nucleic acid from the latter type of nuclei during their preparation. Arginase, aldolase, catalase, cytochrome oxidase, esterase,

and lipase were present in the isolated nuclei. Precise quantitative measurements of the enzyme activity of Behrens type nuclei are difficult to carry out because of agglutination of the nuclei near pH 7.0. Free amino acids were extracted from the nuclei with 76% alcohol and were studied by the method of paper chromatography. The pattern yielded by the nuclei appears to be identical with that yielded by the whole tissue. The spots of maximal intensity are those due to glutamic acid, glycine, alanine, leucine plus isoleucine, β -alanine, and probably glutamine, and also to a spot thought to be due to taurine. Aspartic acid, serine, and valine are present in easily detectable but lower amounts, while most of the essential amino acids were detectable only in trace amounts. Glutathione was uniformly detected in nuclei. Its chromatographic detection in whole tissue was more difficult, perhaps because of greater instability in cytoplasm. Glutathione was found to be about equal in concentration in nuclei and whole tissue by chemical titration with iodate.

Hormonal influences on cytochrome c metabolism and liver regeneration. DAVID L. DRABKIN, *Dept. of Physiological Chemistry, Graduate School of Medicine, Univ. of Pennsylvania, Philadelphia, Penna.* Thyrotoxicosis, induced by thyroxine administration to rats, increased the concentration and content of cytochrome c in their tissues (Drabkin, D. L., *Federation Proc.*, 7: 151, 483, 1948). In a continuation of these studies it has been found that, despite the higher concentration of liver cytochrome c and PNA in such thyroxinized animals, instead of the usual increase in cytochrome c and PNA observed in liver regenerating after partial hepatectomy, there occurred a reduction in the concentration of these constituents, accompanied by an appreciably reduced liver regeneration (43.9% in the thyrotoxic as against 73.9% in the normal). After bilateral adrenalectomy, performed at the time of liver lobectomy in rats on a high (31%) protein diet, liver regeneration in 14 days was consistently greater than that of normal controls and of thyroidectomized animals (89.7% in adrenalectomized, 73.9% in normal, and 65.2% in thyroidectomized). Excluding liver tissue, completely adrenalectomized rats, like thyroidectomized animals, had significant decreases in the cytochrome c concentration and content of heart, kidney and skeletal muscle.

Electrophoretic comparison of plasma and body fluid proteins in certain diseases. ROBERT L. DRYER (by invitation), JACQUELINE H. BUDDE (by invitation), W. D. PAUL (by invitation), and JOSEPH I. ROUTH, *Depts. of Biochemistry and Medicine, College of Medicine, State Univ. of Iowa, Iowa City, Iowa.* We have compared the protein components of plasma and body fluids from patients with a variety of clinically diagnosed illnesses. Blood

samples were withdrawn whenever it was necessary to perform thoracentesis, paracentesis, or joint aspiration. Protein components of the corresponding samples were determined by electrophoresis in a single center section cell. Samples were withdrawn from a number of the subjects at intervals over a considerable period of time. Ten patients with various cardiovascular diseases were studied, and in general it was found that the paracentesis and thoracentesis fluids showed a distribution of protein components which closely paralleled that of the plasma. Both the plasma and the fluid showed a marked drop in albumin with a corresponding increase in globulins, especially γ -globulin. A urine sample from one subject with marked decompensation gave a protein distribution which almost exactly matched the plasma. Eleven patients with neoplastic conditions ranging from gastric carcinoma to reticuloendothelial hyperplasia were also studied. Again the most outstanding characteristic was the marked reduction in albumin. In a case of multiple myeloma a component migrating faster than albumin was found in plasma and urine. In several carcinoma cases the α -globulins rather than the γ -globulin fraction showed a greater increase in the plasma and fluids.

Non lability of choline methyl and the role of choline oxidase in transmethylation. JACOB W. DUBNOFF, *California Institute of Technology, Pasadena, Calif.* The methyl groups of choline are not labile but become available for methionine formation from homocysteine only if the animal can oxidize choline to betaine. *In vitro* studies show that methionine formation is rapid from betaine, dimethylthetin, and certain derivatives of dimethylthetin both aerobically and anaerobically in all animals tested, but choline is effective only in those animals which have choline oxidase. While the aerobic synthesis of methionine from choline in these animals approaches that from betaine, anaerobic methionine formation from choline is relatively small or absent. Rabbit, guinea pig and chick, which do not have choline oxidase, do not form methionine from choline in liver and kidney homogenates aerobically or anaerobically. Inhibition of aerobic methionine formation from choline in rat by choline analogues parallels their effects on choline oxidase. There is no significant aerobic methionine formation from choline at pH 6.7 where its oxidation proceeds only to the aldehyde stage. The importance of choline oxidase may be to enable the animal to divert choline methyl into the general transmethylation scheme.

Thiocyanation of tyrosine. MARIE L. ASTERWOOD (by invitation) and JOHN L. WOOD, *Dept. of Chemistry, School of Biological Sciences, University of Tennessee, Memphis, Tenn.* Thiocyanation has been shown to interfere with the metabolism of tyrosine in the thyroid gland. This physical

activity has been suggested to be an interference of the 'metabolic antagonist' type. Studies on thyroid glands from animals given potassium thiocyanate labeled with radioactive sulfur indicate that the thiocyanate ion itself combines chemically with protein. The data, and the chemical properties of tyrosine and thiocyanogen, are consistent with the hypothesis that administered thiocyanate may react with tyrosine, by a mechanism similar to iodination, to form thiocyano derivatives. This point of view is supported by the thiocyanation of tyrosine *in vitro*. Tyrosine reacts with thiocyanogen (corresponding in state of oxidation to free iodine) in glacial acetic acid to form thiocyanotryosine. The compound has been characterized by ultimate analysis, optical activity, absorption spectrum, and by the formation of derivatives.

On the preparation and preservation of Fraction I from human plasma JOHN T. EDSALL, JOHN D. FERRY, and PETER R. MORRISON (by invitation). *Dept. of Physical Chemistry, Harvard Medical School, Boston, Mass.* In the preparation of Fraction I (the fibrinogen-containing and most insoluble major fraction of plasma as separated by ethanol precipitation, cf. Cohn, et al., *J. Am. Chem. Soc.*, 68: 459, 1946) from human plasma previous conditions of blood processing are particularly important and may be critical. Both short handling times and quick cooling of the blood are needed to insure a stable product. The optimal conditions for the separation from plasma are pH 7.3, 8% ethanol and -3°C . Although the gross purity of the fraction (60-65% fibrinogen) is not sensitive to pH, components are precipitated below pH 7.0 which greatly reduce the stability of the product. Fraction I is most conveniently stored as frozen precipitate, but is much less stable either in this form or as frozen solution than when stored in the dry state. In the latter form, and stored under vacuum, it has been successfully kept at room temperature for more than 4 years. It is less stable in air and more stable at low temperatures. Solutions of fibrinogen are less stable than albumin or globulin at both ordinary and elevated temperatures. They are more like the latter in their response to stabilizing agents. Testing procedures for Fraction I have been followed on a considerable series of bottled 'commercial' preparations. Criteria included total and relative amounts of fibrinogen, pH and buffering capacity, clotting times with thrombin and with calcium, stability of the solutions at 25° , 37° and 45° , and of a clot at 37°C .

Estimation of ergosterol with activated glycerol dichlorohydrin ROMOLA ETTINGER (by invitation) and Albert E. Sobel. *Polytechnic Institute and Jewish Hospital, Brooklyn, N. Y.* The reaction between Ergosterol and GDH (glycerol dichlorohydrin) was studied spectrophotometrically. Activated GDH (Sobel and Werbin, *Anal. Ed.*, 18: 570,

1946) containing 4% acetyl chloride produces a greenish yellow color suitable for the determination of ergosterol. Cholesterol and 7-dehydrocholesterol do not interfere. Ergosterol values obtained on brewer's yeast are similar to those with the SbCl_5 method (Lamb, Mueller and Beach, *Anal. Ed.*, 18: 187, 1946) but lower than the less specific ultraviolet absorption method. A new method of extraction was developed which is simple, reliable, requires small samples (i.e. 25 to 50 mg brewer's yeast) and permits rapid, simultaneous determinations. Specimen is placed in a glass stoppered tube, saponified with 1N alcoholic KOH, extracted three times with petroleum ether in this tube, extracts evaporated to dryness with N₂, dissolved in 1 ml of chloroform, treated with 4 ml of GDH reagent and after 20 minutes read in the spectrophotometer at 410 μ . Quantitative recoveries were made of known amounts of ergosterol added to the brewer's yeast.

The effect of ethionine on fat metabolism and on protein formation E. FARBER (by invitation), M. SIMPSON (by invitation), and H. TARVER. *Div. of Biochemistry, Univ. of California Medical School, Berkeley, Calif.* When DL-ethionine is injected into fasted rats fat rapidly appears in the liver so that in 12 hours the fat content is 14% (Control 6%). Fat continues to accumulate and the animals die within the next 24 hours. The appearance of fat in the liver is prevented by giving D, L or L-methionine but not by other amino acids such as L-lysine, DL-valine, glycine, or L-alanine in equimolar doses. Choline lowers the fat content to only 12.5%. Orally administered sucrose (5 gm) or glucose prevents the fat accumulation. The condition is either cured or at least markedly improved by giving methionine 12 hours after the ethionine. In short term *in vivo* experiments in which ethionine is injected along with labeled methionine (S^{35}) the uptake of the label into liver protein is greatly reduced during the next 3 hours (26 and 53% in different experiments). This inhibition of protein formation is reversed by equimolar doses of methionine. Sucrose given orally does not significantly effect the inhibition. Ethionine also inhibits the conversion of methionine to cystine *in vivo* and this inhibition is relieved by methionine injected in larger doses than are required to relieve the protein inhibition. Probably ethionine prevents the demethylation of methionine. In liver slices the effects of ethionine on protein metabolism are practically duplicated. The inhibition of methionine uptake depends on the ethionine concentration and is eliminated by methionine—best by the L-isomer. Ethionine also inhibits the uptake of glycine both *in vitro* and *in vivo*. Methionine *in vivo* relieves the inhibition.

Quantitative paper chromatography of penicillins II. Development of buffered strips with

organic solvent OLIVER B FARDIG and NANCY D BREED (introduced by S B BINKLEY) *Research Division, Bristol Laboratories, Inc., Syracuse, N Y* A paper strip chromatographic procedure has been developed whereby the commonly known penicillins were completely separated from one another by developing procaine citrate-buffered strips with amyl acetate for 15 to 18 hours. Filter paper strips were buffered the first part of their length with pH 5.2 procaine citrate, spotted with 0.5 to 2.5 μ of penicillin, equilibrated in a chamber saturated with respect to water and amyl acetate vapor, and developed in the same chamber. The dried developed chromatograms were placed on *Staph aureus*-inoculated trays which were then incubated to reveal the position of the individual penicillins. Two chromatograms of different concentrations of the same solution were placed on the same tray. Photographs of the incubated trays were made to facilitate calculation. The amount of penicillin present was determined from measurements of the maximum width of each zone on the photograph. Analysis of penicillin mixtures of known composition gave results in excellent agreement with the known values. No interference was observed in the determination of the penicillins in broth. The rate of movement of the penicillins along the strip increased in the order *p*-hydroxybenzylpenicillin, benzylpenicillin, 2-pentenylpenicillin, penicillin, and *n*-heptylpenicillin. The usual spread of the fast-moving *n*-heptylpenicillin was prevented by allowing this penicillin to move beyond the buffered portion of the paper strip. The separation of the penicillins, their rate of movement, and their compactness on the strips was influenced by the buffer, the solvent, and the conditions of development.

Glycolysis and the Pasteur effect on the sub-cellular level SILVIO FIALA (introduced by DEAN BURK) *National Cancer Institute, Bethesda, Md* Mouse sarcoma 37, when homogenized in 8.5% sucrose solution, yielded on 7 minutes centrifugation at 20,000g a pellet of large particles, and after 2-3 hrs at 20,000g a microsomal gel. Both fractions when washed and re-suspended in 8.5% sucrose, and after addition of fortifying factors (DPN 4×10^{-4} , ATP 8×10^{-4} , Nicotinamid 32×10^{-4} , Na pyruvate 8×10^{-3} , $MgCl_2$ 5×10^{-3} , $KHCO_3$ 2×10^{-2} , and glucose 0.5%, at pH 7.1) glycolyzed HDP but not glucose alone. Use of the Ringer solution results in a lowering of glycolytic activity. Under conditions mentioned there is no oxygen uptake in any fraction. The glycolytic activity of the large granules and microsomal fractions is independent of the presence of oxygen, but a small Pasteur effect in the supernate may occur. When either the large particles or the microsomal pellet is mixed with the supernatant, the aerobic glycolysis (N_2 , CO_2) of the combination

is equivalent to the sum of the glycolysis of each fraction run separately. However, under aerobic conditions (O_2/CO_2) the glycolysis of the combined fractions is lower than of any single fraction. Mixing the particulate elements with the supernatant thus results in the appearance of a pronounced Pasteur effect. Normal mouse liver particulates and the supernatant fluid (after removal of microsomes) showed the same mutual interaction in regard to Pasteur effect but quantitative differences occurred. In the attempt to determine the centrifugal force sufficient to concentrate the remaining glycolytic activity in the supernatant fluid (after removal of formed elements) in the experiments done with Dr G Kegeles (National Cancer Institute) it has been found that after a run of 4 hours at 164,000g the activity in the bottom layer was 2x that of the top layer of the centrifuged sample.

Influence of estrogenic hormones on b-glucuronidase in the human WILLIAM H FISHMAN and LESTER D ODELL (by invitation) *Depts of Surgery and Biochemistry, Obstetrics and Gynecology, Univ of Chicago, Chicago, Ill* In previous work a relationship between the metabolism and action of the estrogenic hormones and b-glucuronidase was indicated. In castrate female mice, the uterine glucuronidase responds by an increase in its activity following the injection of estrogenic substances. In pregnant women, the serum b-glucuronidase becomes elevated. Additional experiments have now been done in humans with a view to exploring the extent of the relationship of estrogen metabolism to b-glucuronidase activity. The following observations have been made. In 15 post-partum women receiving no medication, whether lactating or non-lactating, the serum b-glucuronidase falls from approximately 500 μ /100 cc regularly to non-pregnancy levels by the sixth day after parturition. On the other hand, the decline in glucuronidase was much smaller and more gradual in post-partum women receiving stilbestrol (5 to 25 mg/day for periods of 10 days). Endometrial biopsies were obtained from 33 women at various days of the normal menstrual cycle and assayed for b-glucuronidase activity. The enzymic activity increased during the first two-thirds of the menstrual cycle, followed by a decline, particularly in the last three days of the cycle. The presence or absence of progestational endometrium seemed to bear little reference to these changes. From these and other observations, it is felt that estrogen may be at least one of the factors which influence the b-glucuronidase level in the serum and in uterine endometrium.

Amylase and lipase in the intestinal lymph of the rat E V FLOCK and J L BOLLMA *The Mayo Foundation, Rochester, Minn* Lymph is collected continuously from the small intestine of the rat after cannulation of the main lymphatic vessel with polyethylene tubing. The enzyme activities

24 hours prior to the operation, and on the day following received 2 meals of either a fat-free or high-fat diet by stomach tube. Lymph collected overnight before the first meal and at 2, 4 and 18 hour intervals thereafter was analyzed for amylase and lipase activity. The amylase was determined by the copper reduction method of Somogyi and the lipase (tributyrylase) by the method of Goldstein and Roe. The amylase activity of the intestinal lymph was lower than that of the plasma in the fasting rat. The response to feeding was slight and variable. If an increase was found, the level reached was generally lower than that of the plasma of the fed rat. The average hourly output of amylase in the lymph increased markedly only during the first 2 hours after feeding and this was at least in part due to the increased rate of flow. Higher values for tributyrinase activity were found throughout the 24 hour feeding period studied in the intestinal lymph of rats fed the high-fat diet than during fasting or after feeding of the fat-free diet. The average hourly output of lipase in the lymph of rats fed the high-fat diet was also increased throughout the 24 hour period whereas in rats fed the fat-free meals an increase was found only during the first 2 hours after feeding.

A new P-free and S-free brain lipid. J. FOLCH and S. ARSOVE (by invitation) *McLean Hospital, Waverley, Mass and Harvard Medical School, Boston, Mass.* A new lipid has been found to be present in grain. It appears to be P-free and S-free and to have as a constituent an as yet unidentified primary amine which becomes water-soluble after short-time acid hydrolysis. The new lipid has been partially purified. The preparation obtained is still contaminated by phosphatides (0.15 per cent P) and sulfatides (0.25 per cent S). The constituents of the new lipid appear to be sphingosine (1.1 per cent sphingosine N) or a sphingosine-like substance, carbohydrate (20 per cent as galactose), an unidentified primary amine (1.1 per cent water-soluble NH_2 after acid hydrolysis) and unsaponifiable substances (only small amounts of fatty acids are obtained from it after saponification). The constituent amine is not neuraminic acid (Klenk, E, *Ztschr f physiol Chem* 268 50, 1941 and it is linked to the rest of the molecule by its NH_2 -group, since the intact lipid does not exhibit any free NH_2 -group. The new lipid is soluble in acetic acid, and in CHCl_3 CH_3OH mixtures at room temperature and insoluble in the latter at -10° . The method of isolation which has yielded a partially pure product is based on these solubilities. The new lipid appears to be a contaminant of a large number of cerebroside and sphingomyelin preparations that have been analyzed.

Cholesterol studies on lyophilized chicken serum. J. C. FORBES and G. H. L. DILLARD (by invitation) *Dept of Biochemistry, Medical College*

of Virginia, Richmond, Va. The serum cholesterol of both male and female chickens of varying ages has been fractionated in a manner previously described (*Proc Soc Exper Biol & Med* 68 240, 1948). The results indicate that a large percentage of the cholesterol of lyophilized serum from old hens is extracted by cold chloroform in 3 hours, behaving in this regard very similarly to the serum of nephrotic patients and hypercholesterolemic rabbits. Old hens, however, show this high degree of cholesterol extractability even though the total cholesterol concentration is normal. The sera of young chickens and old roosters show a low value for this 'readily extractable fraction'. Studies on the effect of a high fat diet on these fractions in roosters of various ages will be presented also.

Synthesis of some benzoylamino acid anilides as catalyzed by papain. SIDNEY W. FOX, FREDERICK N. MINARD (by invitation), HARRY WAX (by invitation), CORNELIUS W. PETTINGA (by invitation), and JACQUETTA STRIFERT (by invitation) *Chemical Laboratory, Iowa State College, Ames, Iowa.* The synthesis of anilides from 4-benzoyl-DL-amino acids and aniline, as catalyzed by papain, has been studied in consolidated experiments. The alanine and leucine derivatives were considerably preferred in reactivity over glycine and valine. Benzoylglycine showed relatively moderate reactivity and benzoylvaline barely reacted. Benzoylvaline was the only valine derivative of several (formyl, acetyl, 3,5-dinitrobenzoyl, benzoyl, phthaloyl, and *o*-carboxybenzoyl)-DL-valine, which gave the acylamino acid anilide. The last 2 valine derivatives yielded N-phenylphthalimide, which was also the product isolated from *o*-carboxybenzoyl-DL-valine and aniline in either the absence or presence of enzyme. The relative non-reactivity of benzoylvaline was not due to the presence of the D-isomer, as shown by approximately equal reactivity of the L-component in the L- and DL-forms. These results are of interest in relation to the observation of Roche and Mourgue that papain liberates much more leucine than valine from casein. Roche and Mourgue ascribed this behavior to position of residues in the substrate molecules, in the present work the results can be explained on the basis of greater reactivity of leucine bonds than of valine bonds in papain-catalyzed systems. Hydrolytic data for papain and compounds containing the same residues has been obtained. The retarding effect of various substances, such as iodoacetic acid, p-quinone, and 2,3,5-triphenyltetrazole, upon some anilide syntheses has been studied.

Mechanism of reversible inactivation of lysozyme by iodine and sulfite. H. FRAENKEL CONRAT *Western Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Dept of*

Agriculture, Albany, Calif Meyer et al (J Biol Chem 113 303, 1936) showed that lysozyme loses and regains activity upon treatment with iodine and sulfite, respectively. It has now been found that the effect of a great excess of iodine is partly reversible only after 10–120 minutes at or above neutrality. Reactivation was achieved also with thioglycol and was found associated with a loss in bound iodine, equivalent to 60–100% of the histidine content of lysozyme. Proteins of greatly varying histidine contents, when treated with iodine and sulfite under similar conditions, contained reversibly bound iodine in proportion to their histidine contents. From this and other evidence it is concluded that the reversibly inactivated derivative is formed first and probably contains a labile α -iodohistidine residue, whereas the irreversibly inactivated derivative is formed later by rearrangement of the α -iodohistidine into a stable ϵ -iodo form. The concomitant iodination of some phenolic groups appeared to cause little inactivation, but when over 2 iodine equivalents (per mole lysozyme) were introduced into these groups, the activity was greatly lowered. The incipient oxidative destruction of tryptophan residues (less than 1 per mole) appeared reversible by early reduction. Lysozyme contains no $-SH$ group.

Pteroylglutamic acid deficiency in the dog as produced by 'x-methyl' pteroylglutamic acid
A. L. FRANKLIN (by invitation), T. H. JUKES, E. L. R. STOKSTAD, and M. BELT (by invitation)
Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y. Dogs were kept on wire floors in individual cages and were fed a purified diet with pteroylglutamic acid (PGA) omitted and with a crude PGA antagonist, 'x-methyl' PGA, added. The dogs grew slowly or lost weight and developed a condition marked by emaciation, alopecia, anemia and ulceration of the skin. A slight hemopoietic response with a small reticulocyte rise was obtained when liver extract was injected. However, when PGA was administered a marked response was obtained together with a prompt gain in weight and with growth of hair in the denuded areas. Dogs appeared normal when they received the diet with the antagonist omitted or with sufficient PGA added to reverse the antagonist as judged from experiments with other species.

Observations on the equilibrium between glycine and glycyglycine in the presence of liver peptidase
IAN D. FRANTZ, JR., ROBERT B. LOFTIN, and ANN S. WILNER (introduced by FRITZ LIMANN)
Medical Laboratories of the Collis P. Huntington Memorial Hospital of Harvard Univ., Massachusetts General Hospital, Boston, Mass. Methods have been worked out which permit the study of equilibrium mixtures of glycyglycine and glycine. These are based on the use of long-lived radioactive carbon, for the detection and

measurement of small amounts of the reactants, and chromatography of starch, for their separation. In a typical experiment, labeled glycine is incubated at 38° with a peptidase preparation made from rat liver. The pH is held at approximately 7.6 by means of a borate buffer. After 48 hours the reaction is stopped. Glycyglycine is added as carrier, and the reaction mixture is placed on a column of potato starch. 2.1 normal propanol-water is passed through the column, and the effluent is analyzed by the photometric ninhydrin method of Moore and Stein (*J Biol Chem* 178 367, 1948). The glycine and glycyglycine peaks are each pooled and analyzed for radioactivity. With glycine concentrations as high as 1 molar, the experiment has also been carried out with the addition of carrier, allowing confirmation of the results independent of the use of radioactive carbon. This possibility exists because of the high resolving power of the column, and its large capacity for glycine. Thus, 30 mg of glycine may be completely separated from 60 μ g of glycyglycine, formed during the reaction. The free energy of the peptide bond as determined in this way is in fair agreement with the value calculated by Borsook and Dubnoff, based on thermal measurements (*J Biol Chem* 132 307, 1940).

Purification and properties of ovomucoid
E. FREDERICQ (by invitation) and H. F. DEUTSCH
Depts. of Physical and Physiological Chemistry, Univ. of Wisconsin, Madison, Wis. The solubility of ovomucoid in trichloroacetic acid solution has been utilized for its isolation. In an egg white system of 5% sodium trichloroacetate and pH 3.5, the major portion of the ovomucoid remains in solution while the other proteins are precipitated almost quantitatively. The ovomucoid is then recovered by precipitation at an ethanol concentration of 65% and temperature -7°C . The product consists of 2 components which are closely related electrophoretically. The major component comprises from 90–95% of the total protein. It may be separated in relatively pure form from 1% solution by several precipitations at pH 3.2, ionic strength 0.01, and ethanol concentration 42%. On electrophoresis this protein behaves as a single component over a considerable pH range and has an isoelectric point at pH 3.8. In experiments performed in the region of the isoelectric point some electrophoretic inhomogeneity is evident. Its electrophoretic mobility in diethylbarbiturate buffer at pH 8.6 and ionic strength 0.1, is 4.3×10^{-5} cm² volt⁻¹ sec⁻¹. Ovomucoid which has been isolated by electrophoresis gives a sedimentation constant of $s_{20,w} = 2.9$ s. This value is also obtained with the ovomucoid separated by fractionation. Preliminary carbohydrate analyses of the crude ovomucoid show a level of approximately 10% calculated from competitive antagonists of throxine and structurally related compounds. I. W. F. 1

invitation) and RICHARD J WINZLER *Dept of Biochemistry and Nutrition, Univ of Southern California School of Medicine, Los Angeles, Calif* Thyroxine antagonism by several new compounds has been observed using the amphibian metamorphosis test system described by Frieden and Winzler (*J Biol Chem*, 176 155, 1948) The competitive nature of these inhibitors was shown by the relative constancy of the molar ratio of inhibitor to thyroxine required for reduction of the thyroxine effect by 50%, and by adherence of the data to the more rigorous equation of Lineweaver and Burk (*J Am Chem Soc*, 56 658, 1934) Reduction of the thyroxine effect by 50% by O-benzyl-3,5-diiodo-DL-tyrosine was achieved with molar ratios of 37, while the N-acetyl derivative, described by Woolley (*J Biol Chem*, 164 11, 1946), required a ratio of 550 The benzyl, p-nitrophenylethyl, and methyl ethers of 3,5-diiodo-4-hydroxybenzoic acid required ratios of 8, 75, and 150 respectively for similar effects Specificity of these antagonisms was indicated by the absence of any inhibition of thyroxine by structurally related compounds, goitregens, and other substances Inhibition of the thyroxine-like activity of 3,5-diiodo-4-(3',5'-diiodo-4'-hydroxyphenoxy)benzoic acid was obtained at the same molar ratios with several of the above thyroxine antagonists However, no inhibition of the activity of 3,5-diiodo-4-(4'-hydroxyphenoxy)aniline was observed at very high molar ratios Assuming that thyroxine-like active compounds act by serving as a prosthetic group or coenzyme for some enzyme system or systems, these data lend support to suggestions that the side chain of thyroxine serves as an enzyme-associating locus while the ortho-diiodo hydroxy phenyl group acts as a 'functional' locus of the molecule

Liver regeneration in the adrenalectomized rat CHARLES E FRIEDGOOD (by invitation), HARRY M VARS and JOAN W ZERBE (by invitation) *Harrison Dept of Surgical Research, Schools of Medicine, Univ of Pennsylvania, Philadelphia, Penna* In continuing previous studies of N metabolism and liver protein regeneration male Wistar rats have been adrenalectomized at the time of partial hepatectomy Saline drinking water was available throughout the 2-week postoperative period Total net N balances and new liver protein formed have been determined Adrenalectomy reduced the amount of liver protein regeneration in rats both protein-fed and protein-depleted before operation Whole adrenal extract administered to such rats restored their ability to regenerate new liver protein Rats, protein-depleted for 2 weeks and subjected to the double operation, were given daily injections of desoxycorticosterone acetate, 11-dehydrocorticosterone acetate and 11-dehydro, 17-hydroxycorticosterone (acetate) during the 2 week postoperative period At the dose levels used,

all stimulated hepatic protein metabolism and caused the same or greater regeneration of new liver protein than occurred in animals with intact adrenals DOCA caused the greatest increment, accompanied by evidence of hormonal overdosage 11-dehydrocorticosterone appeared to be slightly more active than 11-dehydro, 17-hydroxycorticosterone

Flavoenzyme catalysis Sulfhydryl character of the d-amino acid oxidase W R FRISSELL (by invitation), E C WEINBACH (by invitation), R C COWGILL (by invitation), and LESLIE HELLERMAN *Dept of Physiological Chemistry, Johns Hopkins Univ School of Medicine, Baltimore, Md* In earlier studies (1940-42) we observed that the activity of the d-amino acid oxidase of lamb kidney is suppressed by p-chloromercuribenzoate (Hellerman in *Cold Spring Harbor Symposia on Quantitative Biology*, 7 165, 1939) and by porphyrindine Notes have appeared from other laboratories on effects of sulfhydryl-binding agents upon such oxidases Of outstanding biochemical importance is the role of 'available' protein-sulfhydryl groups essential to the activity of certain enzymes Although p-chloromercuribenzoate and also iodosobenzoate can attack the separated protein of lamb d-amino acid oxidase, and, less reversibly, pig kidney oxidase, it is demonstrated that these reagents in low concentration may function instead as competitive inhibitors for the substrate It is essential, in these studies, to recognize that certain flavoenzymes dissociate, further, that the aforementioned 'sulfhydryl-reagents' function as such, and, independently, as typical unsaturated anionic inhibitors (*Transactions of the Second Conference, Biological Antioxidants*, 78, 1947) Any labile sulfhydryl groups of these flavoenzymes appear to be 'protected' by FAD Certain reagents attack protein-sulfhydryl irreversibly, in the thermodynamic sense Cogent evidence is revealed in experiments with phenylmercuric acetate Kinetic studies show that the inhibition observed here is not substrate-competitive The action of authentic competitive inhibitors, e.g cinnamate ion, may be superimposed upon that of the mercurial The results in no way support a concept that enzyme-sulfhydryl here is concerned directly with substrate binding or is involved in substrate 'activation'

Preparation of deuterated steroids DAVID K FUKUSHIMA (by invitation) and SEYMOUR LIEBERMAN *Dept of Steroid Biochemistry, Sloan-Kettering Institute for Cancer Research, New York City* For the purpose of studying some problems in steroid metabolism we have investigated the preparation of deuterium labeled steroids Two general methods for the introduction of stably bound deuterium into Δ^4 -steroids have been developed The first of these involved halogenation with bromosuccinimide at C₇ of a steroid ester followed by cataly-

tic reduction with deuterium in the presence of palladium or deuterized Ranney nickel. An alternate procedure, which was advantageous in that 2 deuterium atoms were incorporated, consisted in the conversion of a Δ^5 -7-ketosteroid ester to the corresponding mercaptol by condensation with ethanedithiol. Upon desulfurization with deuterized Raney nickel, the cyclic thioacetal was converted to the Δ^5 -7,7-d₂-steroid. By these methods deuterium labeled cholesterol and Δ^5 -androstenediol-3 β ,17 α -acetate-3,benzoate-17, an intermediate in the synthesis of testosterone, were prepared.

Hepato-renal factors in circulatory homeostasis
XXIV. Conditions leading to aerobic formation of VDM by liver. ROBERT F. FURCHGOTT, BENJAMIN W. ZWEIFACH (by invitation) and EPHRAIM SHORR (by invitation). *Dept. of Medicine, Cornell Univ. Medical College and The New York Hospital, New York City.* Previous experiments from this laboratory have shown that normal liver slices form the vasodepressor, VDM, only under anaerobic conditions, on aerobic incubation, VDM formation is inhibited and VDM added to the incubation medium is inactivated. In contrast, livers from animals in irreversible shock sustain a profound impairment in the capacity to inactivate VDM on aerobic incubation. A similar impairment is shown by normal liver slices following prior exposure to anaerobiosis. In a recent extension of these studies, it has been observed that liver slices from dogs in irreversible shock not only lose their capacity to inactivate VDM aerobically, but also continue to form VDM under aerobic conditions. This was also the case with normal liver following an appropriate period of anaerobic exposure, anaerobic incubation of normal liver (dog, rabbit) for one hour at 37.5°C usually results in the continued elaboration of VDM on the restoration of aerobic conditions. With both types of liver preparations, the rate of aerobic VDM formation is comparable to that of normal liver slices under anaerobic conditions. Respiratory studies of liver slices, treated so as to form VDM aerobically, revealed no relation between the loss of ability to inhibit aerobic VDM formation and changes in oxygen consumption. The significance for shock of this derangement in VDM metabolism resides in the fact that the temporary restoration of aerobic conditions within the liver by transfusions would not, per se, prevent the continued elaboration of VDM and the persistence of the decompensatory effects of this vasodepressor principle on the peripheral circulation.

Chloride diarrhea and systemic alkalosis in potassium deficiency. LYTT I. GARDNER, ELSIE A. MACLACHLAN, MARY L. TERRY, JANET W. McARTHUR and ALLAN M. BUTLER (introduced by RALPH W. MCKEE). *Children's Medical Service, Massachusetts General Hospital, and the Dept. of Pediatrics, Harvard Medical School, Boston, Mass.*

Severe potassium deficiency was produced in 160 to 175-gm rats by use of a diet containing 0.003% K and 0.7% sodium. K added to the diet produced excellent growth in controls. Rats fed the deficient diet gained weight slowly for 35–40 days, then diarrhea began, followed by anorexia, wasting and death between 60 and 120 days. Muscle K was reduced almost 50%. Hypochloremic alkalosis was present. Metabolic studies while on the deficient diet showed negative K and Cl, and positive Na balances. A single injection of 1.0 mEq K as K_2HPO_4 produced transitory reversal of these balances and temporarily arrested the diarrhea and anorexia. The Cl and Na balances lagged 3–4 days in their reversal. Fecal excretion of Cl progressively diminished for 6 days post-injection. Urinary Cl decreased for 3 days post-injection, then abruptly increased on the 4th day, coincident with marked Na diuresis. This may reflect the extrusion of intracellular Na, covered in the urine by the anion Cl, cf. Conway and Hingerty, *Biochem. J.* 42: 372, 1948. On the deficient diet daily Cl excretion was double that on the control diet. There was 4 times as much Cl in the feces of the deficient animal as in the feces of the control. Even during diarrhea there were twice as many mEq of Cl as Na in the feces of the deficient animal. The reported cases of congenital alkalosis with diarrhea, and 2 cases of ulcerative colitis seen at this hospital, show many metabolic similarities to the above findings, which suggest deficit of intracellular K. The etiologic role played in these syndromes by adrenal overproduction of the 11-desoxycorticosteroid (DOCA) type of hormone, known to produce K deficiency when injected, is under investigation.

Inhibition of hyaluronidase by blood serum and bile constituents. DAVID GLICK, LEE W. WATTENBERG (by invitation) and ERICK Y. HAKANSON (by invitation). *Dept. of Physiological Chemistry, Univ. of Minnesota Medical School, Minneapolis, Minn.* In an attempt to determine the origin of the hyaluronidase inhibitor in blood serum, 29 organs and bile, urine, and amniotic fluid of the rabbit, in addition to human saliva, milk, and spinal fluid, and hog synovial fluid were tested. No inhibitory activity was found by the viscosimetric method in any of them except bile and urine. The inhibitor in the latter fluids was heat stable and active in the presence of phosphate, thus differentiating it from that in serum. A variety of hemoglobin derivatives, bile salts, and sterol hormone conjugates have been found to inhibit hyaluronidase. The additive value of the individual inhibitions of any one of the compounds and that of serum was always greater than the observed inhibition of a combination of the two. The data would indicate that the hemoglobin derivatives and bile salts inhibited the serum inhibitor, and that serum also reduced the inhibition

of these compounds. Exceptions to this mutually antagonistic effect were observed with certain of the sterol conjugates. Stercobilin was unique in that it did not inhibit hyaluronidase under the conditions used while it did inhibit the serum inhibitor. Studies of the variations of the human serum inhibitor during the menstrual cycle revealed highest levels during actual menstruation and lowest during subsequent and middle phases of the cycle. No significant change was found during pregnancy or labor, but a marked post-partum rise was observed which reached a maximum from the 2nd to 5th days after delivery.

Amino acid composition of casein, α -casein and β -casein. WILLIAM G GORDON, WILLIAM F SEMMETT (by invitation), and MYRON MORRIS (by invitation). *Eastern Regional Research Laboratory, Philadelphia, Penna.* The investigation of the comparative amino acid composition of whole casein, α -casein and β -casein previously reported in part (*Federation Proc* 6 255, 1947) has been completed. The analytical results, expressed as grams of amino acid yielded by 100 gm of dry, ash-free protein, follow

	Whole Casein	α Casein	β -casein
Glycine	2.7	2.8	2.4
Alanine	3.0*	3.7*	1.7*
Valine	7.2	6.3	10.2
Leucine	9.2	7.0	11.6
Isoleucine	6.1	6.4	5.5
Proline	11.3	8.2	16.0
Phenylalanine	5.0	4.6	5.8
Cystine	0.34	0.43	<0.1
Methionine	2.8	2.5	3.4
Tryptophan	1.2	1.6	0.65
Arginine	4.1	4.3	3.4
Histidine	3.1	2.9	3.1
Lysine	8.2	8.9	6.5
Aspartic acid	7.1	8.4	4.9
Glutamic acid	22.4	22.5	23.2
Amide nitrogen	1.0	1.0	1.0
Serine	6.3	6.3	6.8
Threonine	4.0	4.9	5.1
Tyrosine	6.3	8.1	3.2

* Provisional

Calculation of the data on the basis of N distribution shows that 101.5% of the whole casein N, 101.8% of the α -casein N and 103.4% of the β -casein N have been accounted for in terms of recognized amino acids and amide groups.

Effect of N,N-dibenzyl- β -chloroethylamine (dibenamine) on blood sugar response to insulin and epinephrine. R. LORIMER GRANT. *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.* The subcutaneous injection of dibenzyl- β -chloroethylamine hydrochloride 18-20 hours before the injection of an insulin-containing drug appeared to prolong the hypoglycemic effect of the insulin. Epinephrine injected during the hypoglycemia was significantly inhibited as an antidote to the insulin. Nickerson and Goodman (*J Pharmacol and Exper Therap* 89 167, 1947) found that dibenzyl- β -chloroethyl-

amine produced no significant inhibition of the epinephrine-induced blood sugar rise in rabbits. We repeated their experiment using a 12 rabbit cross-over test and demonstrated a significant inhibition of the epinephrine-induced blood sugar rise following treatment with dibenzyl- β -chloroethylamine. The effect of dibenzyl- β -chloroethylamine on the response to insulin is apparently due to inhibition of endogenous epinephrine or other sympathomimetic factors which increase the rate of glycogenolysis.

Studies on hypoxanthine synthesis in pigeon liver with labeled formic acid. G. ROBERT GREENBERG (introduced by HARLAND G. WOOD). *Dept of Biochemistry, School of Medicine, Western Reserve Univ., Cleveland, Ohio.* It previously has been shown that HC^{14}OOH is incorporated into hypoxanthine in pigeon liver homogenates (*Arch Biochem* 19 337, 1948). Further studies have been conducted to elucidate the intermediate steps in this synthesis using HC^{14}OOH to trace the reaction. Approximately 60-70% of formic acid was accounted for in hypoxanthine but almost 100% of the formic acid disappeared during the same period of time. This suggested that the HC^{14}OOH had either entered into other reactions or was present in intermediate compounds involved in the synthesis of hypoxanthine. Small volumes of the reaction mixture were subjected to filter paper chromatography. The paper strips were cut into 0.5 or 1 cm sections and the radioactivity of each determined. Two distinct radioactive compounds migrated down the paper. One of these has been identified as hypoxanthine and contains the majority of the radioactivity. The other component has a distinctly different absorption spectrum than that of hypoxanthine. The homogenate may be separated into 2 components, the washed particles and the supernatant. Neither fraction shows much activity alone but in combination almost the full synthetic ability is restored. Either component is inactivated by boiling.

Short-term pyridoxine deprivation in two human subjects. LOUIS D. GREENBERG, DAVID F. BOHR (by invitation), HOPE McGRATH (by invitation) and JAMES F. RINEHART (by invitation). *Dept of Pathology, Univ of California Medical School, San Francisco, Calif.* Studies of the blood pyridoxine and the urinary excretion of pyridoxine, 4-pyridoxic acid, xanthurenic acid, tryptophan and N-methylnicotinamide were carried out on two human subjects who persisted on a pyridoxine deficient diet for three weeks. The diet consisted of casein, sucrose, corn oil and salts supplemented with the known vitamins excepting pyridoxine, p-aminobenzoic acid, vitamin K and tocopherols. By the administration of extra pyridoxine prior to the withdrawal of the vitamin, the blood pyridoxine concentration was increased from initial

values of 15 to 20 μg to values of 41 to 71 μg %. At the end of the deficient period the levels had fallen to approximately the initial values or lower. The urinary pyridoxine and 4-pyridoxic acid paralleled each other, rising and falling during periods of administration and depletion of the vitamin, respectively. At the end of 2-3 weeks of depletion, xanthurenic acid excretion following 10 gm doses of DL-tryptophan was markedly increased and returned to low levels after supplementation with pyridoxine (10-15 mg daily) for one week. The urinary excretion of tryptophan as measured microbiologically was not significantly altered by deficiency, but chemical determination of tryptophan revealed that large quantities of tryptophan or an indole derivative appeared in the urine during the depletion period when test doses of DL-tryptophan were fed. Excretion of the metabolite remained high despite supplementation of the experimental diet with pyridoxine for one week. In confirmation of the work of others, an increased excretion of N-methylnicotinamide was observed after the feeding of tryptophan.

Effect of aromatic hydrocarbons on the metabolism of exogenous sulfur amino acids HELMUT R. GUTMAN (by invitation) and JOHN L. WOOD, *Dept of Chemistry, School of Biological Sciences, Univ of Tennessee, Memphis, Tenn.* The changes in urinary sulfur distribution produced by administration of aromatic hydrocarbons to rats were studied after injection of L-cystine or DL-methionine labeled with radioactive sulfur. The labeled total sulfur, inorganic sulfate, and chloroform-extractable sulfur-containing compounds in the urines were estimated by radioactivity measurements. Fifteen % of the sulfur was recovered in the urine 24 hours after injection of 10 mg of L-cystine and was chiefly in the form of inorganic sulfate. When 150 mg of bromobenzene were administered simultaneously, the amount of labeled total sulfur excreted appeared in the urine as chloroform-extractable sulfur at the expense of inorganic sulfate. The radioactive sulfur in this fraction was incorporated into bromophenylmercapturic acid since, following the addition of unlabeled bromophenylmercapturic acid to the urine, radioactive bromophenylmercapturic acid was recovered. Twenty % of S^{35} administered as DL-methionine was found in the urine in the form of inorganic sulfate after 24 hours. The administration of bromobenzene caused an increase in the total excretion of the injected S^{35} to 60% without appreciable change in the inorganic sulfate. The increase in excreted S^{35} was not accounted for by the mercapturic acid found but by other organic sulfur compounds not extractable by chloroform. The studies will be extended and correlated with alterations in sulfur metabolism produced by carcinogenic hydrocarbons.

Study of the carbohydrate-regulating hormone of hog adrenal extract WILLIAM J. HAINES (by invitation), RICHARD H. JOHNSON (by invitation), MARI P. BRUNNER (by invitation), MARVIN L. PABST (by invitation), and MARVIN H. KUIZENGA, *Research Laboratories, The Upjohn Company, Kalamazoo, Mich.* In 1933 Grollman reported that autolysis of beef adrenal tissue caused a significant decrease in the recovery of cortical hormone activity of the survival-growth type. However, subsequent biochemical studies on adrenal extracts, including those resulting in the isolation of 28 pure steroids, appear to have been done with adrenal glands collected by the usual slaughter-house procedures. The large quantities of tissue required in these earlier studies would seem to have prohibited the use of specially-collected glands. More recently we have developed techniques of isolation and identification which allow rather complete characterization of cortical hormone from relatively small amounts of adrenal tissue. These methods have now been applied to the investigation of the carbohydrate-regulating hormone of quick-frozen hog adrenals, in which autolytic changes have been greatly reduced. Comparison of this material, at various stages in its purification, with that obtained routinely from glands collected by the usual procedures, indicates some of the effects of autolysis. The neutral, composite extract from quick-frozen gland contained 120% more bioactivity (liver-glycogen deposition) than that from regular gland. Five hundred mg (10-kg gland) of it gave a normal benzene-water distribution after 2 cycles. Long standing in this system caused a 47% loss of activity. Five hundred fifty mg (11-kg gland) when chromatographed over MgSiO_3 , wherein bands were characterized by optical rotation, formaldehyde-forming capacity, and infrared absorption, yielded 70 mg of 17-hydroxycorticosterone, or 4 times the amount obtained from normal gland. Further characterization of the carbohydrate-regulating hormone are discussed.

Blood changes in acute amino acid deficiencies W. KNOWLTON HALL, LESTER L. BOWLES (by invitation) and V. P. SYDENSTRICKER (by invitation), *Depts of Biochemistry, Microscopic Anatomy and Medicine, Univ of Georgia School of Medicine, Augusta, Ga.* In order to investigate the blood changes which result from amino acid deficiencies, litter mate Wistar-strain rats were placed on diets devoid of one of the indispensable amino acids or on control diets. The deficient diets contained 3 times the minimum requirement of the other indispensable amino acids. Blood was obtained for counts and analyses by sacrifice of the animals and the values obtained with the different groups were compared. Except for some animals which were sacrificed at the beginning of the experimental period, the animals remained on the

diets until the symptoms of the deficiency became extreme. In rats fed a phenyl-alanine-deficient diet there was a reduction in blood volume although the blood counts and plasma protein levels were normal. There was a marked reduction in the number of reticulocytes. In valine deficiency the reduction in blood volume was less marked, there was a slight reduction in plasma protein level and the blood from most of the rats seemed devoid of reticulocytes. In isoleucine deficiency there was a reduction in blood volume, plasma protein levels and the reticulocyte count. These changes may be compared with the extreme hypoproteinemia, reduced blood volume and moderate anemia observed in deficiencies of threonine or protein. There was little difference between control rats fed the mixture containing only the indispensable amino acids and those fed a comparable level of an amino acid mixture similar to casein.

Mode of action of parathormone PHILIP HANDLER, W. J. A. DEMARIA (by invitation) and D. V. COHN (by invitation) *Depts. of Biochemistry and Pediatrics, Duke Univ. School of Medicine, Durham, N. C.* The intravenous administration of commercial parathyroid extract to dogs consistently resulted in an increased flow of a relatively alkaline urine containing considerably more sodium, bicarbonate and phosphate than the control urines. In some animals this phenomenon was immediately apparent while in others there was a lag period of 15-40 minutes. Studies of inulin and p-aminohippurate clearances revealed that the diuresis occurred simultaneously with a marked rise in renal blood flow and glomerular filtration. These in turn appeared to be secondary to a rise in systemic arterial blood and pulse pressures. The lag period occurred only in those dogs in whom parathormone administration induced a temporary shock-like state with low blood pressure, glomerular filtration and renal plasma flow. In no instance did the parathormone function by actually inhibiting renal tubular reabsorption of sodium, bicarbonate, chloride or phosphate, the altered composition of urine being due solely to the increased glomerular filtration consequent upon the rise in blood pressure and renal plasma flow. Studies are now in progress to determine whether the pressor principle in parathyroid extract is identical with the calcium mobilizing hormone or whether it is an artifact created during the extraction procedure.

Enzymatic hydrolysis of carnosine and related peptides H. THEO HANSON (by invitation) and EMIL L. SMITH *Laboratory for the Study of Hereditary and Metabolic Disorders and the Depts. of Biochemistry and Medicine, Univ. of Utah School of Medicine, Salt Lake City, Utah.* Liver, spleen, and kidney of the rat, and swine kidney are excellent sources of an enzyme, carnosinase, which

hydrolyzes carnosine β -alanyl-L-histidine. The swine kidney enzyme has been partially purified. Carnosinase is a metal-protein as indicated by the inhibition produced by cyanide, sulfide, and cysteine. Although both Mn^{++} and Zn^{++} activate the enzyme, it is likely that Zn^{++} is the naturally-occurring metal. This is suggested by the similarity of the Zn^{++} -enzyme and the unactivated preparation with respect to pH of optimal activity, poor stability at 40° in absence of substrate, and insensitivity to phosphate and citrate. At 0.05 M carnosine, the hydrolysis follows the kinetics of a zero order reaction, and the rate is proportional to the enzyme concentration over a range of at least 1-12. The optimal action with Mn^{++} is at pH 8.0-8.4, with Zn^{++} at 7.8-7.9, and without added metal at 7.4-7.5. The hydrolysis of carnosine cannot be attributed to any previously described protease. In addition to L-carnosine, the enzyme rapidly hydrolyzes both L and D-alanyl-L-histidine, and glycyl-L-histidine while L- α -amino-butyl-L-histidine, and glycyl-L-histidinamide are hydrolyzed more slowly. D-Carnosine, β -L-aspartyl-L-histidine, carbobenzoxy-L-carnosine, and carbobenzoxyglycyl-L-histidinamide are relatively resistant to the enzyme. These data indicate that carnosinase is an exopeptidase of dipeptidase or aminopeptidase specificity.

Application of micro methods to the study of steroid metabolism J. D. HARDY (by invitation), HILDEGARD WILSON (by invitation) and KONRAD DOBRINER *Sloan-Kettering Institute for Cancer Research and the Dept. of Physiology, Cornell Univ. Medical College, New York City.* The detailed study of the steroid hormone metabolites in the urine of healthy and diseased persons has been possible in the past with long term collection periods. By the use of infrared spectrometry as a method for the identification of the individual steroids in amounts of 1-3 mg the period of urine collection was shortened. A further improvement of this specific method of analysis was the development of a microcell for routine use. The cell has a volume of 0.03 ml, and is filled by capillary action. It is designed to be used immediately in front of the slit of a Perkin-Elmer spectrometer so that the entire cross section of the cell is effective in producing absorption. With this cell the individual steroids and their mixtures as obtained by chromatographic procedures can be recognized routinely in amounts of 25 μ g and under favorable circumstances 3 μ g suffice. The methods of separation and chromatographic analysis have been adapted for small amounts and estimation of ketosteroids by the Zimmermann reaction has been modified so that 2 μ g can be determined. This development has made possible a precise qualitative and quantitative procedure for the establishment of steroid excretion patterns in collection

periods of 1-10 days. The details of the methods applied and the results obtained will be discussed.

Toxicity of tetramethyl succinonitrile and the antidotal effects of thiosulphate, nitrite and barbiturates. R. N. HARGER and H. R. HULPIEU (by invitation). *Dept. of Biochemistry and Pharmacology, Indiana Univ. School of Medicine, Indianapolis, Ind.* Tetramethyl succinonitrile (T S N) and nitrogen are the chief products formed when azo bis-isobutyro nitrile, 'Porofor N' is heated with rubber to make sponge rubber. T S N, M.P. 167°, is quite volatile, air saturated with it containing 120 ppm at 25° and 7820 ppm at 100°. Animals poisoned with T S N exhibited violent convulsions, with asphyxial death in from 1 minute to 5 hours after the first convulsion. Administered subcutaneously, the LD50 in mg/kg was about 30 for rats and 23 for guinea pigs. Two rats receiving 49 and 56 mg/kg per os first convulsed at 5 hours and died several hours later. Intravenously, the LD50 of T S N for rabbits was 20 mg/kg, but 2.5 mg/kg caused convulsions. Dogs were killed by 20 mg/kg or more, given subcutaneously. When T S N vapor was inhaled by rats, 60 ppm killed in 2-3 hours, and 6 ppm caused death in about 30 hours. Neither artificial respiration, thiosulphate, or thiosulphate followed by nitrite had any effect on the toxicity of T S N. Barbiturates administered as needed saved animals receiving up to 50 mg/kg. The use of a quick-acting barbiturate followed by phenobarbital gave the best results obtained. Preliminary experiments indicate that 10 mg/kg of T S N has a greater analeptic effect than 5 mg/kg of picrotoxin, when tested in rabbits with severe phenobarbital depression.

Bacterial synthesis in the rat of an unidentified growth-promoting factor. A. M. HARTMAN (by invitation), L. P. DRYDEN (by invitation), and C. A. CARY. *Division of Nutrition and Physiology, Bureau of Dairy Industry, U. S. Dept. of Agriculture.* Although rats require a chemically still-undefined vitamin, (X), when 35 young were fed an X-deficient diet containing extraordinarily high levels of the 10 B vitamins, methionine and vitamins C, E, and K, 20 rats grew 169 gm and 15 grew only 87 gm in 4 weeks. Twelve of the latter increased in growth from 13 gm per week before to 16, 36, and 32 gm in successive weeks after being fed a single small dose of feces from the former. Additional slow-growing rats fed sulfasuxidine in the diet did not respond to this feeding of feces. These results suggest very strongly that X may be synthesized by microorganisms which may be induced to thrive in the rat. This synthesis was made possible by the extraordinarily high level of riboflavin, 'inoculations' being ineffective with average intakes of $71 \pm S.D. 16 \mu\text{g/day}$. Material(s) with X activity as well as with animal protein factor and antipernicious anemia activities is (or

are) formed simultaneously microbiologically *in vitro* (Stokstad, *J. Lab. Clin. Med.* 33: 860 1948). Crude preparations of B₁₂ and of the 'cow manure' factor are active as X. These results and those of Ott (*J. Biol. Chem.* 174: 1047, 1948) and Bird (*J. Biol. Chem.* 176: 1477 1948) with B₁₂ suggest that all of these factors are either very closely related or identical. X occurs in different combinations in different feeds, all are active in the normal rat.

Isotopic studies of the biosynthesis of nucleic acid components. I. Purines and pyrimidines. MILTON R. HEINRICH (by invitation), D. WRIGHT WILSON and SAMUEL GURIN. *Dept. of Physiological Chemistry, School of Medicine, Univ. of Pennsylvania, Philadelphia, Penna.* The incorporation of carbon dioxide, acetate, and glycine into the nitrogenous bases of nucleic acids and nucleotides in the rat has been studied. After administering C¹⁴-labeled compounds to rats, nucleotides were extracted from the carcasses with acid, hydrolyzed, and adenine isolated. Lipids were removed, nucleic acids extracted with 10% NaCl, and the latter hydrolyzed to individual purines and pyrimidines. Within 7 hours after injecting labeled NaHCO₃ into a rat, a very small amount of radioactive carbon was found in the nucleotide adenine and the nucleic acid adenine, guanine, and uracil. Permanganate degradations showed the isotope to be in the non-ureide carbon of guanine, and the ureide carbon of uracil. When an approximately equivalent total amount of isotope in the form of carboxyl-labeled sodium acetate was injected into rats for 5 days, about the same degree of incorporation was observed. No isotope appeared in the ureide or guanidino carbon of guanine. The highest concentration was found in the ureide carbon of uracil, probably due to incorporation of carbon dioxide. Glycine labeled in the carboxyl position was fed to a group of rats for 10 days, using about twice the quantity of isotope given in each of the previous experiments. Incorporation into the purines of nucleic acids and nucleotides was about 20 times that observed with either bicarbonate or acetate. Very little isotope was found in the uracil or thymine. A sample of the nucleic acid guanine was hydrolyzed with concentrated HCl to give glycine in which the carboxyl group is derived from carbon 4 of guanine. This glycine carboxyl, obtained with ninhydrin, contained essentially all the isotope incorporated into the guanine.

Cytochrome c reductase (DPN). LEON A. HEPPEL (introduced by B. L. HORECKER). *Experimental Biology and Medicine Institute, National Institutes of Health, Bethesda, Md.* The reduction of cytochrome c by reduced diphosphopyridine nucleotide (DPN) has been observed in freshly prepared pig heart suspensions (Lockhart and Potter, *J. Biol. Chem.* 137: 1, 1941) and a partially purified preparation of the enzyme has been ob-

tained from baker's yeast (Altschul, Persky and Hogness, *Science* 94 349, 1941) Lack of information regarding the properties of the cytochrome *c* reductase (DPN) of animal tissues has prompted this investigation The enzyme could be stored for long periods in hog liver acetone powder, from which it was extracted with 0.06 M phosphate, pH 7.4 The turbid extract was incubated at room temperature with crude steapsin After this treatment a considerable portion of the activity was precipitated between 0.4 and 0.5 saturation with ammonium sulfate and on addition of water a clear solution was obtained With extracts not treated with steapsin almost all of the activity was precipitated below 0.3 saturation with ammonium sulfate and could not be redissolved The ammonium sulfate fraction had a pH optimum at 6.8 and the enzymatic activity was precipitated below pH 5.3 By lyophilization a stable powder was prepared which is being subjected to further purification

Degradation of polysaccharides by muscle phosphorylase SHLOMO HESTRIN (introduced by C. F. CORI) *Dept. of Biological Chemistry, School of Medicine, Washington Univ., St. Louis, Mo.* Branched polysaccharides (glycogen and amylopectin) could only be degraded partially ($40 \pm 5\%$) by recrystallized muscle phosphorylase β -amylase limit dextrans of the branched polysaccharides were not degraded to a significant extent by the recrystallized muscle phosphorylase Like β -amylase and potato phosphorylase, the phosphorylase of muscle is thus incapable of degrading starch and glycogen chains beyond their branchpoints The phosphorylase limit dextrin of glycogen differed, however, from the β -amylase limit dextrin of glycogen in respect to priming activity for muscle-phosphorylase Since the phosphorylase limit dextrin afforded roughly an equivalent of one mole maltose per calculated end group when treated with β -amylase, the outer chains of the phosphorylase limit dextrin are probably three or more glucose units long The role of this dextrin in the equilibrium mediated by phosphorylase is discussed

Ataxia and loss of equilibrium in manganese-deficient rats (motion picture) ROBERT M. HILL, DORSEY E. HOLTKAMP (by invitation), A. R. BUCHANAN (by invitation) and ENID K. RUTLEDGE (by invitation) *Depts. of Biochemistry, Anatomy, and Pathology, Univ. of Colorado Medical Center, Denver, Colo.* Shils and McCollum (*J. Nutrition* 26 1, 1943) reported the development of ataxia and poor equilibrium in rats on a manganese-deficient diet Symptoms increased in severity to the 18th day of life when the rats either died or 'recovered to a great extent' We have seen these symptoms in the 2nd, 3rd, and 4th generations of rats on a very low manganese intake, about 0.006 mg/rat/

day In animals placed on low manganese diets after maturity no symptoms appeared Symptoms appeared in 5 female rats in the 2nd generation, 2 before 6 months and 3 between 6 and 14 months of age The latter 3 of these animals raised litters, although poorly Of 3 males and 4 females that were, allowed to reach maturity in the 3rd generation, 1 male and 2 females developed symptoms before weaning One of these females that did not show symptoms was bred and successfully raised one of a litter This one 4th-generation rat, a female, is now 80 days of age and exhibits marked symptoms The symptoms appeared earlier in this rat than in those of the 3rd generation All 8 females with symptoms showed shoulders leaning to the right with the head rotated to the left The 1 male showed shoulders leaning to the left with the head rotated to the right After rotation on a turntable all low-manganese animals showed extreme ataxia and loss of equilibrium Post-rotational nystagmus was normal in direction but slow and of shorter duration than normal

A 16-oxygenated steroid from adrenocortical tumor urine H. HIRSCHMANN and FRIEDA B. HIRSCHMANN (by invitation) *Dept. of Medicine, Western Reserve Univ., and the Lakeside Hospital, Cleveland, Ohio* The triacetate (I) ($[\alpha]_D^{25} = -101^\circ$) of a triol $C_{27}H_{48}O_6$ (provisionally designated as Compound A (Hirschmann and Hirschmann, *J. Biol. Chem.* 157 601, 1945)) isolated from the urine of a boy with an adrenocortical tumor has been reduced catalytically to the triacetate $C_{27}H_{48}O_6$ (II) (m.p. 177-179°, $[\alpha]_D^{25} = -58^\circ$) The same substance has been prepared from the triacetate (III) of the allopregnanetriol-3,16,20 of the urine of pregnant mares (allopregnanetriol-3(β),16(α),20(β) (IV) (Hirschmann *et al.* *J. Biol. Chem.* in press)) The 20-monoacetate of IV was tritylated in positions 3 and 16 and the ditrityl ether (m.p. 170-172°) saponified in position 20 The 3(β),16(α)-ditritoxyallopregnanol-20(β) (m.p. 168-171°) was oxidized with chromic acid in acetone to the 3(β),16(α)-ditritoxyallopregnanone-20 (m.p. 269-270°) which was reduced with lithium aluminum hydride, then hydrolyzed with acid and acetylated to yield 2 triacetates which were identified as compounds II and III Substance II, therefore, is allopregnanetriol-3(β),16(α),20(α) triacetate In view of the change in molecular rotation accompanying the reduction of I it is most probable that Compound A is Δ^5 -pregnenetriol-3(β),16(α),20(α) As this triol was obtained from adrenal tumor urine in a yield of about 6 mg/l it is derived most likely from compounds formed by adrenal tumor cells This finding seems to warrant an inquiry into the corticoid activity of suitably substituted 16-oxygenated steroids and into the possible presence of such compounds in the normal adrenal cortex

Dietary manganese and susceptibility to pneumococcal infection GEORGE H. HITCHINGS, ELVIRA A. FALCO (by invitation) and MARION B. SHERWOOD (by invitation) *The Wellcome Research Laboratories, Tuckahoe, N. Y.* The chief component of the dietary factor which increases the susceptibility of mice to pneumococcal infection (*Proc Soc Exper Biol & Med*, 61: 54, 1946, *Federation Proc* 6: 261, 1947) has been identified as manganese. By the use of a highly purified diet and a minimally infective dose of the pneumococcus (*Type I* SV1 strain) it can be shown that the susceptibility of mice to infection steadily increases with increasing manganese content of the diet to levels as high as 0.6 mg Mn/gm of diet. The effect of manganese in some cases can be partially antagonized by a large excess of dietary cobalt. Similar tests with other types and strains of pneumococci and hemolytic streptococci have resulted in the discovery of several with which the same dietary effect on susceptibility is demonstrable.

Effect of dietary manganese on the growth of white rats DORSEY E. HOLTKAMP (by invitation) and ROBERT M. HILL, *Dept of Biochemistry, Univ of Colorado Medical Center, Colo.* Several laboratories have shown that manganese is an essential dietary factor for growth of young rats, but hitherto no uncomplicated dietary study has been made to determine the optimal manganese intake for this function. In the work reported here, 55 female rats, just before breeding, were divided into 7 groups and put on identical manganese 'free' (6 µg/rat/day) but otherwise adequate basal diets with supplements of manganese in the respective groups added at the following average daily intakes per rat: none, 0.1 mg, 1.0 mg, 5.0 mg, 10.0 mg, 20 mg and 40 mg. These diets were maintained throughout gestation, lactation and the subsequent growth period of the young. Forty-eight litters were obtained of which 38 (194 young) were raised beyond 18 days of age. In the Mn 'free' group 5 litters were born from the 8 females bred, and 3 litters (16 rats) were raised to weaning age. The other 6 groups showed normal birth records with no significant differences between them. Growth records of the young were started on the second and third days and were continued through the 50th day of life. Growth was better with increased manganese supplements up to 5 mg/mother/day. This intake was optimal. Growth was progressively poorer with the higher supplements of 10, 20 and 40 mg/mother/day. At the 40 mg level growth was similar to that on the Mn 'free' diet. The advantage of the optimal level of manganese intake over both higher and lower levels becomes more apparent the longer the diets are maintained.

Quantitative paper chromatography of penicillins I. Use of aqueous solution as developing agent IRVING R. HOOPER, DAVID L. JOHNSON,

AND HAROLD D. TAYLOR (introduced by S. B. BINKLEY) *Research Division, Bristol Laboratories Inc., Syracuse, N. Y.* The commonly known penicillins were separated in paper strip chromatography by development with a sodium citrate solution. 2-Pentenylpenicillin and *n*-pentylpenicillin, moving fastest, were not separated. Benzylpenicillin and *p*-hydroxybenzylpenicillin, appearing together, were not completely separated from the 2-pentenylpenicillin and *n*-pentylpenicillin. *n*-Heptylpenicillin moved slowest and was completely separated. Schleicher and Schuell 589 Blue Ribbon filter paper strips, $\frac{1}{4}$ inch wide were spotted with 0.5 to 2.5 units of penicillin in 0.5 to 5 microliters of solution and then were developed by allowing 40% sodium citrate to flow over the strip in a humidified chamber. After development the strips were placed on *Staphylococcus aureus* inoculated trays which were incubated to demonstrate the position of the penicillins on the strip. The results were photographed. By this procedure benzylpenicillin in mixtures has been determined quantitatively without purification or concentration. The percentages of the penicillins present were determined from the maximum widths of the inhibition zones of two strips with different volumes of a penicillin solution placed on one tray. A statistical analysis was made on the accuracy of the quantitative determination of benzylpenicillin in a series of solutions prepared with known amounts of benzylpenicillin, *n*-pentylpenicillin and *n*-heptylpenicillin. For benzylpenicillin content it was concluded that the observed average for two strips should be within 8.5% of the true value 95 times out of 100 and within 4.2% two times out of three.

Cytochrome *c* reductase (TPN) from liver B. L. HORECKER, *Exptl Biology and Medicine Institute, National Institutes of Health, Bethesda, Md.* Haas, Horecker and Hogness (*J Biol Chem* 136: 746, 1940) have isolated an enzyme from yeast which catalyzes the reduction of ferricytochrome *c* by reduced triphosphopyridine nucleotide (TPNH₂). Cytochrome reductase (TPN) has now been purified from hog liver by extraction of acetone powder with Na₂HPO₄, digestion with trypsin, fractionation between 0.45 and 0.70 saturated (NH₄)₂SO₄, fractionation with alcohol at pH 5.0, adsorption on Al(OH)₃, C γ gel, elution with Na₂HPO₄, adsorption on Ca₃(PO₄)₂ gel and elution with Na₂HPO₄. This procedure yields a pale yellow solution which is 170-fold purified compared to the acetone powder extract. The purified enzyme has a specific activity in the reduction of cytochrome *c* with excess TPNH₂ which is about one sixth that of pure cytochrome reductase from yeast. In the acetone powder extracts the bulk of the activity is associated with insoluble particles from which it is liberated by the digestion with trypsin. The activity is found in the mitochondrial fraction

prepared from fresh rabbit liver by the method of Hogeboom, Schneider and Pallade (*J Biol Chem* 172 619, 1948) The specific activity in these granules is identical with that of the liver acetone powder extracts With purified preparations no reduction of ferricytochrome *c* occurs with reduced diphosphopyridine nucleotide

Penicillin and amino acid assimilation in staphylococci ROLLIN D HOTCHKISS *Rockefeller Institute for Medical Research, New York City* Penicillin is known to cause death of growing, but not of non-growing, bacteria From the work of Gale it appears that those strains of staphylococci which are susceptible to penicillin are dependent upon pre-formed amino acids for growth Gale has also shown that staphylococci grown in penicillin are subsequently unable to take up glutamic acid normally It is difficult to know whether this effect is a cause, or a result, of the primary biochemical damage to these growing cells An effect upon washed cells could not be demonstrated The author has reported (*Federation Proc* 6 263, 1947) that washed, respiring staphylococci can take up amino acids and couple them into cell protein In the presence of crystalline penicillin G (50 u/mg of cells), however, the nitrogen compounds produced are recovered largely or entirely in the suspending medium This is true although the rates and extents of disappearance of amino groups have always been found to be identical in the presence and absence of penicillin, from a variety of simple and complex mixtures of pure amino acids Similar small amounts of ammonia, esterified phosphate and other side products are produced in the two cases Under appropriate conditions, simultaneous utilization of simple amino acids by washed staphylococci leads to the disappearance of uracil from the environment, this disappearance is markedly increased by low concentrations of penicillin Other pyrimidines or purines are not materially affected The uracil utilized cannot be recovered by hydrolyzing the bacteria

Purification, crystallization and properties of circulin, an antibiotic from the bacillus circulans group SPACEY F HOWELL *Biochemistry Section, V D Research Laboratory, U S Public Health Service, Staten Island, N Y* Circulin is an antibiotic of polypeptide nature and has been isolated in crystalline form from the culture fluid of *Bacillus circulans* It is purified by acidifying the culture fluid to pH 2.0, adding acetone to 30% followed by saturation with ammonium sulfate The circulin transfers to the acetone layer Part of the acetone is removed by distillation *in vacuo* The circulin is precipitated from the acetone layer by shaking with chloroform The circulin is dissolved in water and precipitated with ammonium sulfate The antibiotic is dissolved in 70% ethyl alcohol and crystallized at reduced temperature

Circulin can be recrystallized from a number of solvents including 30% ethyl alcohol, methyl alcohol, 70% isopropyl alcohol, 80% acetone and molar sodium acetate solution The form of crystal obtained varies with the solvent Circulin is very stable Water solutions or dry preparations can be stored at room temperature without inactivation Circulin has the following properties M P 235 C, α_D^{20} -93.3, isoelectric point in 0.02M citrate buffer is at pH 7.0 and absorption bands are at 2520, 2580, and 2640 Å The elementary analysis and molecular weight of 1050 indicate a molecular formula of $C_{61}H_{83}O_{15}N_9 \cdot SO_4$ Crystalline circulin is soluble in water (0.3%), 0.7M ammonium acetate solution (2.0%), ethylene glycol (5.0%), and 70% ethyl alcohol (1.5%) at 25 C Although the antibacterial activity of circulin is low, 200,000 *E coli* u/gm, the spectrum is very wide Circulin is toxic to animals

Phosphate turnover of various nucleotides in respiring liver homogenates J P HUMMEL and OLOV LINDBERG (introduced by H A MATTELL) *Wenner-Gren's Institute, Univ of Stockholm, Sweden* To determine the phosphate turnover in the pyridine and flavin nucleotides, a respiring liver homogenate, with glutamate as substrate, was briefly incubated with P^{32} orthophosphate After deproteinization with trichloroacetic acid, the filtrate was saturated with ammonium sulfate and the liver acid-soluble nucleotides (flavin and pyridine nucleotides, adenylic acid, and small amounts of ATP) were extracted into phenol The phenol-extracted material was transferred to water by adding excess ether The water extracts were analyzed by paper chromatography against the following solvents butanol-acetic acid, butanol-phenol, pyridine, phenol, and 2,4,6-collidine Radioactivity on the paper strips was measured directly under a Geiger counter Location of the nucleotides was established by analysis of extracts from the paper Excellent separations of the components were obtained with phenol and collidine Radioactivity was found in a fraction corresponding to ATP, another radioactive fraction was associated with the position of flavin adenine dinucleotide No radioactivity was found with the adenylic acid, flavin mononucleotide, or cozymase fractions On the assumption that only one of the two phosphorus atoms of flavin adenine dinucleotide can exchange phosphate, the specific activity of this phosphorus atom was of the same magnitude as that of the easily hydrolyzable P of ATP This suggests that flavin adenine dinucleotide is intimately associated with aerobic phosphorylation

Anaerobic phosphorylation due to a coupled oxidation-reduction between α -ketoglutaric acid and α -iminoglutaric acid F EDMUND HUNTER, JR *Washington Univ Medical School, St Louis, Mo* When α -ketoglutarate is added to washed

particles from rat liver tissue in anaerobic experiments, essentially no dismutative reactions occur. If ammonium ions are added along with the α -ketoglutarate a rapid reaction takes place. With bicarbonate buffer evolution of carbon dioxide indicates the formation of acid groups. Since the reaction stops when approximately one molecule of carbon dioxide has been evolved per molecule of ammonium ion added, the ammonium ion must participate stoichiometrically rather than catalytically. If ammonia is in excess, the reaction stops when the α -ketoglutarate concentration has fallen to a very low level. These data suggest the almost exclusive occurrence of the reaction $2 \alpha\text{-ketoglutarate} + \text{NH}_3 \rightarrow \text{succinate} + \text{CO}_2 + \text{glutamate}$. This dismutative reaction was observed by Krebs and Cohen (*Biochem J* 33 1895, 1939), but they reported negative results for rat liver. When hexokinase and glucose are added there is considerable phosphorylation of glucose coupled with the oxidation-reduction. The inorganic phosphate removal approaches one molecule per molecule of ammonium ion added, which would be equivalent to one per molecule of α -ketoglutarate oxidized to succinate in the proposed reaction.

Interaction of 9-aminoacridines with proteins and nucleic acids J. LOGAN IRVIN and ELINOR MOORE IRVIN (by invitation) *Department of Physiological Chemistry, Johns Hopkins University, School of Medicine, Baltimore, Md.* The proton acceptor species of 2-methoxy-6-chloro-9-(1'-methyl-8'-diethylamino-octylamino)-acridine (SN-12868) and of quinacrine combine reversibly with bovine plasma albumin and with the proteins of fraction III-1 of bovine plasma. This interaction can be evaluated spectrophotometrically with the equation $\text{pH} = \text{pK}'_1 + \log \frac{(D_1 - D)}{(D - D_2)} + \log \frac{(D_1 - D)}{(D - D_2) + k'_1 [p]}$ in which pK'_1 is the apparent ionization exponent for the group on the aromatic nucleus, k'_1 is the equilibrium constant for the binding of the first acridine molecule to the protein, $[p]$ is the molar concentration of unbound protein at equilibrium, and D_1 , D_2 , D_3 , and D are the optical densities, at a selected wavelength, when the acridine is completely in the form of the proton-donor species (D_1), the free proton-acceptor species (D_2), the protein-bound acceptor species (D_3), and an intermediate equilibrium state (D). For SN-12868 versus bovine albumin in the presence of 1M NaCl to minimize uncertainties regarding ionic strength, $\log k'_1 = 4.37$ (pH 6.7 to 8.3). Binding of this compound by the mixed proteins of fraction III-1 is about one-half the strength of binding by albumin on the basis of total weight of this fraction, and the data suggest a different type of binding. Strong interaction between a cationic species of SN-12868 and sodium ribonucleate (yeast) or desoxyribonucleate (sperm) has been observed spectrophotometrically

in buffered aqueous solutions from pH 6 to 8.6. At pH 6.4 and a concentration of the acridine of $4 \times 10^{-5} \text{M}$, the mid-point of the equilibrium is attained at a concentration of 0.011 gm of sodium ribonucleate per 100 ml.

Difference in electrophoretic behavior of sickle cell hemoglobin and normal human hemoglobin HARVEY A. ITANO and LINUS PAULING (introduced by CARL G. NIEMANN) *Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena, Calif.* The nature of the phenomenon of sickling of erythrocytes in sickle cell anemia suggests that this disease involves a pathological state of the hemoglobin molecule. The electrophoretic behavior of hemoglobin from individuals with sickle cell anemia and from normal individuals has been studied with the Tiselius apparatus with the collaboration of Dr. S. J. Singer. Ferrohemo-globin and carbonmonoxyhemoglobin from sickle cell blood and from normal blood were studied in phosphate buffers of 0.1 ionic strength at pH values of 5.7, 7.0, and 8.0. A significant difference was found between the electrophoretic mobilities of hemoglobin derived from sickle cell anemia blood and from normal blood in both ferrohemoglobin and carbonmonoxyhemoglobin. The curves of mobility against pH are roughly parallel, the isoelectric point of normal hemoglobin being lower than that of sickle cell hemoglobin for each compound. At pH 7.0 sickle cell carbonmonoxyhemoglobin moves as a positive ion while normal carbonmonoxyhemoglobin moves as a negative ion. The most plausible explanation for the observed differences in mobility is that there is a difference in the ionizable groups in the two hemoglobins. With use of the titration data of German and Wyman it is possible to calculate the approximate difference in net charge between the two hemoglobins. This calculation indicates that the sickle cell hemoglobin molecule in the pH range studied has between 2 and 4 less net negative charges than the normal hemoglobin molecule.

Mechanism of action of phosphoglucomutase VENKATARAMAN JAGANNATHAN (by invitation) and JAMES MURRAY LUCK *Dept. of Chemistry, Stanford Univ., Stanford, Calif.* Phosphoglucomutase was prepared from rabbit muscle by extraction, heating and fractionation with acetone and manganous sulfate. The purified enzyme required Mg^{++} , serum albumin, and sodium sulfite for maximum activity at pH 7.5. It contained non-dialysable phosphorus. When the enzyme acted on glucose-1-phosphate labelled with P^{32} the enzyme became radioactive while the specific activity of the ester-P decreased. The observed values for the subsequent radioactivity of enzyme and substrate corresponded with those calculated on the hypothesis that an exchange between enzyme-P and substrate-P took place. The radioac-

tivity of the enzyme could not be removed by dialysis but after reaction with non-labelled glucose-1-phosphate the enzyme lost its radioactivity on dialysis. After inactivation by iodine the radioactivity of the enzyme was not removed by a similar treatment. An exchange between enzyme-P and substrate-P is postulated as an essential step in phosphoglucomutase action.

Interaction between diisopropyl fluorophosphate (DFP) and proteins of brain tissue. BERNARD J. JANDORF and PRISCILLA D. MCNAMARA (by invitation) *Biochemistry Section, Medical Division, Army Chemical Center, Md.* Rabbit brain homogenates were incubated with 10^{-7} to 10^{-9} M DFP solutions. With these concentrations, the inhibition of brain cholinesterase progresses at a slow rate and requires more than 4 hours at 38°C to become maximal. When DFP containing 15–20 mc P^{32} /gm was used, the initial rate of uptake of P^{32} by the tissue was far higher than the rate of cholinesterase inactivation, near-maximal binding of P^{32} was attained at a time when more than 50% of the cholinesterase activity was still unimpaired. A small but significant uptake of P^{32} was obtained when a brain homogenate, previously treated with a 100-fold excess of unlabelled DFP, was incubated with labelled DFP. When radio-DFP was subjected to mild alkaline hydrolysis, the resulting mixture added to brain homogenates produced no inhibition of cholinesterase activity, and no binding of P^{32} by protein.

Mode of inhibition of chymotrypsin by DFP. II. Elimination of fluorine and introduction of isopropyl. EUGENE F. JENSEN, M-D. FELLOWS NUTTING (by invitation), ROSIE JANG (by invitation) and A. K. BALLS *Enzyme Research Division, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Dept. of Agriculture, Albany, Calif.* It has previously been shown by us that the esterase and proteinase activities of crystalline trypsin and chymotrypsin are inhibited by diisopropyl fluorophosphate (DFP). Since both activities were inhibited at the same rate and to the same extent, the activities were considered to be due to the same active groups of the enzymes. Only 17 moles of inhibitor per mole of enzyme (using 40,000 as the molecular weight) were needed for complete inhibition of chymotrypsin, whereas chymotrypsinogen was not affected by DFP. After complete inhibition the resulting inert protein was obtained in crystalline form. After treatment of crystalline chymotrypsin with DFP containing P^{32} , analysis of the crystalline inert protein showed that the chymotrypsin had accepted 16 moles of P per mole of enzyme. Hence all of the phosphorus of the DFP needed for complete inhibition was introduced into the enzyme. Zeisel analyses showed that the isopropyl moiety was also introduced, but only 2.8

equivalents per mole of crystalline chymotrypsin were found. Whether the difference between the amount of phosphorus and that of isopropyl introduced is real or reflects analytical difficulties is not apparent. Fluorine was shown to be absent by spectrographic analysis. Most probably the inhibition reaction is $2 \text{DFP} + \text{XtH}_2 \rightarrow \text{Xt}(\text{DP})_2 + 2 \text{HF}$ where XtH_2 represents chymotrypsin containing two peculiar, active hydrogens. It is felt that a complete knowledge of the mode of DFP inhibition may lead to an understanding of the mode of action of certain enzymes.

Lipids of the nervous system during *in vitro* degeneration. A. C. JOHNSON (by invitation), A. R. McNABB (by invitation) and R. J. ROSSITER *Dept. of Biochemistry, Univ. of Western Ontario, London, Canada.* The changes in the concentration of lipids during the *in vitro* degeneration of cat brain slices and sciatic nerve have been determined after incubation in bicarbonate buffer for periods of time from 1 to 14 days. In brain slices incubated for 14 days there was a great decrease in the concentration of total phospholipid with no change in the concentration of cerebroside or total cholesterol. Of the individual phospholipids, there was a decrease in the concentration of sphingomyelin and cephalin and no significant change in the concentration of lecithin. In sciatic nerve incubated for 8 days there was a significant decrease in the concentration of cephalin and a decrease of borderline significance in the concentration of total phospholipid. There was no significant change in the concentration of cerebroside, free and total cholesterol, sphingomyelin or lecithin. Thus, in confirmation of the work of others, there is a phospholipid-splitting mechanism, possibly enzymatic in nature, in tissue of the nervous system. There are many differences between the degeneration of tissue from the nervous system *in vitro* and the *in vivo* degeneration of peripheral nerve (*Rev. Canad. Biol.* 1949 in press).

Chromatographic separation of bile acids. I. Separation of mixtures of cholic and desoxycholic acids. ARTHUR F. JOHNSON (introduced by ARTHUR H. SMITH) *Dept. of Surgery, College of Medicine, Wayne Univ., Detroit, Mich.* The quantitative separation of milligram quantities of cholic and desoxycholic acids has been accomplished by a chromatographic technique. Hydrated magnesium sulfate (Epsom Salts), in the 40–200 mesh particle size range, is employed as the adsorbent. The adsorbed bile acids decrease the intensity of the fluorescence of the magnesium sulfate making it possible to follow the progress of the chromatogram under ultra-violet light. Empirical sectioning of the completed chromatogram is more convenient for routine determinations. Standard 18-mm diameter chromatographic tubes are packed with dry adsorbent under suction to give

a column 100 mm in length. The acids are introduced in benzene solution and development carried out with an additional 100-200 ml of benzene. After development the column is allowed to dry under suction and divided into four equal portions which are eluted with alcohol. Aliquots of the eluates and of the filtrate are analysed by colorimetric or fluorimetric methods. The cholic acid is found in the eluate from the top quarter of the column and the desoxycholic acid in the bottom quarter and in the filtrate.

Inhibitory action of some antioxidants on rat kidney phosphatase. CARTER D. JOHNSTON (introduced by EDWIN P. LAUG) *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.* The inhibition of alkaline rat kidney phosphatase by several compounds proposed as antioxidants in food has been studied in an exploratory manner. The enzyme system consisted of a crude kidney extract, glycerol phosphate substrate, barbitol buffer (pH 9.4) and added Mg ions. Hydroquinone (HQ), nordihydroguaiaretic acid (NDGA), propyl gallate, and thioldipropionic acid inhibit this system 20-25% in concentrations of 0.001 to 0.005M. While 0.005M HQ produces 30% inhibition aerobically, it shows no or very little effect when the experiment is carried out anaerobically in evacuated Thunberg tubes. Quinone, however, inhibits to the same extent under either condition, the degree of inhibition ranging from 80% at 0.005M to 20% at 0.0002M. Quinhydrone lies in an intermediate position. The inhibitory action of HQ thus appears to result from its partial conversion to quinone in the alkaline buffer. Propyl gallate and NDGA show inhibition both in the presence and absence of oxygen. At 0.001M there was a greater inhibition aerobically than anaerobically, but still an appreciable effect in the absence of air (25-40% inhibition). The extension of these studies to related compounds and under a variety of experimental conditions is in progress.

Effects of metabolic inhibitors on membrane potentials. NORMAN R. JOSEPH, ERVIN KAPLAN (by invitation) and C. I. REED *Univ. of Illinois, Chicago Professional Colleges, Chicago, Ill.* Determinations of the membrane potential across the synovialis of the knee in dogs have been made with various electrolytes and nonelectrolytes. With numerous ions the potentials agree with theoretical values calculated from ionic mobilities. With metabolic inhibitors or activators, however, the potentials are of a greater order of magnitude, and appear at lower concentrations. Inhibitors or activators have been classed under five groups: 1) cytochrome inhibitors, 2) heavy metals and other sulphhydryl inhibitors, 3) thiols, 4) inhibitors or activators of succinic dehydrogenase, and 5) electron acceptors, etc., iodine or H_2O_2 . In general inhibitors produce

positive and activators negative potentials. Sulphide yields approximately 400 millivolts, about twice the effect given by cyanide. From the effects of the reagents on the respiratory enzymes, a theoretical explanation of the sign and magnitude of the potentials can be advanced. Hydrogen transfer systems terminate metabolically at one or another of the cytochromes, which are electron acceptors. The electrons are carried to oxygen via cytochrome oxidase, the component having the highest standard redox potential. The mean difference of potential between this component and the original electron acceptor depends on the potential at which electrons are transferred from hydrogen transport systems. The potential difference depends on facilitation or blocking of hydrogen or electron pathways. Asymmetrical increase of the source potential (blocking electrons at cytochrome b) or decrease of the terminal potential (sulphide, cyanide) result in high positive potentials. Decreasing source or increasing terminal potentials produce negative potentials. Experiments on the gastrocnemius of the rat are in essential agreement.

Acetate oxidation by a washed rabbit kidney cortex preparation. GEORGE KALNITSKY *Dept. of Biochemistry, State Univ. of Iowa, Iowa City, Ia.* A rabbit kidney cortex, homogenized and washed in several volumes of NaCl-KCl solution and finally suspended in one volume of NaCl-KCl solution, oxidizes acetate practically completely. Acetate is utilized, oxygen is taken up, and carbon dioxide is evolved, according to the following equation: $CH_3COOH + 2O_2 \rightarrow 2CO_2 + 2H_2O$. The activity of the tissue suspension is proportional to the concentration up to 0.9 ml of the suspension. Veronal, borate and glycine buffers do not inhibit acetate oxidation by this preparation. The pH optimum is in the alkaline range, with the peak at approximately pH 8.6. This preparation actively oxidizes acetate, lactate, caproate and succinate. Citrate is oxidized at a slightly lower rate. Pyruvate, glucose and glutamate are not appreciably oxidized. Mg^{++} and inorganic phosphate increase the rate of oxidation of acetate. Mn^{++} cannot replace Mg^{++} . Kidney cortex was found to be the best source of this acetate oxidation system. Under the same conditions, liver oxidized acetate only slightly. Brain, spleen, lung, heart muscle and skeletal muscle exhibited little or no activity on acetate. Fluoroacetate and malonate at 0.005M concentrations almost completely inhibit acetate utilization, oxygen uptake and carbon dioxide evolution.

Acetyl precursor in aromatic amine acetylation in pigeon liver extracts. NATHAN O. KALLAN and MORRIS SOODAK (introduced by LEWIS L. L. L.) *Biochemical Research Laboratory, New York City General Hospital and Dept. of Biological Chem.*

istry, Harvard Medical School, Boston, Mass The product of the reaction between ATP and acetate in extracts of *E. coli* which appears to be an acetyl precursor in pyruvate synthesis (Kaplan and Lipmann) was tested for activity in the enzymatic acetylation of sulfanilamide. A partially purified fraction was found to promote acetylation under conditions in which monoacetyl phosphate and diacetyl phosphate were ineffective. Coenzyme A is necessary for the acetylation with this compound. Compared with ATP plus acetate the yields of acetylated amine were 50 to 90% at 25°, at 37° the yield was 25% only, presumably due to the much faster rate of destruction. The reactivity as acetyl precursor in pigeon liver extract is apparent but less pronounced than in *coli* extract. This may in part be due to a greater stability of the compound in the latter. Present results suggest however that ordinarily no free acetyl precursor may spill into solution. This is indicated furthermore by experiments on the differential competition for the acetyl donor between the acetyl-trap hydroxylamine and aromatic amines of varied acetyl-affinity. The nature of the primary acetyl donor in pigeon liver and in *E. coli* extracts will be discussed.

Influence of pteroylglutamic acid on glycine and on porphyrin metabolism CECILIA K. KEITH (by invitation) and JOHN R. TOTTER *Dept of Biochemistry, School of Medicine, Univ of Arkansas, Little Rock, Ark* The effect of pteroylglutamic acid (PGA) on growth and on fecal porphyrin production of weanling albino rats receiving standard purified rations or similar rations with 3 per cent sodium benzoate was investigated. The addition of 0.5 mg of PGA/100 gm of purified diet did not influence the growth rate of the animals but elevated the average daily fecal porphyrin excretion from 42 μ g for the control group (12 animals, 28 days) to 50 μ g (12 animals, 28 days) in one experiment and in a second experiment from 68 μ g (8 animals, 24 days) to 94 μ g (8 animals, 24 days). The inclusion of 3% sodium benzoate in the highly purified diets reduced the weekly gain in weight from an average of 21 gm/animal to 5.6 gm/animal. The addition of 0.5 mg of PGA/100 gm of the benzoate-containing diet resulted in an increase in growth rate to 15.5 gm/week/animal. The average daily fecal porphyrin excretion by these two groups were respectively 27 μ g (13 animals) and 37 μ g (12 animals) per rat over a 28-day period. The addition of 2% glycine to the benzoate-containing diet prevented the reduction in growth rate and in porphyrin production brought about by the benzoate.

Effect of dietary pteroylglutamic acid on liver D-amino acid oxidase of young rats BARBARA KELLEY (introduced by PAUL L. DAY) *Dept of Biochemistry, School of Medicine, Univ of Arkansas, Little Rock, Ark* Weanling male albino

rats were given a standard purified diet, with and without pteroylglutamic acid (PGA), for several weeks. The oxygen consumption of the liver homogenates from such animals was measured by the Warburg technic using DL-alanine as the substrate. The inclusion of 5 mg of PGA per kilo of diet resulted in a decreased enzyme activity. The average oxygen uptakes (μ l/gm wet weight of tissue) for 4 groups of control animals receiving the basal diet and for 4 groups receiving the basal diet with added PGA were respectively at the end of one hour, 136 and 111, at the end of 2 hours, 262 and 213, and at the end of 3 hours, 705 and 525. The addition of an excess of coenzyme (FAD) to the reaction flasks resulted in an increase in the oxygen uptake of both groups. The amount of coenzyme present appears to be a limiting factor in this reaction. Data on the activity of other flavin-containing enzyme systems will also be presented.

Demonstration of specificity of a simplified method for the determination of total cholesterol FORREST E. KENDALL, LIESE LEWIS ABELL (by invitation), BETTY B. LEVY (by invitation), J. MURRAY STEELE (by invitation) and BERNARD B. BRODIE *Dept of Medicine, College of Physicians and Surgeons, Columbia Univ, Depts of Medicine and Biochemistry, New York Univ College of Medicine, and New York Univ and Columbia Research Services, Goldwater Memorial Hospital, New York City* Although it has been recognized that neither digitonin precipitation nor color development with the Lieberman-Burchard reagent is specific for cholesterol, one or both of these reactions form the basis of all methods for cholesterol determination. Studies on cholesterol metabolism and its relationship to arteriosclerosis made it imperative to know the identity of the substances being measured by these reactions. A simple method which avoids digitonin precipitation has been devised for cholesterol. Serum is treated with alcoholic KOH to saponify cholesterol esters. After dilution with water the cholesterol is extracted into petroleum ether. The cholesterol content of an aliquot of the petroleum ether phase is determined by the Lieberman-Burchard reaction. Accuracy of the method was checked by recovery experiments. Specificity was assayed by application of Craig's countercurrent distribution technique to the material extracted into the petroleum ether. At least 98% of the material which gave color with the Lieberman-Burchard reagent was cholesterol. This degree of specificity pertained to sera of abnormal as well as of normal cholesterol levels. The new procedure gave results on 100 sera that agreed with the values obtained with the Schoenheimer-Sperry method. Indirectly this demonstrates also the specificity of the latter procedure.

Oxidation of higher fatty acids by isolated rat liver mitochondria EUGENE P. KENNEDY (by invitation) and ALBERT L. LEHNINGER *Depts of Biochemistry and Surgery, Univ of Chicago, Chicago, Ill* It has been difficult to study the enzymatic oxidation of the higher, physiologically occurring fatty acids by preparations of mitochondria from rat liver due to their extreme insolubility and the fact that the fatty acids are themselves potent inhibitors of the oxidase system. By standardizing techniques of preparing substrate solutions it has been found possible to study the oxidation of the normal saturated acids from C_8 to C_{18} . With increase in length of the carbon chain the tendency to form acetoacetate diminishes greatly while the tendency for complete oxidation to CO_2 increases, as indicated by measurements of R. Q., and acetoacetate formed. Purified normal saturated fatty acids (C_{14} , C_{16} , C_{18} , C_{17}) and oleic acid are oxidized essentially completely to CO_2 at high rates as judged by R. Q. measurements. The oxidation of oleic acid has the same cofactor requirements as octanoic acid. Linoleic, linolenic, and vaccenic acids are also oxidized at high rates by the mitochondria. α -Monopalmitin and the more soluble phosphatidic acid prepared therefrom are readily oxidized. It appears probable that oxidation of esterified forms of higher fatty acids is preceded by enzymatic hydrolysis.

Analysis of protein hydrolysates by group separation and paper chromatography of isotopically labelled derivatives ALBERT S. KESTON (by invitation), SIDNEY UDENFRIEND (by invitation) and MILTON LEVY *Dept of Chemistry, New York Univ College of Medicine, New York City* The formation of p-iodophenyl sulfonyl (pipsyl) derivatives of amino acids can be carried out quantitatively (Keston, Udenfriend and Cannan, *J Am Chem Soc* 68: 1390, 1946). The derivatives are separable by paper chromatography (Keston *et al* *J Am Chem Soc*, 69: 315, 1947). The analytical reagent is labelled with I-131. An indicator reagent labelled with S-35 is used to prepare known pure S-35 pipsyl amino acids. When these are added in known quantities to the I-131 labelled unknowns the estimation of the unknown becomes independent of complete resolution and depends only on the S-35/I-131 ratio in any pure fraction. The possibility of errors in identification and of overlapping chromatographic bands is greatly reduced by preliminary separation of the pipsyl derivatives into groups. This may be accomplished by simple counter current techniques. The most hydrophilic pipsyl derivatives (those of aspartic and glutamic acids, hydroxyproline, serine and threonine) constitute group I and are obtained from the aqueous end of a $CHCl_3$ -0.2N HCl extraction system. Group II which consists of glycine and alanine appear predominately in the organic end of a sym-

dichloroethane-0.2 N HCl system. For maximum separation chromatographic development on paper is best done with butanol-isopropanol mixtures for glutamic and aspartic acids (Group Ia), with butanol for hydroxyproline, serine and threonine (Group Ib) and with amyl alcohol for glycine and alanine. In each case the solvent is saturated with aqueous NH_3 solution before use. The bands on the paper are located by radioautography and separated into strips. Constant ratios of counts with and without a filter in successive strips indicates purity. The corresponding S-35/I-131 values are used in the calculations. Several proteins have been analysed by the technique.

Immunochemical studies of factors modifying interaction of egg albumin and rabbit anti-egg albumin WALTER J. KLEINSCHMIDT (by invitation) and PAUL D. BOYER *Division of Biochemistry, Univ of Minnesota, St Paul, Minn* The effect of ionic strength and of pH on inhibition of the EA-antiEA reaction and on dissolution of preformed EA-antiEA precipitates has been investigated by quantitative immunochemical procedures. Inhibition of precipitate formation increases with concentration of NaCl up to 1M but decreases with higher concentrations. With KBr the inhibition increases with concentration up to 3M. KBr produces slight and NaCl no dissolution of preformed precipitates. Definite limits of pH have been established for the EA-antiEA reaction, complete precipitation occurs between pH 6.25 and 8.45. Inhibition of precipitate formation is practically complete at pH 4.2 and 9.5, and disappearance of preformed precipitate occurs in buffer solutions of pH beyond the optimal range. Knowledge of the amino acid composition of egg albumin and γ -globulin allows approximation of the changes of reactive groups with pH. Such calculations indicate that aspartic acid, glutamic acid and lysine play a prominent role in antigen-antibody reactions, while histidine is relatively unimportant. Preliminary experiments have been made on the effect of soluble amino acid derivatives, in particular acetyltyrosine, on the EA-antiEA interaction with the view of further defining the importance of various protein groups in antigen-antibody reactions. Sodium acetyltyrosinate at neutral pH shows a marked inhibition at 0.15M and nearly complete inhibition at 0.5M concentration. Approximately 50% dissolution of preformed precipitate is effected by 0.575M. These effects are much greater than those due to ionic strength alone and may be related to the importance of tyrosyl residues in the interaction.

Penicillin-cytochrome complexes I. M. KLOTZ and W. W. WELSH (by invitation) *Dept of Chemistry, Northwestern Univ., Evanston, Ill* Spectrophotometric investigations have been made which gave direct evidence of complex formation between cytochrome c and penicillins G, K, and X.

tively In the presence of any one of these antibiotics at a concentration of 2×10^{-3} molar, in a phosphate buffer at pH 7.0, the spectrum of the heme protein (7×10^{-5} molar) in the ferristate, develops 2 horns at 520 and 550 m μ , on top of the characteristic broad absorption with a peak at 530 m μ The sample of cytochrome which gave the most striking results was assayed by the method of Rosenthal and Drabkin (*J Biol Chem* 149 437, 1937) and found to be 97% pure Experiments with another sample assaying 54% cytochrome c gave much less pronounced effects It seems unlikely, therefore, that the observed effects are due to spectral changes in hemin impurities All of the penicillins were crystalline samples obtained from the Antibiotics Study Section of the National Institute of Health Of the penicillins examined, K was the most effective in producing the spectrophotometric change, and hence presumably the most strongly bound in this complex As in other anion-protein complexes, penicillin can be displaced from its cytochrome complex by the addition of a competing anion such as sodium dodecyl sulfate (2×10^{-3} molar) This displacement by another anion indicates the importance of electrostatic attraction in the formation of the complex with the antibiotic

Effect of arsenic on the metabolism of selenium

HARLAN L KLUG (by invitation), DONALD F PETERSEN (by invitation) and ALVIN L MOXON *Chemistry Dept, South Dakota Agricultural Experiment Station, Brookings, S D* The alleviation of selenium poisoning by arsenic was first reported from this laboratory in 1938 Since then, the study has been extended to various laboratory and farm animals and the use of arsenic remains the only practical way of counteracting selenium poisoning Relatively little is known about the mode of action of this selenium-arsenic antagonism although it has been suggested that the selenium might be bound by the arsenic in a non-toxic form in the tissues Three groups of rats were kept in metabolism cages and given controlled dosages *per os* for 2 ten-day periods One group received 1 mg/kg of arsenic (arsenite) daily, the second group received 1.4 mg/kg selenium (selenite) daily and the third group received 1 mg/kg arsenic plus 1.4 mg/kg selenium daily Urine and feces samples for each period were analyzed and at the end of the 20-day period the rats were sacrificed and various tissues analyzed The results show that arsenic had little effect on the absorption, excretion or tissue deposition of selenium The conclusion concerning tissue content is also supported by other data from this laboratory Metabolism studies indicate that the lungs may be an important pathway of elimination

Enzymes of liver oxidizing tryptophan to a kynurenine-like compound W E KNOX, KATHLEEN MERO (by invitation), W I GROSSMAN (by invitation), and V H AUERBACH (by invitation)

Rheumatic Fever Research Institute, Northwestern Medical School, Chicago, Ill The metabolic conversion of tryptophan to kynurenine and ultimately to nicotinic acid is evidence for the existence of a new system of enzymes which act on tryptophan Such a system has been obtained, from liver only, of several animal species The soluble enzyme preparation carries out the aerobic oxidation of l-tryptophan in the α -position of the indole ring, d-tryptophan is not oxidized The product of the reaction is a compound similar to kynurenine, but has not yet been completely identified The properties of the enzymes and the nature of the transformations do not agree with those reported by Kotake *et al* for the reactions forming kynurenine The reaction occurs in air, and the rate is doubled by addition of methylene blue The product is a diazotizable amine with a free α -amino group Analyses show the reaction to be 1 l-tryptophan + 2 O — 1 amine No CO₂ is produced The conversion of tryptophan to kynurenine requires the further removal of one atom of carbon One atom is found as formic acid upon hydrolysis of the reaction product Simple fractionation does not separate the preparation into different enzymes On dialysis the need for two co-factors becomes apparent These have been identified as Mg⁺⁺ and adenylic acid

Metabolism of testosterone and androstanediol-3 α , 17 α by liver and kidney homogenates CHARLES D KOCHAKIAN, N PARENTE (by invitation) and H V AROSHIAN (by invitation) *Dept of Physiology and Vital Economics, School of Medicine and Dentistry, Univ of Rochester, Rochester, N Y* In previous reports it was shown that testosterone is metabolized by rabbit liver slices (*J Biol Chem* 170 23, 1947), and homogenate (*Federation Proc* 7 (1) 42, 1948) to Δ^4 androstenedione-3,17, *cis*-testosterone and traces of unidentified compounds Also that rabbit kidney homogenate (*Federation Proc* 7 (1) 42, 1948) converts testosterone in part to Δ^4 -androstenedione-3,17 The homogenates of guinea pig liver and kidney also convert testosterone to Δ^4 -androstenedione-3,17 but in smaller yields than rabbit tissues Furthermore, other as yet unidentified ketosteroids are formed in small amounts These same homogenates convert androstanediol-3 α , 17 α to one or more ketosteroids The metabolites, however, account for less than 5% of the original steroid of which approximately 90% was recovered as crystalline material

Micro molecular distillation of fatty acids ALFRED E KOEHLER and ELSIE HILL (by invitation) *Santa Barbara Cottage Hospital and The Sansum Clinic Research Foundation, Santa Barbara, Calif* Distillation of the neutral fat fatty acids of the blood from a film at 0.03 micron Hg for 30 minutes indicates that some of the fatty acids distill readily at 25° As the temperature is increased more fatty

acids are distilled but at about 60° the curve flattens markedly with only about 75 per cent distilled. This was at first believed to be due to the presence of higher homologues with low boiling points but it was later found that some pure fatty acids undergo changes at the higher temperature that retards their distillation. In general the fatty acids from caprylic to linoleic distill in proportion to their molecular weights at 30°. The following percentages were found: caprylic 100, capric 75, myristic 60, palmitic 32, oleic 20, linoleic 12, and stearic 4. At higher temperatures (40° to 100°) this relationship no longer holds. Palmitic and stearic acid have conventional types of distillation curves but myristic, lauric, oleic, and linoleic acids do not. In fact, myristic and oleic acids at certain higher temperatures may distill less completely than at lower levels. This phenomena is probably explained on the basis of polymorphism. This may occur in the solid phase below the melting point as in the case of myristic acid or in the acids that are liquid throughout the temperature range as in oleic and linoleic acid.

Adaptive enzymatic conversion of l-malate to lactate and CO₂. SEYMOUR KORKES (by invitation), ALICE DEL CAMPILLO (by invitation), and SÉVERO OCHOA, *Dept. of Pharmacology, New York Univ. College of Medicine, New York City*. As previously reported (Korkes and Ochoa, *J. Biol. Chem.* 176: 463, 1948) *Lactobacillus arabinosus* can be adapted to convert malate to lactate and CO₂. The isolated enzyme system requires Mn⁺⁺ and catalytic amounts of diphosphopyridine nucleotide (DPN). The reaction appears to be the combined result of the two reactions (1) l-malate + DPN_{ox} ⇌ pyruvate + CO₂ + DPN_{red} and (2) pyruvate + DPN_{red} ⇌ lactate + DPN_{ox}. The second reaction is catalyzed by lactic dehydrogenase while the first is catalyzed by an enzyme which, except for its DPN specificity, is identical to the one previously isolated from pigeon liver (Ochoa *et al.*, *J. Biol. Chem.* 174: 979, 1948). A study of extracts of acetone powders from either unadapted or malate-adapted cells shows that: 1) Approximately equal amounts of lactic dehydrogenase are present in either case, 2) no 'malate' activity can be extracted from unadapted cells while very high activity is obtained from adapted ones, 3) simultaneously with the appearance of activity toward malate the extracts become able to catalyze the decarboxylation of oxaloacetate to pyruvate and CO₂, a weak fumarase activity also appears. Adaptation to malate can be obtained in a short time with suspensions of resting cells in Warburg vessels if glucose and an allanoin growth medium are also present. Both a supply of glycolytic energy and of anoxia are essential for the adaptation. The partial purification and properties of the enzyme system will be described.

Inorganic pyrophosphate in the enzymatic synthesis of DPN. ARTHUR KORNBERG (introduced by W. H. SEBRELL, JR.), *Experimental Biology and Medicine Institute, National Institutes of Health, Bethesda, Md.* The purification of an enzyme which catalyzes the reaction: nicotinamide mononucleotide (NMN) + adenosine triphosphate (ATP) ⇌ diphosphopyridine nucleotide (DPN) + inorganic pyrophosphate (P-P), has been reported recently (*J. Biol. Chem.* 176: 1475, 1948). An autolyzate of dried brewer's yeast was fractionated twice with ammonium sulfate and precipitated at pH 4.80. The resulting preparation was free of phosphatase activities and was about 1000 times more active per milligram of protein than the original extract. With 50 micrograms of the purified preparation in 1 cc of incubation mixture, equilibrium was reached in 30 minutes at 38° from either direction starting with approximately 2 micromoles each of NMN and ATP or of DPN and P-P. The equilibrium constant, $K = \frac{(\text{DPN})(\text{P-P})}{(\text{NMN})(\text{ATP})}$ is approximately

0.4. These findings indicate a mechanism for the synthesis of DPN and for the origin and function of inorganic pyrophosphate. Current studies with partially purified preparations from brewer's yeast suggest that the synthesis of triphosphopyridine nucleotide involves a direct phosphorylation of DPN by ATP.

Fate of the infecting virus particle. LLOYD M. KOZLOFF (by invitation) and FRANK W. PUTNAM, *Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.* The origin of virus phosphorus and the fate of the infecting virus particle have been investigated using P³² to label various components of the *E. coli*-T₂ bacteriophage system. Previous study in synthetic lactate medium revealed that the medium itself is the ultimate source of 70% of the virus P (Putnam and Kozloff, *Science* 108: 386, 1948). Extension of these experiments to nutrient broth has yielded substantially similar results. In experiments on the fate of the infecting virus particle T₂ bacteriophage was labeled by growth on *E. coli* in broth containing P³² (2-3 μc/ml). Bacteria in isotope free broth were infected with the purified radioactive virus (60,000 cts/min) under conditions producing a single generation of virus. The phage was purified by concentration in the supercentrifuge followed by differential centrifugation. Prior to infection the radioactivity of the labeled phage was 95% sedimentable at 20,000 g. Upon infection 60% of the counts were absorbed by the host bacterium. After lysis 60% of the radioactivity was non-sedimentable in the supercentrifuge (50,000 g). The low radioactivity of the phage could be accounted for as undischarged P³² used for infection. Disruption of the phage particle is observed in the presence of a high concentration of phosphate.

60 to 400 times that of the progeny. The data indicate that the integrity of the infecting virus particle is destroyed in the course of reproduction.

Pteridines and xanthine oxidase activity EDWIN G. KREBS (by invitation) and EARL R. NORRIS, *Dept of Biochemistry, Univ of Washington, Seattle, Wash.* An enzyme preparation active in the oxidation of either xanthopterin or xanthine was obtained from whey. Spectrophotometric methods as described by Kalckar were used in studying these oxidations. The 'natural' substrate for the enzyme appeared to be xanthine, which was converted to uric acid at a rate about $25\times$ greater than found in the conversion of xanthopterin to leucopterin. Xanthopterin was found to be an effective inhibitor of xanthine oxidation, the inhibition being of the competitive type. The dissociation constant of xanthine with the enzyme was estimated to be 7×10^{-6} , while that of xanthopterin with the enzyme was around 2×10^{-6} . Pteridines with a substitution in the 7-position, such as xanthopterin-7-carboxylic acid, inhibited xanthopterin oxidation as well as xanthine oxidation. These findings may be of significance in explaining some of the observations of Norris and Majnarić on the effects of pteridines in bone marrow cultures or in rats made anemic on a diet containing sulfathiazole. Xanthopterin in low concentrations or dosages was found to stimulate hematopoiesis, but was inhibited in this action by 7 substituted pteridines. In higher concentrations xanthopterin inhibited blood cell formation. This latter effect might be attributed to its possible role as a structural antagonist in purine reactions such as the oxidation reaction discussed here.

Effect of diet composition on rehabilitation following starvation in albino rats W. A. KREHL and W. P. McNULTY, JR. (by invitation), *Yale Nutrition Laboratory, Dept of Physiological Chemistry, Yale Univ., New Haven, Conn.* Experiments conducted with growing and adult albino rats to determine the effect of diet composition on their ability to rehabilitate following 30% loss of body weight as a result of complete starvation led to the following observations: 1) The efficiency of rats in regaining their original weight bore little or no significant relationship to the re-fed diets, the composition of which varied in protein content from 10 to 60% and in isocaloric substitution of carbohydrate with fat. 2) Two of three groups of weanling rats held at constant weight for 5 weeks by chronic inanition were re-fed, respectively, on an optimum artificial ration and on an identical diet, except for the isocaloric substitution of fat for carbohydrate. No significant difference in the rate of gain on the two diets was observed. The third group, placed on a self-selection regimen (after Richter, exhibited a distinctly inferior growth rate, in fact, half the animals failed to select a diet which was satisfactory

for growth by any criterion. A control group of weanling rats placed on self-selection from the start either grew poorly or failed to grow at all. 3) Adult rats placed on dietary self-selection after starvation fell into two groups, a) those that regained at a reduced rate and b) those completely unable to gain. 4) Adult rats starved a second time after recovery either on a prepared diet or on self-selection made a more rapid recovery on this second trial. Furthermore, 100% of the animals placed on the self-selection regimen the second time rehabilitated successfully.

Some properties of Chymotrypsinogen B and Chymotrypsin B VIRGINIA KUBACKI (by invitation), KENNETH D. BROWN (by invitation), and M. LASKOWSKI, *Dept of Biochemistry, Marquette Univ. School of Medicine, Milwaukee, Wis.* Chymotrypsinogen B and Chymotrypsin B were prepared according to previously described methods (*J. Biol. Chem.* 170: 227, 1947; 173: 99, 1948). For the purpose of comparison Chymotrypsinogen alpha and Chymotrypsin alpha were prepared according to Kunitz and Northrop (*J. Gen. Physiol.* 18: 433, 1934-35). Each protein has been recrystallized a minimum of 4 times. Electrophoresis was carried out in a standard 15 ml. cell using a 1% solution of protein in 0.1 M buffers at 3°C. Each of the 4 proteins showed a single component, indicating purity of the protein. A 1:1 mixture of Chymotrypsinogen B and Chymotrypsinogen alpha showed two widely separated components in approximately equal amounts. The non-identity of proteins of the B type with proteins of the alpha type was further proved by pH mobility curves. The zero mobility point for Chymotrypsin B was found at pH 4.7, for Chymotrypsinogen B at pH 5.2, for Chymotrypsin alpha at pH 8.3, and for Chymotrypsinogen alpha at pH 9.1. Solubility studies using Chymotrypsinogen B were conducted according to Kunitz (*J. Gen. Physiol.* 13: 788, 1930). Before the experiment, the material was equilibrated 5 times with the solvent (0.1 M acetate, pH 5.0, 0.27 saturation with ammonium sulfate). The slight deviation from the theoretical curve for one component might have been due to the experimental conditions, or the presence of a small amount of contaminant.

Bixin and the rate of autoxidation of methyl linoleate H. O. KUNKEL and WALTER L. NELSON (introduced by JAMES B. SUMNER), *Dept of Biochemistry and Nutrition, Cornell Univ., Ithaca, N. Y.* The rate of oxidation of methyl linoleate dissolved in triacetin in an oxygen atmosphere was measured in the Warburg manometric apparatus at 25°C. The addition of 130 γ or less of bixin to 50 mg of linoleate in 2 ml of solution decreases this rate of oxidation of the linoleate, while at the same time the bixin is rapidly decolorized. The destruction of bixin proceeds as an apparent first order reaction with respect to oxygen uptake as

contrasted to a first order reaction with respect to time. When a larger amount of bixin (325 γ to 50 mg of linoleate) is added the rate of oxidation is considerably increased, while the bixin is destroyed at the same rate as in the first case. In the presence of light from a mercury lamp, bixin at all concentrations used has been found to produce a more rapid oxidation of the linoleate. This is true even when α -tocopherol has been added to the system at the start. In the presence of α -tocopherol, the bixin is not appreciably destroyed until the end of the induction period, after which its destruction is as rapid as in the experiments first described. Preliminary experiments indicate that carotene acts in a manner similar to that of bixin. Data have also been obtained to show that bixin will enhance the antioxidant action of α -tocopherol.

Certain organic anions and amide and peptide linkages. GRANVIL C. KYKER and SANFORD L. STEELMAN (by invitation) *Dept. of Biological Chemistry and Nutrition, School of Medicine, Univ. of North Carolina, Chapel Hill, N. C.* The rates of hydrolysis at 65°C of the amide and peptide linkages of certain proteins (egg albumin, serum albumin, gelatin and hemoglobin) by hydrochloric acid in the presence of certain organic anions depend on the concentration of the protein, the anion, and the hydrochloric acid and on the nature of the protein. Most important of all is the ratio of the concentration of the protein to the concentration of the anions. The differences in the hydrolytic effectiveness of the organic anions do not parallel, in all cases, the differences in the affinities of the anions of the acids for proteins as suggested by Steinhardt (*J. Research NBS* 29: 315, 1942). A maximum rate of amide hydrolysis is reached with sodium dodecyl sulfonate and egg albumin. This is not the case with the other proteins studied. Sulfosalicylic acid, which has an 'intermediate' anion affinity for wool protein, inhibits amide hydrolysis of egg albumin. The catalytic effect of organic anions on the peptide hydrolysis is limited to the initial stages of hydrolysis and when the protein is further split, the rate tends to approach that of HCl. For the ones studied, when the protein was approximately 25% hydrolyzed the rate began to closely approach that of the HCl. The mechanism for the catalytic effect is uncertain but may be due to the denaturing action of the anion on the protein and to the combination with it. As the hydrolysis proceeds, there is evidently less tendency of the anion to combine and the rate of hydrolysis approaches that of HCl.

Photoelectric colorimetry of pH. GRANVIL C. KYKER *Dept. of Biological Chemistry and Nutrition, School of Medicine, Univ. of North Carolina, Chapel Hill, N. C.* Since the advent of photoelectric colorimeters, many procedures for the colorimetric measurement of pH have appeared as adaptations to various instruments. Such procedures

presume the accurate determination of one color of an indicator in the presence of its other. The colors vary reciprocally with pH. The selectivity of filters employed by photometric instruments to yield monochromatic light is generally inadequate to separate quantitatively the two colors. The effect of one color on the other is compensated by the present method to permit accurate measurements of pH. The choice filter is selected for each of the colors of an indicator. The indicator is added to a sample and instrumental readings are taken using each filter. The two readings are expressed as a ratio. The ratio varies with pH. The procedure affords certain distinct advantages. A source of error is eliminated since the concentration of indicator has no effect over generous limits. When comparing 8, 10, and 12 drops of bromphenol blue, respectively, in 10 ml samples of standard buffers (pH 3.0-4.6) readings varied greatly with concentration (Klett-Summerson blue and green filters) while the ratio remained constant with pH. Indicators, for which neither color is accurately determinable by an instrument depending on filters, may be acceptable. The method has given excellent results in the teaching laboratory, students encountering it the first time obtain titration curves from 5 ml 0.1M lactic acid which superimpose smoothly upon the theoretical. Calibration is done in the usual manner.

Studies on the metabolism of radioactive sucrose in the rat. ALVIN R. LAMB and GEORGE O. BURR *Experiment Station, Hawaiian Sugar Planters' Association, Honolulu, Hawaii.* Recrystallized sucrose, isolated from a sugarcane leaf after photosynthesis in radioactive CO_2 , was given by stomach tube to fully-fed mature rats. Immediately after feeding the 200-mg dose, the rat was placed in a desiccator cage through which air was passed from a tank at a constant rate of 400 cc/min. The CO_2 in the effluent air was trapped in NaOH solution. By means of a by-pass, samples were taken at intervals in Ba(OH)_2 , beginning 1 minute after the sucrose was given, and each 10 minutes thereafter, with increasing intervals later. The BaCO_3 was filtered rapidly on 20 mm paper, dried and counted, using an area of 2.65 sq. cm. In one experiment net counts per minute were 18.9 at 11 minutes, 92.8 at 21 minutes, 285.1 at 51 minutes, 116.8 at 225 minutes and 15.3 at 10.5 hours. Sucrose, therefore, has been absorbed and oxidized within 11 minutes of ingestion, by a fully fed animal. The highest points of the curves of respiratory elimination of radioactive CO_2 were reached in 72-81 minutes. The curves dropped quite rapidly thereafter. Radioactive carbon appeared in the urine within 2 hours. The dried sample registered 223.0 counts/min. Uncontaminated feces registered 25.7 counts/min. The animals were killed after 24 hours. A few p

cal counts muscle glycogen, 43.4/min, bone marrow, 10.9 to 24.7, brain, 19.3, plasma proteins, 32.5, plasma lipids, 5.8, mesenteric fat, 8.7 to 16.6, total carcass residue fat, 7.1

A new metabolic regulator in mammalian spermatozoa HENRY A. LARDY and D. GHOSH (by invitation) *Dept. of Biochemistry, College of Agriculture, Univ. of Wisconsin, Madison, Wis.* Bovine epididymal spermatozoa have comparatively low rates of respiration and aerobic glycolysis and a high rate of anaerobic glycolysis. In contrast, ejaculated spermatozoa separated from seminal fluid by centrifugation have much higher rates of respiration and anaerobic glycolysis and a lower rate of aerobic glycolysis. Thus the Pasteur effect exhibited by the epididymal cells has almost completely disappeared in seminal spermatozoa. These metabolic differences result from the liberation of an extremely labile, lipid-soluble substance from a bound form occurring in the epididymal sperm cell. Until the substance has been characterized we shall refer to it as a 'Metabolic Regulator'. Additions of minute quantities of the purified regulator to epididymal spermatozoa immediately stimulates their respiration and anaerobic glycolysis and abolishes the Pasteur effect. An assay for the regulator has been developed based on its ability to stimulate the fermentation of Bakers' yeast. Using this assay the regulator has been concentrated about 2000-fold from bull semen. It is present also in the spermatozoa of the boar, ram, rabbit and man. In low concentrations the regulator stimulates the oxidation of pyruvate + fumarate and of succinate by washed residue of rat kidney homogenate. It inhibits glutamate oxidation. The regulator functions by uncoupling phosphorylation from enzymatic oxidations.

Esterification of phosphate coupled to electron transport between dihydrodiphosphopyridine nucleotide and oxygen ALBERT L. LEHNINGER and MORRIS E. FRIEDKIN (by invitation) *Depts. of Surgery and Biochemistry, Univ. of Chicago, Chicago, Ill.* Previous work in this laboratory has shown that inorganic phosphate labeled with P^{32} is incorporated into an esterified fraction when dihydrodiphosphopyridine nucleotide ($DPNH_2$) is incubated aerobically with washed rat liver suspension, ATP, Mg^{++} , and cytochrome-c. In order to study this phosphorylation more closely, a system was devised in which $DPNH_2$ was continuously generated by action of β -hydroxybutyric dehydrogenase on β -hydroxybutyrate. The oxidation of the latter by molecular oxygen is not complicated by further oxidation of acetoacetate, which is inert in the system. Oxidation of β -hydroxybutyrate by this system in the presence of ATP, Mg^{++} , catalytic amounts of DPN and cytochrome-c, and inorganic P^{32} causes esterification of the latter. Omission of ATP or Mg^{++} has no effect on the rate

of oxidation but abolishes the esterification, which is therefore not obligatory for the oxidation. Aging of the enzyme at 30° causes loss of phosphorylating activity without affecting oxidative activity. The phosphorylation can also be decoupled from oxidation by arsenite, dinitrophenol, Ca^{++} , and other substances. Phosphorylation does not occur if cytochrome-c is replaced by methylene blue, brilliant cresyl blue, or ferricyanide. Adenylic acid has been used as an acceptor to study the efficiency of the phosphorylation in non-isotopic experiments. The maximum P/O ratio observed has been 1.10, indicating that for each pair of electrons passing from $DPNH_2$ to oxygen, at least one molecule of phosphate is esterified.

Mammalian tyrosinase. Action on compounds structurally related to tyrosine and dihydroxyphenylalanine A. BUNSEN LERNER (by invitation), T. B. FITZPATRICK (by invitation), and W. H. SUMMERSON *Biochemistry Section, Medical Division, Army Chemical Center, Md.* Study of the ability of tyrosinase preparations from the Harding-Passey mouse melanoma to catalyze the oxidation of a variety of monohydroxy and dihydroxy phenolic compounds reveals that, of the various monohydroxy compounds, only tyrosine and its ethyl ester possess significant substrate activity. Compounds such as phenol, dihydroxytyrosine, 4-methoxyphenylalanine, and tyramine are relatively or completely inert as substrates, even in the presence of catalytic amounts of added dihydroxyphenylalanine (dopa), which we have shown promotes the oxidation of tyrosine by mammalian tyrosinase (*Federation Proc.* 7:167, 1948). The 3-amino, 3-fluoro, N-formyl, and N-acetyl derivatives of tyrosine proved to be potent inhibitors of tyrosinase activity. Of the dihydroxy phenolic derivatives, only L-dopa and DL-dopa are significantly effective as substrates, although certain of them (arterenol, catechol, homogentisic acid) resemble dopa in their ability to shorten the induction period in the action of the enzyme on tyrosine, and others (epinephrin, hydroquinone, cobefrin) are rapidly oxidized if dopa oxidation is proceeding simultaneously. The bearing of these findings on the question of substrate specificity of mammalian tyrosinase will be discussed.

Physical stress and liver fat content of the fasted mouse LOUIS LEVIN *Dept. of Anatomy, College of Physicians and Surgeons, Columbia Univ., New York City.* The present experiments confirm previous findings of other investigators that administration of crude pituitary extract to fasting mice causes mobilization of fat to the liver. There are good indications that this fat mobilizing effect is mediated by the adrenal cortex. Data will be presented to show that pure adrenocorticotrophin (Armour) is as effective as crude pituitary extract. Certain physical stresses thought to activate

the pituitary-adrenal system have been tested with respect to liver fat mobilization. Some of these (exposure to cold, severe exercise) are very effective in causing increase in liver fat. The fat increase may be detected within one hour after beginning of exposure to the stress and increases in intensity as the exposure period is prolonged. Other stresses (exposure to high temperature or to low barometric pressure), though indicated to be very severe by the inability of many of the animals to survive, do not increase the liver fat content but instead have a tendency to cause a loss of liver fat. Parenteral administration of glucose during exposure to an otherwise effective stress or during treatment with pituitary extract prevents most of the liver fat increase. However, comparison with the liver fat content of glucose injected, non-stressed mice indicates that the glucose treatment does not completely inhibit the fat mobilizing effect. All procedures which cause increase in liver fat also produce a relative and absolute liver enlargement but the increased liver weight cannot all be ascribed to the accumulation of fat.

Investigation of phospholipid composition in minute amounts. CELIA LEVINE (by invitation) and ERWIN CHARGAFF, *Dept. of Biochemistry, College of Physicians and Surgeons, Columbia Univ., New York City*. The procedures for the chromatographic separation of nitrogenous lipid constituents described recently (Chargaff, *et al.*, *J. Biol. Chem.* 175: 67, 1948) have been extended to the quantitative micro estimation of the bases. Serine and ethanolamine, separated by means of butanol-diethylene glycol-water (4:1:1), were determined in aqueous propanol extracts of the respective adsorbates on filter paper by a modification of the photometric ninhydrin method of Moore and Stein (*J. Biol. Chem.* 176: 367, 1948). Parallel guide strips, developed by means of ninhydrin, indicated the position on the chromatogram of the various amino compounds. Between 2 and 15 γ of each component in 0.01 cc. of solution served for the determination which had an accuracy of $\pm 3\%$. Choline (25 to 100 γ) was estimated with a precision of about 5% in separate chromatograms by the planimetric measurement (Fisher *et al.*, *Nature* 161: 764, 1948) of the molybdenum blue spots produced, as described previously, by the successive treatment of the choline adsorbates with phosphomolybdic acid and stannous chloride. Choline standards were run with each series of determinations. The use of these techniques for the study of the composition of various cellular lipid fractions and for the investigation of novel lipid constituents will be discussed.

Isolation and physicochemical characterization of pituitary follicle-stimulating hormone. CHON HAO LI, *Institute of Experimental Biology, Univ. of California, Berkeley, Calif.* A method for the isolation of follicle-stimulating hormone (FSH)

from sheep pituitaries has recently been described (Li, Simpson and Evans, *Science*, in press). Ca(OH) extract of fresh whole sheep glands was used as the starting material. The extract was next fractionated with $(\text{NH}_4)_2\text{SO}_4$ and FSH activity concentrated in the fraction 0.50–0.75 saturated $(\text{NH}_4)_2\text{SO}_4$. This fraction was extracted with 40% ethanol, the extractable material was then precipitated by adding ethanol to 80%. The alcohol precipitate was finally fractionated again with $(\text{NH}_4)_2\text{SO}_4$ at pH 4.7, FSH precipitated out in the fraction 0.55–0.70 saturated $(\text{NH}_4)_2\text{SO}_4$. The hormone thus prepared behaves as a single protein in the ultracentrifuge and in electrophoresis. It contains approximately 1.3% mannose, 0.6% hexosamine, 0.6% tyryptophane and 4.5% tyrosine. Qualitative analysis indicated that the hormone contains cystine but no cysteine. The results of elementary analysis of the preparation were carbon 44.93%, hydrogen, 6.67%, nitrogen, 15.10%. Electrophoretic analysis of FSH solutions at different pH buffers gave the following mobilities: $+4.8 \times 10^{-5}$ at pH 4.00, $+2.2 \times 10^{-5}$ at pH 4.25, -1.5×10^{-5} at pH 4.60, -1.9×10^{-5} at pH 4.81, -3.0×10^{-5} at pH 5.10, -4.2×10^{-5} at pH 5.60, -6.5×10^{-5} at pH 7.00. These experiments were conducted at 2°C in buffers of 0.10 ionic strength. From these values, the isoelectric point of FSH is estimated to be approximately at pH 4.5. Ultracentrifugal study of one specimen of FSH in a Spinco ultracentrifuge at 165,000 times gravity gave a sedimentation constant, S_{20}^w , to be 4.3 S. Without the values of diffusion constant and partial specific volume, an approximate molecular weight of FSH may be estimated to be 70,000.

Relative size of adrenocorticotropically active peptide fragments. CHON HAO LI, *Institute of Experimental Biology, Univ. of California, Berkeley, Calif.* In previous studies we found that the adrenocorticotrophic hormone (ACTH) isolated from sheep glands preserved its hormonal activity after partial acid or peptic hydrolysis. The active peptide fragments were not precipitated by trichloroacetic acid and were readily dialyzable. We have now found that the average peptide length in the active hydrolysates is approximately 8. In 6 M HCl at 120°C for 24 hours, the hormone hydrolysate contained 6.5% of the total nitrogen as imide nitrogen and 79.4% of the total nitrogen minus the imide nitrogen as the amino nitrogen. These values represent an average of 8 determinations from 1 different ACTH preparations. When the hormone was digested at 37.4°C to the extent of 48–51% with crude pepsin and the hydrolysate fractionated, the corticotropically active material was estimated to be approximately 10% of the total nitrogen.

Experiment No	% of Digestion	Total N of Hydrolysate	NH ₂ -N	N in Peptides	A _v Peptide length
		mm	mm	mm	
83	48	1.04	0.111	0.830	7.5
81	48	1.09	0.099	0.865	8.7
86	50	1.07	0.093	0.850	9.1
87	51	1.07	0.113	0.850	7.5

age peptide length in the hydrolysates was computed and found to vary from 7-9. We are concerned with the isolation of the adrenocorticotropical active peptide(s) in these hydrolysates.

Studies on the biological synthesis of cholesterol from acetic acid. HENRY N. LITTLE (by invitation) and KONRAD BLOCH, *Dept. of Biochemistry and Institute of Radiobiology and Biophysics, Univ. of Chicago, Chicago, Ill.* Studies on the formation of cholesterol in animal tissues carried out with the aid of deuterium have shown that acetic acid is employed in the synthesis of both the isooctyl side chain and the cyclic moiety of the sterol molecule (*J. Biol. Chem.* 145: 625, 1942). Experiments have now been carried out in which cholesterol was synthesized in liver slices from either $\text{CH}_3\text{C}^{14}\text{OOH}$ or $\text{C}^{14}\text{H}_7\text{COOH}$. Degradation of cholesterol obtained from these 2 sources shows that the isotope concentration is higher in the nuclear portion than in the side chain when $\text{CH}_3\text{C}^{14}\text{OOH}$ is the precursor and that the isotope concentration in the side chain at least equals that of the cyclic moiety in cholesterol synthesized from $\text{C}^{14}\text{H}_7\text{COOH}$. Cholesterol formed from acetic acid labeled by C^{13} in the methyl and C^{14} in the carboxyl group contains C^{13} and C^{14} in a ratio of about 1:4 indicating that decarboxylations occur at some stage of the synthetic process. Cholesterol obtained by biosynthesis from labeled acetic acid was converted into cholestane and the latter oxidized with chromic acid to yield the isopropyl group of the side chain in the form of acetone. Isotope analysis of this fraction indicates that the isopropyl group is also derived from acetic acid.

Experiments on the mechanism of coupling between respiration and phosphorylation. W. F. LOOMIS (introduced by Fritz Lipmann), *Biochemical Research Laboratory, Massachusetts General Hospital and Department of Biochemistry, Harvard Medical School, Boston, Mass.* Some aspects of the kidney homogenate technique will be discussed. Preparations, when quick frozen in dry ice, were found to retain practically intact respiration and phosphorylation. Freezing and storage has to occur at around -80°C . At higher temperatures fast deterioration occurs. Phosphorylation coupled anaerobically with ferricyanide reduction was studied and a quotient P/2 ferricyanide of approximately 1 was found. Aerobically a minimum P/O ratio of 2.3 was found in analogous preparations. The already reported inhibition of

the coupling by m-dinitrophenol is also observed with p-nitrophenol but not with p-nitrophenol phosphate. Similar activity was found with di- and trihalophenols but none with diiodo-tyrosine or thyroxine. The apparent substitution in the respiration of tissue dispersions of phosphate by nitro or halophenols has been further explored.

Determination of gamma globulin in blood serum. JOSEPH M. LOONEY and MARY O. AMDUR (by invitation), *Research Laboratory, Veterans Administration Hospital, West Roxbury, Mass.* A study of the method proposed by Kunkel (*Proc. Soc. Exper. Biol. & Med.* 66: 217, 1947) for the turbidimetric determination of gamma globulin with zinc sulfate indicated that the turbidity varied with the albumin concentration. When albumin was added to a 2.0% solution of γ -globulin the turbidity readings obtained were 5.0, 14.0, 10.8 and 8.5 μ , at albumin concentrations of 0.0, 1.0, 3.0 and 8.0% respectively. Irrespective of the γ -globulin concentration on the maximum turbidity was obtained at an albumin concentration of approximately 1.0%. By adding a solution of zinc sulfate to a buffered serum dilution in the presence of gum ghatti a stable opalescent suspensoid, which was proportional to the γ -globulin concentration, was obtained. For the determination, 0.3 ml of serum was mixed with 4.0 ml of barbital buffer pH 7.5 (187 mg barbital and 140 mg Na-barbital/l.) and 2.5 ml of 2% gum ghatti, and then 1.0 ml of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (192 mg/l.) was added. After 30 minutes the turbidity was read at 650 μ in a Coleman Jr. spectrophotometer against a blank containing saline in place of the serum. The concentration was determined from a standard curve prepared from pure γ -globulin. The turbidity produced by a given amount of γ -globulin remained constant when the albumin concentration was varied from 0.0-8.0%. Recoveries of γ -globulin added to sera were made in a series of 27 experiments. The mean value of the γ -globulin as calculated was 2.20 gm/100 ml, and that found was 2.15 gm/100 ml. The standard deviation of the individual readings was 0.15 gm. The difference between values exceeded 0.20 gm in only 3 cases. The mean value of γ -globulin found in 32 patients with normal serum proteins was 0.89 gm/100 ml.

Comparative study of growth factors for some lactic acid bacteria. CARL M. LYMAN and J. M. PRESCOTT (by invitation), *Dept. of Biochemistry and Nutrition, Agricultural and Mechanical College of Texas, College Station, Texas.* By means of electrolysis of a dilute solution of liver extract prepared for the treatment of pernicious anemia (reticulogen), the growth factors required by *Leuconostoc citrovorum* 8081 and *Lactobacillus leichmannii* 4797 were separated. The factor for *Leuconostoc citrovorum* migrated toward the positive electrode indicating the acidic nature of the sub-

stance The factor for *Lactobacillus leichmannii* was found in the electrolysis cells nearest to the negative electrode Separation of the factor for *Lactobacillus leichmannii* from the other factor was also accomplished by treatment of a dilute liver extract solution with the anion exchange resin IRA 400 The factor for *Leuconostoc citrovorum* was quantitatively retained on the column while almost 100% of the other factor passed through A striking difference in the stability of the 2 factors toward heating in dilute alkaline solution was found On autoclaving with 0.05 N NaOH at 15 pounds pressure for 30 minutes, the factor for *leichmannii* was completely destroyed while the *citrovorum* factor showed 100% recovery When *Lactobacillus leichmannii* 313 was used as the test organism, the activity of the various fractions indicated above followed very nearly the same pattern as that obtained with *Lactobacillus leichmannii* 4797 Although the factors for *Leuconostoc citrovorum* and *Lactobacillus leichmannii* appear to be distinctly different compounds, they are very probably related In a comparative test on the activity of enzymatic digests of various animal tissues, liver and spleen gave the highest values and muscle tissue gave the lowest values with both organisms The presence of thymidine may have influenced these results

D-serine inhibition of *E. coli* and its reversal by other amino acids and by mutation WERNER K. MAAS (by invitation) and BERNARD D. DAVIS *U S Public Health Service, Tuberculosis Research Laboratory, Cornell Univ Medical College, New York City, and the Naval Medical Research Institute, Bethesda, Md* D-serine produces a nephrotoxic effect in the rat (Artom *et al*, *Proc Soc Exper Biol & Med* 60:284, 1945) Animals can be protected against the action of D-serine by several amino acids (Wachstein, *Arch Path* 43:515, 1947) We have found that growth of normal *E. coli* is delayed considerably by concentrations of D-serine as low as 5 γ /ml In the presence of D-serine the bacteria undergo a few divisions at normal rate Further multiplication is then retarded for a period whose length depends upon the concentration of D-serine, but eventually the organism overcomes the inhibition This adaptation is due not to the formation of an adaptive enzyme, but rather to an adjustment of the environment Of 11 other DL-amino acids tested at 200 γ /ml, none proved to be inhibitory L-aspartic acid, although not inhibitory by itself, greatly enhances D-serine activity Complete antagonism is provided by DL-alanine and glycine Most other amino acids, even in high concentration, produce only partial reversal of the inhibition L-cysteine, L-cystine, L-hydroxy proline and, curiously, L-serine have no effect Spontaneous mutants which are resistant to D-serine inhibition occur with rela-

tively high frequency The mutation rate is greatly increased by ultraviolet irradiation Resistant colonies when grown on D-serine medium together with sensitive bacteria produce a zone of satellite growth of the sensitive bacteria The simple structure of D-serine permits this phenomenon to serve as a model for studying the mechanism of drug resistance

Evaluation of six standard proteins by growth of young dogs DOROTHEA MABEE (by invitation) and AGNES FAY MORGAN *Laboratory of Home Economics, Univ of California, Berkeley, Calif* This experiment was part of a cooperative effort made by several laboratories to standardize methods of assessing the nutritive value of proteins Either 2 or 3 comparable young dogs from our purebred cocker spaniel colony were used for each diet Growth and intake were observed for 120 days, and blood composition and N retention regularly determined Raw egg yolk was used as the control diet The proteins were fed at the 18% level except for the egg yolk and whole egg powder which were fed at the 12% level and the gluten and dried egg albumin at both 18 and 40% levels The apparent absorbabilities were gluten 96, casein 93, casein plus 10% methionine 91, beef 89, peanut 88, egg yolk 87, whole egg powder 82, heated (15 minutes at 140°) casein plus 0.6% lysine 78, heated casein 76, heated egg powder 61, egg albumin 45 If the gain per gm protein eaten for raw egg yolk be taken as 100, that of whole egg powder was 77, beef muscle 73, casein 71, heated casein plus lysine 67, heated casein 65, heated egg powder 61, casein plus methionine 55, peanut flour 41, gluten 28 (18% level) and 34 (40% level), dried egg albumin 25 (18% level) and 9 (40% level) The dried egg albumin contained an active antitryptic agent and the gluten produced running fits The egg albumin was well utilized by rats showing no evidence of antitryptic activity

Determination of hemochromogen in serum WILLIAM M. McCORD (introduced by J. Roy Dorr) *Dept of Chemistry, Medical College of South Carolina, Charleston, S C* Slight hemolysis of red cells interferes with the determination of hemochromogen in serums By determination of 'oxyhemoglobin hemin' and 'total hemin' the serum hemochromogen may be calculated by difference By determining the optical density of a sample of serum, with a Beckman Spectrophotometer, at 576 m μ and at 588 m μ , using water as the blank, the concentration of the hemin, representing the oxyhemoglobin present, may be calculated by using the formula $Hemin_{(O_2)} = (O.D._{576} - O.D._{588}) \times 6.86 \text{ mgs/100 ml}$ Total hemin is determined by adding 8.5 ml of 10% ammonium hydroxide and 0.5 ml pyridine to 1 ml of serum Five ml of the mixture is used as a blank, to 3 ml of the remainder, in the absorption cell, is added 0.1 ml of freshly prepared

20% sodium hydrosulphite ($\text{Na}_2\text{S}_2\text{O}_4$) The optical density is read at $555 \text{ m}\mu$ The total hemin concentration is calculated by using the formula $\text{Hemin}_{(\text{total})} = \text{O D}_{555} \times 34.72 \text{ mgs/100 ml Serum}$ hemochromogen, in terms of hemin, is then calculated by difference Standardization is accomplished by the use of crystalline hemin Sensitivity of the method is of the order of 1 mg hemin per 100 ml with an accuracy of about 5% Bilirubin does not interfere

Stability and stabilization of testicular hyaluronidase D ROY McCULLAGH, FLORENCE VALENTINE (by invitation), and SIBYLLE TOLKSDORF (by invitation) *Dept of Biochemistry, Schering Corporation, Bloomfield, N J* The stability of hyaluronidase which has been dried from the frozen state has been investigated It has been found to decrease as the purity increases Bulk enzyme assaying 150 Turbidity Reducing Units per mg does not measurably decrease in potency on standing at room temperature for several months 500 TRU/mg material has a half life of approximately 3 months Hyaluronidase can be sterilized by filtration without destruction When it is packaged in vials, losses are occasionally observed during drying The lability is greater when the amount of enzyme in the vial is very small The enzyme in vials is stable for several months at 5°C , but at room temperature it decreases considerably in potency in 2-3 months At 60°C the aqueous solution is almost immediately destroyed In the dry state at 60°C the half life may vary from a few hours to a few days according to the amount of enzyme in the vial It has been found that the addition of small quantities of gelatin will greatly increase the stability of hyaluronidase However, when gelatin is included in the solution to be dried, the final product is not readily soluble Therefore, a polypeptide derived from gelatin has been used as a stabilizer When this material is used the potency decreases only 15-20% in 7 weeks at 60°C The product is very soluble in water Various bacteriostatic agents have been used in conjunction with hyaluronidase and do not appear to destroy the activity

Further studies on enzymatic benzoylations R W MCGILVERY (by invitation) and P P COHEN *Laboratory of Physiological Chemistry, Univ of Wisconsin, Madison, Wis* The study of enzymatic benzoylations has been extended to the conjugation of ornithine by the fowl The washed residue of chicken tissue homogenates couples p-aminobenzoic acid (PAB) with N^6 -benzoyl-L-ornithine to form p-amino-L-ornithuric acid The product has been isolated as the p-(p-hydroxyphenyl) azo derivative N^a -benzoyl-L-ornithine also reacts with PAB, presumably forming p'-amino-L-ornithuric acid, but this reaction has not been studied in detail Adenosine triphosphate (ATP) is required for the synthesis Other properties of the systems will be reported

Growth stimulatory factors for the malarial parasite, Plasmodium knowlesi RALPH W MCKEE and QUENTIN M GEIMAN (by invitation) *Depts of Biological Chemistry, and of Comparative Pathology and Tropical Medicine, Harvard Medical School, Boston, Mass* Submaximal growth and multiplication of *Plasmodium knowlesi* are obtained when a nutrient medium of known composition (*J Exper Med*, 84 607, 1946), simulating plasma, is used The addition of at least 2, apparently different, factors stimulate *in vitro* growth and multiplication of the malarial parasites One factor is a dialyzable component (or components) present in horse, monkey and human plasmas In a washed erythrocyte culture system, to which are added the nutrient medium and purified serum albumin or globulin, growth and multiplication are retarded The addition of either plasma or the dialyzable component restores the activity This growth stimulatory substance is organic and appears to be quite heat stable as deproteinization with heat at pH 5 does not destroy the activity The other active material is a non-dialyzable substance (or substances) present in 15 unit antipernicious anemia concentrate of liver The liver concentrate (0.005 ml per culture) increases the rate of growth and the numbers of parasites to as much as 150% of that obtained when plasma alone is present The addition of folic acid or of additional p-aminobenzoic acid appear to have no stimulatory action Vitamin B_{12} remains to be tested Further chemical and isolation studies are under way on these factors

Rate and mechanism of the Liebermann-Burchard reaction on various derivatives of cholesterol HERBERT MCKENNIS, JR, JOSEPH Y THOMAS (by invitation) and J C FORBES *Dept of Biochemistry, Medical College of Virginia, Richmond, Va, Dept of Physiological Chemistry, The Johns Hopkins Univ School of Medicine, Baltimore, Md and Laboratory for Surgical Research, Medical College of Virginia, Richmond, Va* During the course of our investigations of the effect of various derivatives of cholesterol on growth it became desirable to know the comparative behavior of these substances in the Liebermann-Burchard reaction The results of rate studies at bath temperatures of 10° and 22° appear to indicate a process which is kinetically of the first order In view of the recent isolation of dicholesteryl ether from the spinal cords of cattle (Silberman, N, and Silberman-Martyncewa, S, *J Biol Chem* 159 603, 1945) it was particularly interesting to study this ether On a molar basis the amount of color produced was essentially twice that from cholesterol itself This finding gives indirect support to Yoder's conception of cholesteryl ether cleavage by a cholesterol-cholesterilene route It further opens to question the view (Nath, M C, Chakravorty, M K, and Chowdhury, S R, *Nature* 157 104, 1946) that

dicholesteryl ether is not an intermediate in the production of the Liebermann-Burchard color

Formation of formaldehyde in the biological oxidation of the methyl group of sarcosine COSMO G MACKENZIE and VINCENT DU VIGNEAUD *Dept of Biochemistry, Cornell Univ Medical College, New York City* In the course of studies on the biological oxidation of the methyl group in S- and N-methyl compounds, radioformaldehyde has been obtained as an oxidation product of sarcosine labeled in the methyl group with C¹⁴. The formaldehyde has been isolated as the dimedon derivative from the trichloroacetic acid filtrate of rat liver homogenates incubated with the labeled sarcosine. This derivative of formaldehyde has also been isolated by direct distillation of the incubation mixture from 0.1 M H₂PO₄ into aqueous dimedon. Free formaldehyde is formed in the course of the oxidation of the methyl group as can be shown by blowing it out of the incubated homogenate-sarcosine mixture with a stream of N₂ at 37° and trapping it in a solution of dimedon. The formation of free formaldehyde from the methyl group of radiosarcosine by respiring liver slices may similarly be demonstrated by drawing a stream of O₂ through the reaction vessel and into a dimedon scrubber. A positive color test for formaldehyde in liver homogenates incubated with sarcosine has been reported by Handler, Bernheim and Klein (*J Biol Chem* 138: 211, 1941). The homogenate described by these authors was employed in the present experiment. Radioactive CO₂ has also been obtained as a product of the oxidation of the tagged methyl group of sarcosine both in liver preparations and in the whole animal.

Specific volume of α - and β -casein and relationship of specific volume to amino acid composition T L McMEEKIN, M L GROVES (by invitation) and N J HIPP (by invitation) *Eastern Regional Research Laboratory, Philadelphia, Penna* The specific volume of a protein is of great importance in determining its molecular weight by sedimentation and diffusion. It has been suggested by Cohn *et al* (*J Biol Chem* 100: Proc. xxviii, 1933) that the apparent specific volume of a protein is largely determined by the volume increments of the amino acid residues of which it is composed. The fairly complete amino acid analyses of α - and β -casein reported by Gordon *et al* (*Federation Proc*, this issue), which show considerable differences in the composition of these proteins, offer the opportunity of testing the accuracy of the hypothesis that the specific volume of a protein is determined by its amino acid composition. The apparent specific volumes of α - and β -casein, as well as unfractionated casein, have been determined in dilute alkali and in acid solutions. The following data illustrate the agreement of the experimentally determined specific volumes with the specific volumes calculated from the amino acid analyses of Gordon *et al*.

	Apparent Sp Vol Calculated from Amino Acid Residues	Apparent Sp Vol Determined at 25 C
α Casein	0.728	0.724
Unfractionated casein	0.732	0.727
β Casein	0.744	0.740

Lipids of peripheral nerve during Wallerian degeneration A R McNABB (by invitation), A C JOHNSON (by invitation) and R J ROSSITER *Dept of Biochemistry, Univ of Western Ontario, London, Canada* It has previously been shown (*Rev Canad Biol* in press) that when a cat peripheral nerve undergoes Wallerian degeneration there is a steady decrease in the concentration of the myelin lipids, i.e. cerebroside, free cholesterol and sphingomyelin, the substances which we have previously suggested are the principle lipid components of the myelin sheath (*Biochem J* 43: 573, 578, 1948), and an increase in the concentration of the ester cholesterol. Further observations on the concentration of neutral fat and total fatty acid make it possible to piece together the changes in the distribution of lipids in a nerve when it undergoes Wallerian degeneration. There is an early increase in water content and a reduction in the concentration of neutral fat. After 8 days, corresponding in time to the appearance of macrophages along the course of the degenerating nerve, there is a steady decrease in the myelin lipids, i.e. cerebroside, free cholesterol and sphingomyelin. These substances are presumably slowly hydrolysed, and the products of hydrolysis, e.g. glycerol, fatty acid, choline, galactose, sphingosine and phosphate are removed. There is, in addition, a hydrolysis of cephalin and a slower hydrolysis of lecithin. Some of the fatty acids liberated during hydrolysis presumably combine with free cholesterol to form ester cholesterol while others may be converted into neutral fat. Such a process would explain many of the histochemical observations made on degenerating nerve tissue.

Distribution of radiophosphorus in rabbit tissues after injection of labelled disopropyl fluorophosphate (DFP) PRISCILLA D McNAMARA (by invitation) and BERNARD J JANDORF *Biochemistry Section, Medical Division, Army Chemical Center, Maryland* Sublethal doses of DFP, containing 15-20 mc of P³²/gm, were injected intravenously into rabbits. Upon sacrifice of the animals from 10 minutes to 24 hours after injection, the specific radioactivity (P³²/unit tissue weight), in decreasing order, was found to be high in kidney, plasma, lung, and liver, moderate in skeletal muscle, heart, and erythrocytes. The specific activity of cerebral tissue was low even after injection of DFP into the carotid artery. Thus, no correlation was found to exist between the cholinesterase activity of these tissues and their ability to retain P³² derived from DFP. The magnitude of specific

activity of rabbit organs depended on the time interval between injection and sacrifice of the animal, while their order of relative activity was independent of this interval. Kidney and liver tissue of animals injected with DFP and sacrificed at intervals showed a decrease in total specific activity with time, however, the proportion of protein-bound to total radioactivity in these organs increased steadily during the same interval. In lung tissue, a maximum value for total and protein-bound P^{32} was attained within 10 minutes after injection, these values decreased only slightly during the subsequent 24-hour period. When DFP was replaced with an equivalent dose of sodium diisopropyl phosphate, little or no P^{32} was retained, 24 hours after injection, in the tissues mentioned.

Micro permanganate acid ashing method for determination of serum iodine EVELYN B MAN and DOROTHY A SIEGFRIED (by invitation) *Yale Univ School of Medicine, New Haven, Conn*. Protein bound iodine in duplicate 1 cc aliquots of blood serum is precipitated with solutions of zinc sulfate and sodium hydroxide in micro flasks which fit into 250 cc centrifuge cups. The centrifuged precipitate, after decantation of supernatant fluid, is washed about 3 times with iodine free water to remove as much inorganic iodine as occurs during Lugol's administration. The precipitate is heated with solutions of potassium permanganate and sulfuric acid. Without transfer from the precipitation flask, the digest is reduced with acid arsenite solution and iodine distilled through a micro condenser into a dilute acid arsenite solution. Iodine in the concentrated distillate is measured by recording, during about 5 minutes, the color change with ceric sulfate in Chaney's constant recording colorimeter. The average of duplicate recoveries of inorganic iodine added to zinc hydroxide blanks after centrifugation but before digestion was 2.2 for 2.0, 4.8 for 5.0, 9.2 for 10.0, and 15.1 for 15.0 gamma % of iodine. Serum hormonal iodine determined by this microprocedure has checked with values obtained by the Riggs, Man macro permanganate acid ashing method. This method requires only 2 cc of serum for duplicate determinations. Precipitation, digestion and distillation are continued in the same micro flask, without transfer. Different concentrations of acid arsenite solution are used for reduction of the digest, collection of iodine in distillate, and quantitation of iodide by ceric sulfate. Constant records of color change eliminate the problem of exact timing and facilitate recognition of irregularities in color fading produced by contaminating substances in the distillate.

Effect of methyl-bis(beta-chloroethyl)amine on blood constituents GEORGE H MANGUN *Dept of Laboratories, Henry Ford Hospital, Detroit, Mich*. Hartman and Mangun demonstrated that the nitrogen mustards are capable of inactivating viruses

and bacteria in the presence of blood plasma and whole blood, and that a variety of plasma and red blood cell constituents are essentially unaffected or only slightly affected by the sterilizing dosage of Methyl-bis(beta-chloroethyl)amine (HN2) (500 mg/l). An exception is the prothrombin time of citrated plasma. When undiluted citrated plasma is treated with 500 mg/l of HN2 at pH 7.0, the apparent prothrombin concentration (Quick's one-stage method) drops progressively over a 3 day period at 4°C to about 20-30% of its original value. The effect of HN2 has been studied on separated preparations of bovine thrombin, prothrombin, fibrinogen and accelerator globulin. When treated with 500 mg/l of HN2 at pH to 7.5 at 4°C the following changes were noted. Thrombin (0.5 mg/ml of Seeger's preparation #480904, 17,800 U/mg of tyrosine) is slowly inactivated, showing about 1/3 of its original activity at 3 hours and 2-3% at 20 hours. Prothrombin (Seeger's #481025, about 500 U/mg) is rapidly inactivated in 0.5 mg/ml saline solution, about 1% of the control activity being present at 2 1/2 hours and less than 0.5% at 20 hours. Accelerator globulin (not purified, treated in 1:10 saline) after 20 hours was about 5% of the control activity. The clotting rate and total fibrinogen (Seeger's #470712, 92% clottable) precipitated by thrombin were unaffected by the sterilizing dosage of HN2.

Enzymatic degradation of thymidine L A MANSON (by invitation) and J O LAMPEN *Dept of Biological Chemistry, Washington Univ School of Medicine, St Louis, Mo*. In a previous study we obtained both phosphorolysis and arsenolysis of hypoxanthine desoxyriboside by enzyme preparations from calf thymus and rat liver. A desoxypentose-5-phosphate was isolated as a product of phosphorolysis and evidence was obtained for its formation by way of the 1-phosphate ester (*Abstracts*, Sept 1948 meeting of the American Chemical Society). The mechanism of the action of nucleosidase on the desoxyriboside appears identical with that demonstrated for the riboside by Kalckar, Deutsch and Laser (*Ztschr f physiol Chem* 186 1, 1930) and Klein (*Ztschr f physiol Chem* 231 125, 1935), using phosphate and arsenate buffers, showed the existence of a separate pyrimidine nucleosidase. We have studied the degradation of thymidine by preparations from dog bone marrow and calf kidney and have again observed both an arsenolysis and a phosphorolysis. The products of the arsenolysis are free thymine and the free sugar. Free pyrimidines inhibit this reaction. The kinetics of the arsenolysis will be discussed. Thymidine was not degraded by a hemolysate of rabbit erythrocytes or a purine nucleoside phosphorylase preparation from rat liver. The products of the phosphorolysis of thymidine are free thymine and a phosphorylated form of the sugar. The evidence for

the latter is that an uptake of inorganic phosphate occurs and little free sugar is found. Also the addition of hypoxanthine increases the rate of formation of free thymine. The preparations contain purine nucleoside phosphorylase, hence this result is in agreement with the formation of a phosphate ester which is removed by reaction with the hypoxanthine. We have not observed any requirement of a dialyzable cofactor (other than phosphate or arsenate) by the pyrimidine nucleosidase.

Groups involved in the Zimmerman and Kober reactions. H. W. MARLOW (introduced by H. C. TIDWELL) *Dept of Biochemistry, Southwestern Medical College, Dallas, Tex.* The colored compounds formed by the Zimmerman and the Kober reagents are still unknown, hence it has not been possible to determine the exact groups involved in these tests. In the Zimmerman reaction, it has been postulated that a methylene group adjacent to a carbonyl is necessary for color development. In the Kober reaction the presence of an α -diketone was at one time believed to be responsible for the specific color development. With the synthesis of various 16 substituted steroids, it is now possible to shed further light on these color tests relative to the groups involved. Present data indicate that in the Zimmerman reaction a carbonyl group at C₁₇, with adjacent methylene group at C₁₆, is necessary for the highest color development. Steroids in which a carbonyl group is present at C₁₆ with two adjacent methylene groups, however, give no color with the Zimmerman reagent. This finding is not in agreement with the contention that all $-\text{CH}_2-\text{CO}-\text{CH}_2-$ groupings (as in acetone, in ethyl acetoacetate and in cyclopentanone) yield strongly positive Zimmerman reactions. 16,17-di-ketosteroids also give a negative Zimmerman. With the Kober reagent, 16-diketosteroids produce no color. However, 16-keto, 17-hydroxysteroids give an intense Kober reaction. Also 16,17-diketosteroids produce no color with the Kober reagent.

Incorporation of C¹⁴-formic acid into nucleic acid fractions of rat and pigeon. WALTON H. MARSH (introduced by HARLAND G. WOOD) *Dept of Biochemistry, School of Medicine, Western Reserve Univ., Cleveland, Ohio.* While other workers have shown that labeled formate is incorporated into uric acid and hypoxanthine in pigeons, it has not previously been demonstrated to enter the nucleic acid fractions of birds or mammals or to be oxidized by the bird. Radioactive formate was fed to a pigeon and injected intraperitoneally into rats, the time of sacrifice being 3 days in the former and 14 hours in the latter. The nucleic acids of both the rat and the pigeon were radioactive, although the activity was only a small per cent of that administered. The adenine and the guanine from the pigeon nucleic acids were radioactive. It was also found that the formate which was given to a pigeon

at the rate of 0.7 mm/hr, was oxidized to CO₂ on the average of 6.5% for the first 12 hours. The rate of oxidation increased very rapidly throughout this period. This oxidation probably was not entirely due to intestinal bacterial action since pigeon liver homogenates were shown to oxidize formic acid at the rate of 0.2 $\mu\text{m/hr/gm}$ wet weight of tissue. From the respiratory C¹⁴O₂, only 5% of the activity in the purines could have come from the incorporation of CO₂ whereas, in the rat, on the basis of the high formate oxidation (W. Sakami, *J. Biol. Chem.* 176:995, 1948), all the radioactivity of the purines could be accounted for by CO₂ fixation. The location of isotope in the purines may shed light on the mechanism of synthesis.

Determination of fumaric acid in animal tissues by partition chromatography. LAWRENCE M. MARSHALL (by invitation), JAMES M. ORTEN and ARTHUR H. SMITH *Dept of Physiological Chemistry, Wayne Univ. College of Medicine, Detroit, Mich.* A method for examining the fumarate content of tissues has been devised. It employs column partition with silica gel as the mechanical support for the aqueous phase over which passes an amyl alcohol-chloroform mixture, the non-aqueous, mobile phase. The sequence of organic acids liberated by the mobile phase from the silica gel column is directly related to the distribution coefficients of the solutes with reference to the system employed. Citric, malic, lactic, succinic, fumaric, beta-hydroxybutyric, acetic and benzoic acids represent a partial list of acids arranged in inverse order of their release from the column. Glutamic, aspartic, and nicotinic acids, like citric, are delivered after fumaric acid. The effluent acids are titrated with 0.004N sodium hydroxide. When the fumaric acid emitted from the column is collected in successive fractions having geometrically increasing volumes, the concentrations of fumaric acid plotted against the fraction number describes a curve which approximates the normal curve of error. The observed curve agrees closely with a theoretical curve calculated from the binomial law. Recovery studies demonstrated the applicability of the technique to animal tissues. The fumarate concentrations, expressed in mg %, in several tissues of the fasted adult rat maintained on a stock diet show the following values: brain, 15; kidney, 9.5; liver, 7.8; gastrocnemius muscle, 2.3; and blood, less than 0.3. Preliminary studies indicate the applicability of the foregoing procedure to the determination of other related organic acids in animal tissues.

Protein changes in the developing chick embryo. MARGARET E. MARSHALL (by invitation) and H. F. DEUTSCH *Dept of Physiological Chemistry, Univ of Wisconsin, Madison, Wis.* The proteins of the blood serum and amniotic fluid of chick embryos have been studied as a function of time of incubation. No electrophoretic changes in the

residual egg white proteins are apparent in pH 8.6 diethylbarbiturate buffer of ionic strength 0.1. The proteins of the amniotic fluid from the 12-19th day of incubation gave patterns identical with those of the egg white proteins. Analyses of the blood serum of embryos, age 10-19 days, revealed marked changes. On the 13th day of incubation the serum contains 102 mg % protein nitrogen and 8.3 mg % lipid phosphorus. A large amount of the protein possesses an electrophoretic mobility higher than that of the serum albumin. These faster migrating proteins carry the lipid phosphorus as evidenced by analyses of electrophoretically isolated material and correlating sedimentation studies. By the 19th day of incubation they are present in much lower concentration. Simultaneously the lipid phosphorus appears with slower migrating proteins. Sedimentation analyses of embryo serum in sodium chloride solutions of varying density shows the presence of at least 3 molecular components.

Metabolism of dehydroisoandrosterone in a woman before and after removal of an adrenocortical tumor. HAROLD L. MASON, JOHN J. SCHNEIDER (by invitation) and EDWIN J. KEPLER (by invitation). *Division of Biochemistry and Division of Medicine, Mayo Clinic, Rochester, Minn.* Dehydroisoandrosterone acetate (150 mg/day for 8 days) was administered to a woman with a virilizing adrenocortical tumor. The daily amount of 17-ketosteroids excreted increased from an average of 258 to 274 mg, and the proportion of the β -fraction did not change. After removal of the tumor, administration of the same amount of dehydroisoandrosterone acetate increased the excretion of 17-ketosteroids from 2.8 to 52.3 mg/day (average). The β -fraction increased progressively to 38 and 36 per cent of the total 17-ketosteroids on the 7th and 8th days. Androsterone, dehydroisoandrosterone, etiocholan-3(α)-ol-17-one, 3-chlorodehydroandrosterone, Δ^6 androstene-3(β), 17(α)-diol, pregnane-3(α), 20(α)-diol and a new alcohol, $C_{21}H_{34}O$, were isolated from the urine during a control period, and during the first period of administration of dehydroisoandrosterone acetate. The quantitative relations of these compounds were essentially the same in the 2 periods. During the period after operation, androsterone, dehydroisoandrosterone, etiocholan-3(α)-ol-17-one and pregnanediol were isolated. It was expected that administration of an amount of dehydroisoandrosterone comparable to the amount being excreted would affect the relations of the various urinary steroids, and that perhaps most of the dehydroisoandrosterone would be excreted unchanged. The only effect, however, was a small increase in the total amount of 17-ketosteroids. After operation considerable dehydroisoandrosterone was recovered from the urine, and androsterone and etiocholanolone were isolated in amounts (43 and 32 mg, respectively) which were

considerably greater than the total of the endogenous 17-ketosteroids. This last result is in accord with the conclusion that dehydroisoandrosterone normally is converted largely to androsterone and etiocholanolone before excretion.

Manometric determination of methyl alcohol in blood. M. F. MASON, *Parkland Hospital and Southwestern Medical College, Dallas, Texas.* Methyl alcohol, alone or in the presence of ethyl alcohol, in blood may be determined with accuracy sufficient for forensic purposes by the following manometric procedure which is independent of standardized reagents. The distillate obtained from 10 cc or 20 cc of tungstate filtrate in the Harger method for blood alcohol (*J. Lab. Clin. Med.* 20: 746, 1935) is made to 25 cc. Apparatus 'C' described by Van Slyke *et al.* (*J. Biol. Chem.* 141: 627, 1941) for determining carboxyl- CO_2 is modified by introduction of a side arm equipped with a stop-cock and cup at the shoulder of the tube. Silicone lubricant is used sparingly in the stop-cocks, phosphoric acid as a joint seal. Five cc of a bichromate-sulfuric acid mixture (e.g. Anstie's reagent) are placed in the tube and the apparatus evacuated (water pump) several times with shaking. Five cc of distillate and 0.5 cc of water for washing are admitted via the cup without loss of vacuum, the apparatus shaken, and boiled for 10 minutes. After cooling to about 45°C the apparatus is connected to the chamber of the Van Slyke-Neill manometric analyzer and the CO_2 evolved is determined as described by Van Slyke *et al.* (*vide supra*). The blank is determined on acidified, boiled water, treated similarly. Methyl alcohol is calculated from the CO_2 determined. Recoveries of added methyl alcohol with or without ethyl alcohol ranged from 97 to 100%. In interpreting results the specificity features of the bichromate oxidation should be kept in mind.

Preliminary communication on the effect of growth hormone on tissue enzymes. JAMES C. MATHIS (introduced by OLIVER H. GAEBLER), *Edsel B. Ford Institute for Medical Research, Henry Ford Hospital, Detroit, Mich.* Growth hormone might promote nitrogen storage by reducing amino acid deamination, or by increasing reutilization of D-amino acids, therefore its effect on D-amino acid oxidase activity was investigated. The oxidase was assayed according to Axelrod *et al.* (*J. Biol. Chem.*, 134: 749, 1940), but a pyrophosphate buffer of pH 8.3 was employed. *In vitro* experiments involving five different oxidase sources and various pH values showed growth preparations to be without effect. Growth hormone also did not counteract the oxidase inhibitor effect. Experiments on the intact rat, using two strains, several levels of hormone treatment, groups of both sexes, with both *ad libitum* and paired feeding were performed. The data for one representative series

follow Five 'plateaued' Sprague-Dawley female rats were given 6 mg hormone intraperitoneally per day for 7 days and 12 mg on the 8th day, following which they and their pair-fed controls were killed for enzyme assay. Oxidase activities, and standard deviations, are given in cmm oxygen uptake per organ/hr/100 gm final body weight. The essentially constant enzyme activity would indicate that no specific activating or deactivating relationship exists between the hormone and the

Organ	Controls	Treated
Kidney	-1170 \pm 740	-1220 \pm 170
Liver	-730 \pm 470	-1100 \pm 490

oxidase. The total amount of functioning enzyme in the liver appears roughly equal to that in the kidney. Effects of growth preparations on other enzyme systems will also be discussed.

Formazyl color reaction as a possible biochemical analytical tool. NORMAN L. MATTHEWS (introduced by EARL R. NORRIS) *Dept of Pharmacology, Univ of Washington, Seattle, Wash, Emory Univ School of Medicine, Emory University, Ga, and Univ of Rochester School of Medicine and Dentistry, Rochester, N Y*. Although the formazyls were well known in the older literature, apparently no attempt has been made to use the formation of these highly colored substances for analytical purposes. The following classification serves to indicate the possible wide range of application of the formazyls in this respect. I. Substances which yield formazyls by direct reaction with benzene diazonium salts in a) mild acid medium (oxalacetic, acetoacetic, and malonic acids) and b) only alkaline medium (pyruvic acid, acetaldehyde, acetone, etc.), II. Substances whose phenylhydrazones yield formazyls when reacted with diazonium salts in alkaline medium (aldehydes, aldose sugars, and certain α -ketoacids). Formazyls apparently have not been prepared from aldoses, and only formazyl itself could be isolated from the reaction mixture when mannose phenylhydrazone and glucose phenylhydrazone were studied. The advantages of the formazyls as possible analytical agents are that they are easily formed, the sensitivity is great and the colors are characteristic. Disadvantages are that a mixture of formazyls is often formed from one compound, the reaction is not specific, and there is a certain amount of blank color. Quantitative studies have been made with glucose. It was found possible to achieve excellent color development by simply treating with iodine a solution prepared by adding NaOH to a briefly heated mixture of the glucose sample and buffered phenylhydrazine solution. With proper conditions it is possible to minimize the blank color, quantitate the amount of color

with the amount of glucose and limit the effect of interfering substances.

Deamination of crystalline egg albumin. PAUL MAURER (by invitation) and MICHAEL HEIDELBERGER *Depts of Biochemistry and Medicine, College of Physicians and Surgeons, Columbia University, New York City*. Water solutions of crystalline egg albumin (Ea) were treated at 0-2°C in acetate buffer with NaNO₂ at pH 4.0 for varying periods. The mixtures were neutralized, dialyzed nitrite-free, and were treated with thioglycollic acid at pH 7.5 to reduce any -S-S- linkages formed. After dialysis to remove thioglycollate, two fractions were separated at the pH of maximum insolubility, 3.9 to 4.0, one soluble, the other insoluble. Both fractions gave homogeneous electrophoretic and diffusion patterns. The soluble fraction was homogeneous in the ultracentrifuge and showed the properties to be expected of an undenatured, partly deaminated Ea. Complete deamination was not achieved under the mild conditions used. In spite of the care taken, the insoluble fraction, which increased with time of exposure to the deaminating solution, was denatured as shown by its insolubility at the isoelectric point and other physical properties and by its chemical and immunological reactions. Data are presented on the percentage of deamination, viscosity, $[\alpha]_D$, and cross reactivity of each fraction with calibrated anti-Ea and anti-denatured Ea sera.

Depolymerase activities of calf spleen and thymus cathepsin preparations. MARY E. MAVER and ANTOINETTE GRECO (by invitation) *National Cancer Institute, Bethesda, Md*. In a study of the role of cathepsins in nuclear metabolism it was found that these intracellular proteinases from calf thymus and calf spleen hydrolyzed thymus nucleoproteins and rendered the nucleic acid dialyzable. This dipolymerizing function of the cathepsin preparations differed from that of the pancreatic dipolymerases in showing optimal activity at pH 4.5 for both the desoxyribonucleic acid (DNA) and ribonucleic acid (RNA). In view of the close association of proteolytic activity with the crystalline depolymerases of the pancreas the correlation of proteinase activity with the dipolymerase activity of the cathepsins is of interest. The spleen enzymes which show the highest catheptic activity of any tissue studied show the greatest depolymerase activity. The same activators and inhibitors which affect the catheptic activities of these enzymes also affected their depolymerase activities. The proteinase activity of the calf spleen cathepsin was found previously to be sensitive to the sulfhydryl reagent, *p*-chloromercuribenzoate. The *K* value of 22×10^{-3} for the first order reaction in the depolymerization of DNA as determined by viscosity measurements was reduced 48% by the addition of 2.5 micromoles

of *p*-chloromercuribenzoate per mg enzyme. The same reagent decreased the amount of soluble phosphorus liberated from both DNA and RNA. No inorganic phosphorus was liberated by the cathepsins. Cysteine caused a definite increase in the rate of depolymerization of DNA and in the amount of soluble phosphorus liberated.

Hepato-renal factors in circulatory homeostasis

XXI Quantitative immunochemistry of ferritin and apoferritin ABRAHAM MAZUR and EPHRAIM SHORR (by invitation) *Dept of Medicine, Cornell Univ Medical College and The New York Hospital, New York City*. During a study which resulted in the identification of ferritin as the hepatic vasodepressor, VDM, which is concerned with the regulation of the peripheral circulation (*J Biol Chem*, 176: 771, 1948), the immunochemical behavior of ferritin and its iron-free protein moiety, apoferritin, was investigated. Using the quantitative-precipitin technique, it was shown that horse liver and spleen ferritin are identical. Similar immunochemical identity was established for horse ferritin and apoferritin. The iron in the specific precipitates accounted quantitatively for all of the antigen added in the region of excess antibody and up to the end of the equivalence zone. Thus, in these zones, the antibody N in the precipitate may be calculated by subtracting the added antigen N from the total N of the precipitate. Calculation of the molar ratios of rabbit serum antibody (\bar{M} 150,000) to apoferritin (\bar{M} 465,000) gave values corresponding to the formulae, Ab_1Apo in the zone of extreme antibody excess, $Ab_{1.4}Apo$ at the antibody excess end of the equivalence zone and Ab_7Apo at the antigen excess end of the equivalence zone. Inhibition of precipitation was observed with large excess of antigen. By the micro precipitin technique, using the Folin color reagent to estimate the precipitated protein, 1 to 2 gamma ferritin N could be determined. By means of the complement-fixation test, 0.05 gamma ferritin N was detected. Immunochemical cross reactions were obtained between horse and dog ferritins and to a lesser extent between horse and human ferritins.

Hepato-renal factors in circulatory homeostasis **XXII Chemical properties of ferritin and apoferritin** ABRAHAM MAZUR and EPHRAIM SHORR (by invitation) *Dept of Medicine, Cornell Univ Medical College and The New York Hospital, New York City*. The identity of ferritin with the hepatic vasodepressor, VDM, which is concerned with the regulation of the peripheral circulation (*J Biol Chem*, 176: 771, 1948) led to a study of some chemical characteristics of ferritin and its iron-free protein moiety, apoferritin. Fractionation with varying concentrations of $(NH_4)_2SO_4$ yielded preparations with widely differing Fe

content. The original ferritin contained 1.88 mg Fe/mg N. The fraction precipitated at 23% saturated $(NH_4)_2SO_4$ had 2.20 mg Fe/mg N, whereas that precipitated between 40-50% saturated $(NH_4)_2SO_4$ had 0.55 mg Fe/mg N. All fractions gave typical crystals with $CdSO_4$. The ration of Fe/P in all fractions as constant. A similar fractionation was obtained by centrifuging a ferritin solution at 13,000 rpm. Electrophoresis studies with ferritin and apoferritin yielded identical mobilities at pH values from 3.95 to 8.6. An approximate isoelectric point of 4.4 was thus obtained. Amino acid analyses of apoferritin and ferritin yielded identical values for both on the basis of N content. Some amino acid values for apoferritin were (in %) glutamic (17.0), leucine (19.1), arginine (9.0), lysine (7.5), histidine (4.8), phenylalanine (5.9), tyrosine (4.8), valine (4.3), cystine (1.7), methionine (1.8). The total S of apoferritin (0.89%) was accounted for by cystine and methionine. The action of crystalline pepsin and trypsin on ferritin and apoferritin was also studied.

Comparison of the electrophoretic components of human serum at pH 4.5 and 8.4 JOHN W. MEHL, FLORENCE GOLDEN (by invitation), and JANE HUMPHREY (by invitation) *Dept of Biochemistry and Nutrition, University of Southern California School of Medicine, and the Los Angeles County General Hospital, Los Angeles, Calif*. At a pH of 4.5, two components with isoelectric points below those of serum albumin may be distinguished. One of these has been identified as a mucoprotein, and has been found to increase in cancer, pneumonia, and a number of other conditions. This component contributes to the α globulin fraction at pH 8.4. The other acid component is also increased in conditions in which the most acid fraction is increased, and may be also a mucoprotein. Three globulin fractions are obtained at pH 4.5, one of which can be identified with γ -globulin, and a second with β globulin. The third component must be made up of part of the α -globulin indicated by electrophoresis at pH 8.4. The quantitative relations between the components observed at pH 4.5 and pH 8.4 will be discussed, and possible applications to the study of pathological sera considered.

Metabolism of 2,4- and 3,5-diketo acids ALTON MEISTER (introduced by JESSE P. GREENSTEIN) *National Cancer Institute, Bethesda, Md*. The enzymatic hydrolysis of 2,4-diketo acids yielding pyruvic and fatty acids, which was previously reported, may be followed by an ultraviolet absorption technique. Such a procedure was employed in following the metabolism of 3,5-diketohexanoic acid and its δ -lactone. The lactone was enzymatically converted to free 3,5-diketohexanoic acid and the free acid was hydrolyzed to yield aceto-

acetic and acetic acids by homogenates of rat liver and kidney. Lactonase activity was associated with the sedimentable fraction of liver homogenate and had a pH optimum at about 6, while the activity toward 2,4- and 3,5-diketo acids remained in the supernatant after high speed centrifugation. The pH optimum for hydrolysis of 2,4- and 3,5-diketoheptanoic acids was 7.3 to 7.9. Hydrolysis of 2,4- and of 3,5-diketo acids are analogous in that both reactions involve splitting of a C-acyl group. 2,4-diketoheptanoic acid was hydrolyzed 11 to 14 times more rapidly than was 3,5-diketoheptanoic acid by liver and kidney of several species, by mouse hepatoma, and by partially purified enzyme preparations. Under conditions where n-hexanoic, β -ketoheptanoic, and 2,4-hexadienoic acids were oxidized rapidly by a sedimentable fraction of rat liver fortified as described by Lehninger, both 2,4- and 3,5-diketoheptanoic acids were metabolized at negligible rates. This suggests that these polyketo acids as such are not intermediates in the oxidation of n-hexanoic acid in this system, but are probably involved in other metabolic pathways.

Synthesis and microbiological testing of some alpha-aminosulfonic acids. JULIUS MENDEL and DONALD W. VISSER (introduced by HARRY J. DELEZ, JR.) *Dept. of Biochemistry and Nutrition, Univ. of Southern California School of Medicine, Los Angeles, Calif.* Several alpha-aminosulfonic acids have been prepared which competitively inhibit the naturally-occurring alpha-aminocarboxylic acids. McIlwain has reported considerable overlapping in the growth-inhibiting ability of the aliphatic alpha-aminocarboxylic acids. It was of interest, therefore, to synthesize an aliphatic aminosulfonic acid having no structural analog among the naturally-occurring amino acids. Alpha-aminopropanesulfonic acid and alpha-amino-beta-bromobutanesulfonic acid were synthesized by the addition of ammonium bisulfite to a slight excess of the appropriate aldehyde. The alpha-aminopropanesulfonic acid was antagonistic toward *Lactobacillus casei* at levels from 10^{-2} to 10^{-4} M. Slight reversal of antagonism was observed in the presence of alpha-aminobutyric acid, but no reversal of growth occurred with any of the other amino acids or mixtures of amino acids. Alpha-amino-beta-bromobutanesulfonic acid inhibited the growth of *Lactobacillus casei* completely at 10^{-4} M concentration.

Quantitation of the growth-retarding and anemia-producing effects of hexahomoserine. E. T. MERTZ, R. H. WALTZ, JR., D. C. SHELTON, L. P. DOYLE, and A. L. DELEZ (introduced by F. W. QUACKENBUSH) *Dept. of Agricultural Chemistry and Veterinary Science, Purdue University, Lafayette, Ind.* Young white rats were divided into groups of 6 and maintained for 28 days on a basal diet containing zein, tryptophan, histidine, and

2% DL-lysine HCl. Each group received, in addition to the basal diet, a different level of DL-hexahomoserine (α -amino, ϵ -hydroxy caproic acid). When the diet contained 0.08% to 0.33 DL-hexahomoserine, the growth rate was retarded without the simultaneous development of anemia. When the diet contained 1% DL-hexahomoserine, the growth rate was either retarded or reduced to zero, and the animals developed anemia. The rats were autopsied at the end of the experiment, and the autopsy findings will be discussed.

Electrometric method for the determination of red blood cell and plasma cholinesterase activity. HARRY O. MICHEL (introduced by WILLIAM H. SUMMERSON) *Biochemistry Section, Medical Div., Army Chemical Center, Md.* Red blood cell and plasma cholinesterase activity is determined in terms of the change in pH produced at 25° by the action of the enzyme on acetylcholine in a standard veronal-phosphate buffer, initially at pH 8.00. The pH change is measured with the glass electrode, and the reaction time is 1-1½ hours. Separate buffer solutions are used for red cells and plasma, the capacity of the buffers is such that relatively large variation in buffer capacity of added red cells or plasma do not affect results, and the decrease in enzymatic activity with decrease in pH is compensated for by an equivalent decrease in buffer capacity over the same range. Small correction factors are provided for non-enzymatic hydrolysis and for slight deviation in rate of pH change with pH. Only 1 ml. of a 1:50 dilution of plasma or hemolyzed red cells is required for a determination. The method compares favorably with the standard manometric procedure, and is much more suitable for routine use.

Metabolic conversion of the carbon chain of DL-lysine- ϵ -C¹⁴ to L-arginine in the dog. LEON L. MILLER *Dept. of Radiation Biology, Univ. of Rochester, School of Medicine and Dentistry, Rochester, N. Y.* DL-Lysine- ϵ -C¹⁴ (2.00 gm, 1.24×10^3 disintegrations/min) was fed mixed with meat protein to a normal dog. The dog was killed with perfusion 24 hours later. Tissue and blood proteins were hydrolyzed with 20% HCl, and the basic amino acids separated with the ion exchanger Amberlite IRC-50. L-Arginine was isolated as the monofluoride, after several reprecipitations from

	C ¹⁴ DISINTEGRATION/MINUTE/MILLIMOL $\times 10^{-4}$			
	Arginine mono- fluoride	Guanidi- nium moiety	Ornithine moiety	Glutamic acid
Liver	0.329	0.13	0.19	0.750
Plasma protein	0.323	0.15	0.13	0.60

solution containing added inert L-lysine. Failed to change its activity. The fluoride was used.

such and a portion decomposed with barium hydroxide. The arginine was converted to ornithine, carbon dioxide and ammonia by further treatment with baryta in a sealed tube. Ornithine was isolated as a diparatoluenesulfonyl derivative which was assayed as such. The ornithine moiety contained about 50% of the activity present in the arginine flavanate. The rest of the activity was found in the CO_2 of the guanidine moiety. The activity of glutamic acid isolated from the same tissues is included for comparison. Because the glutamic acid had only about 4 times the activity of the ornithine, it seems more likely that both were derived from a common precursor, rather than that ornithine is derived from glutamic acid through several intermediates postulated by others for such a conversion.

Metabolic conversion of the carbon chain of DL-Lysine- $\epsilon\text{-}^{14}\text{C}$ to L-glutamic and L-aspartic acids in the dog. LEON L. MILLER and WILLIAM F. BALE (by invitation). *Dept. of Radiation Biology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.* DL-Lysine- $\epsilon\text{-}^{14}\text{C}$ (2.00 gm with 1.24×10^8 disintegrations/min) was fed with meat protein to a normal dog. When killed 24 hours later, approximately $\frac{1}{3}$ of the activity had appeared in the expired CO_2 , $\frac{1}{3}$ in the urine and the rest incorporated in tissue and blood proteins. The proteins were hydrolyzed with 20% HCl, and the dicarboxylic amino acids separated with the ion exchanger Amberlite IR-4. Glutamic acid was isolated as the hydrochloride and aspartic acid as the copper salt. Paper partition chromatography was used routinely to control the separations and as an added criterion of purity of isolated amino acids. No evidence for the occurrence of α -amino-adipic acid was found. The most likely interpretation of the data must postulate more or less direct conversion of the carbon chain of lysine to that of glutamic acid by a hitherto unrecognized mechanism.

Podophyllotoxin on enzymes and tissue metabolism. ZELMA BAKER MILLER, CLARKE DAVISON (by invitation) and PAUL K. SMITH. *Dept. of Pharmacology, George Washington Univ. School of Medicine, Washington, D. C.* Podophyllotoxin, a carcinoclastic agent from podophyllin, was investigated against various metabolic systems. Oxygen uptake of rat kidney slices is progressively inhibited by 10^{-3}M podophyllotoxin (74% at 3 hours). Podophyllotoxin at 10^{-4}M inhibits to a less degree. The inhibition is greater in the absence of added substrate than in the presence of alanine, pyruvate, succinate and glutamate. Acetate oxidation is inhibited. The oxygen uptake of rat brain and liver slices, and of rat testes is only slightly reduced. The respiration of whole 5-day chick embryo is inhibited in the absence of, but not in the presence of, glucose. Oxygen uptake is stim-

ulated by podophyllotoxin below 10^{-3}M . Anaerobic glycolysis of 5-day chick embryo is inhibited 30-60% by 10^{-3}M podophyllotoxin, that of rat testes is inhibited 40-75%. Stimulation is sometimes observed at lower concentrations. Rat brain glycolysis is not affected. Carbohydrate synthesis from kidney slices and pyruvate is inhibited 40% by 10^{-3}M podophyllotoxin. Acetate and butyrate, but not glucose, oxidation by rabbit kidney homogenates is markedly inhibited by 10^{-3}M podophyllotoxin. Succinoylase (pigeon breast), choline and hypoxanthine oxidases (liver), choline esterase (brain), leucine oxidase (*Proteus vulgaris*), adenosine triphosphatase (liver), serum acid and alkaline phosphatase, ribonuclease (spleen), and thymonucleodepolymerase (dog serum) are not affected by this concentration. The viscosity of highly purified thymonucleic acid is not decreased by 30^{-3}M podophyllotoxin.

Prolamines treated with nitrogen trichloride. III. Sulfated zein. FERNANDA MISANI (by invitation), PHILIP WEISS (by invitation), M. G. CORNASCO (by invitation), T. W. FAIR (by invitation), and L. REINER. *Research Laboratories of Wallace & Tiernan Products, Inc., Belleville, N. J.* The NCl_3 -treated zein was sulfated according to the procedure of Reitz *et al.* (*J. A. C. S.* 68: 1024). The resulting product contained 1.9% sulfate sulfur and was soluble in water at neutral pH values. It possessed the typical fit producing toxicity of the parent compound. If zein was first sulfated and then treated with NCl_3 , a non-toxic product resulted. The NCl_3 consumption by sulfated zein was at least as great as that of the native zein. It appears that sulfation blocked in some manner the reactive group. Sulfamation is hardly responsible for the blocking effect since it has been shown previously that $-\text{NH}_2$ groups are not involved in the toxigenic reaction. Esterification of $-\text{OH}$ and $-\text{SH}$ groups, sulfonation and denaturation through dehydration might have occurred. One of the groups involved in these reactions must also be involved in the production of a toxic residue through reaction with NCl_3 .

Glycogen content of the liver of fasted alloxan diabetic rats. YOSHIKAZU MORITA (by invitation) and JAMES M. ORTEN. *Dept. of Physiological Chemistry, Wayne Univ. College of Medicine, Detroit, Mich.* In the course of investigations on the effect of inorganic and organic phosphate upon the glucose utilization of alloxan diabetic rats, it became necessary to determine the liver glycogen levels in fasting diabetic animals. A survey of the literature showed little information on this question. Male Sprague-Dawley rats, 150-250 gm in weight were rendered diabetic by injecting alloxan. Liver glycogen and blood sugar levels were determined on 24-hour and 48-hour fasted animals, and the results compared with values found

in normal controls Twenty rats were used in each group In the 24-hour fasted animals, the diabetic group averaged $2.15 \text{ gm} \pm 0.277$ (the standard error of the mean) per 100 gm of liver, while the normals averaged $0.324 \pm 0.053 \text{ gm} \%$, a highly significant difference In the 48-hour fasted group, the diabetic animals showed $0.679 \pm 0.090 \text{ gm} \%$, also a highly significant difference The fasting blood sugar for the 24-hour fasted group of diabetic animals averaged $153 \pm 22 \text{ mg} \%$, for the 48-hour fasted animals, $90 \pm 7 \text{ mg} \%$ The blood sugar values for the control group of rats were within the normal range ($55\text{--}90 \text{ mg} \%$) Studies on the effect of inorganic phosphate and hexose phosphate on the glycogen content of the liver in the alloxan diabetic rat are now in progress

Position of the oxygen dissociation curve in cyanotic congenital heart disease MINERVA MORSE, DONALD E CASSELS (by invitation) and MELBA HOLDER (by invitation) *Dept of Pediatrics, Univ of Chicago, Chicago, Ill* In spite of decreased arterial oxygen saturation, the arterial oxygen content in cyanotic congenital heart disease is usually as high or higher than normal because of a compensating polycythemia It is possible, however, that the tissues suffer anoxia due to the low oxygen tension associated with lowered saturation The position of the oxygen dissociation curve has been examined to determine whether the affinity of hemoglobin for oxygen has been altered by compensatory processes in such individuals Data were derived from Van Slyke analyses of blood samples equilibrated at 37°C with gas mixtures containing approximately 5% CO_2 and different tensions of O_2 Data were corrected to a pH of 7.1 As a control corresponding data were determined for normal individuals The position of the O_2 dissociation curve for healthy young adult males as determined by us was found to differ somewhat from reports in the literature Furthermore the curve for normal children lay to the right of that for adult males Positions of the curve for patients with cyanotic congenital heart disease, compared with those of normal individuals of approximately the same age, indicate a trend toward the right, i e, toward decreased affinity of hemoglobin for oxygen This is more marked in adults than in children, and is especially marked in cases complicated by pulmonary stenosis This is interpreted as an adaptation to increase the oxygen tension of the tissues

Hemostatic effects of normal blood transfusions in hemophilia-like swine M E MUHRER (introduced by A G HOGAN) *Depts of Agricultural Chemistry and Animal Husbandry, University of Missouri, Columbia, Mo* Evidence has been accumulated to show that the hemophilia-like swine at the Missouri Experiment Station have a blood vessel defect in addition to the previously ob-

served coagulation defect A number of treatments have been effective in reducing the coagulation time to near the normal level but these treatments have not been effective in reducing the prolonged saline bleeding time The injection of hemolytic agents or hemolyzed cells will sharply lower the coagulation time but has no effect upon the saline bleeding time An intravenous injection of 5–50 ml of normal blood in a 50-pound bleeder pig lowers the coagulation time but the bleeding time of over 600 seconds is not affected However, if the amount of normal swine blood transfused into a 50-pound bleeder animal is increased to 500 ml both the coagulation time and saline bleeding time are lowered Ten animals ranging from 13 pounds–140 pounds in weight were given transfusions ranging from 175 ml–1000 ml All animals had a saline bleeding time of more than 600 seconds before transfusion and an average of 195 seconds after transfusion It appears that there are two blood factors involved in the hemostatic defect in hemophilia-like swine, one that affects coagulation time and another that affects bleeding time If there is a capillary defect, as is usually postulated with prolonged bleeding times, then the capillaries may be affected by the blood that is flowing through them and failure of the capillaries to constrict could be caused by a blood defect

Relation of the electrolyte compositions of serum and skeletal muscle EDWARD MUNTWYLER, GRACE E GRIFFIN (by invitation), GEORGE S SAMUELSEN (by invitation) and LOIS E GRIFFITH (by invitation) *Dept of Biochemistry, Long Island College of Medicine, Brooklyn, N Y* The electrolyte content of the serum and skeletal muscle was determined in rats and dogs maintained on diets deficient in potassium, and following repeated injections of desoxycorticosterone acetate In rats, the loss of skeletal muscle potassium and gain of intracellular sodium was found to be accompanied by an increased serum bicarbonate and a lowered serum chloride concentration These observations are in agreement with those reported recently by Darrow, *et al* (*J Clin Invest* 27:198, 1948) In the present experiments the change in the composition of the muscle with respect to potassium and sodium was generally found to be less pronounced in dogs than in rats Dogs with a deficit of muscle potassium and gain of intracellular sodium failed to exhibit the large increase in the serum bicarbonate concentration observed in rats Similarly, there was a smaller decrease in the serum chloride concentration However, in certain experiments with dogs the serum bicarbonate concentration tended to be increased In these instances the serum chloride concentration appeared to show a relatively greater fall

New colorimetric reaction for the determination of epinephrine SAMUEL NATELSON and JOSEPH

B PINCUS (introduced by ALBERT E SOBEL) *Dept of Chemistry and Pediatrics, Jewish Hospital, Brooklyn 16, N Y* 100 μ g of epinephrine heated in a boiling water bath for twenty minutes with 3 ml of a 2% selenic acid solution in 1 N sulfuric acid will reduce the selenic acid to produce a pale blue color turning to yellow and then orange. An orange precipitate forms on standing. When this solution is cooled to room temperature and acid arsenomolybdate is added the molybdate blue develops in a few minutes. Acid Phosphotungstate and acid Phosphomolybdate will also produce blues but of lesser intensity. Glucose, Tryptophane, Glycine, Methionine and Tyrosine do not interfere in this procedure. Catechol gives the same reaction. The color read at 730 $m\mu$ in the Beckman Spectrophotometer yielded an $E_{1\%}^{1cm}$ of approximately 2000 at 730 $m\mu$ based on the amount of epinephrine present. If the color is developed in 1 ml and the micro cuvettes with a 1 cm light path are used the procedure is capable of determining 0.2 μ g of epinephrine.

Concentration of bound pantothenic acid J B NEILANDS (by invitation), HARVEY HIGGINS (by invitation), and F M STRONG *Dept of Biochemistry, College of Agriculture, Univ of Wisconsin, Madison, Wis*. The development of Lipmann's enzymatic method for liberation of pantothenic acid from coenzyme A preparations (*J Biol Chem*, 167: 869, 1947) offered a convenient guide for attempts to purify the bound (*i.e.* microbiologically unavailable) form of pantothenic acid present in certain fresh biological materials. Preliminary work with hog liver and fresh brewer's yeast involving hot water extraction, precipitation with 70% alcohol, and fractional precipitation with heavy metal salts led to preparations containing only about 0.1% pantothenic acid in the bound form. Better results were obtained when livers from freshly killed rabbits were homogenized with 0.9% KCl, and the easily sedimentable fraction ('cyclophorase', Green *et al*, *J Biol Chem*, 172: 389, 1948) was used as starting material. When this material was deproteinized with trichloroacetic acid, and precipitated with barium and alcohol, the precipitate contained 0.2-0.4% of bound pantothenic acid. Counter-current fractionation (Craig) of such concentrates with phenol and aqueous buffer solutions raised the purity to about 2%. This represents approximately a 150-200-fold concentration as compared to the original liver. Such preparations contain no detectable free pantothenic acid.

Effect of pH and temperature on carboxypeptidase activity HANS NEURATH and ELAINE ELKINS-KAUFMAN (by invitation) *Dept of Biochemistry, Duke Univ School of Medicine, Durham, N C*. Kinetic measurements of the hydrolysis of carbobenzoxyglycyl-L-phenylalanine by carbo-

xyptidase were carried out at pH 6.5, 7.5, 8.3 and 9.0, and at temperatures ranging from 9° to 32°. Hydrolysis rates were evaluated according to the two step reaction mechanism and were expressed in terms of K_m , the enzyme-substrate dissociation constant, and k' , the specific rate constant for the activation process (Elkins-Kaufman and Neurath, *J Biol Chem* 175: 893, 1948). K_m was minimal at pH 7.5 and increased about 5 fold between pH 7.5 and 6.5, and about 2 fold between pH 7.5 and 9.0. k' was constant between pH 7.5 and 8.3, with a sharp increase below and a moderate decrease above this range. The calculated maximum proteolytic coefficient was highest at pH 7.5. Measurements of the temperature effects at each pH were interpreted in terms of the classical thermodynamic constants, ΔH , ΔS and ΔF , for the equilibrium reaction (step 1) and in terms of the corresponding constants for the activation process (step 2), as previously described for chymotrypsin (Kaufman, Neurath and Schwert *J Biol Chem* 177, in press). ΔH decreased markedly with increasing pH. However, a compensatory decrease in ΔS , the entropy of the equilibrium reaction, rendered ΔF , the free energy change, nearly independent of pH (about 1,600 cal/mole). Similar relations were found for the corresponding thermodynamic constants of the activation process (ΔF^* about 15,000 cal/mole). The significance of these data is discussed.

Histochemical demonstration of three groups of alkaline (pH 9.2) phosphatases using various substrates and inhibitors WILLIAM NEWMAN (by invitation), IRWIN FLIGIN (by invitation), ABNER WOLF (by invitation), and ELVIN A. KABAT *Veteran's Hospital, Kingsbridge, Departments of Pathology and Neurology, Columbia University and the Neurological Institute, New York City*. The Gomori histochemical technique for localizing alkaline phosphatases was studied using glycerophosphate, glucose-1-phosphate, creatine phosphate, yeast nucleic and adenylic acids, thiaminepyrophosphate hexosediphosphate, muscle adenylic acid and adenosinetriphosphate as substrates and with various inhibitors. Three distinct groups of alkaline (pH 9.2) phosphatases were recognized. **Group I** enzymes were seen with all substrates in cytoplasm and nuclei of many organs, notably, intestine, renal tubules and adrenal cortex. Enzyme action is inhibited by heat, M/4 glycine or arginine and M/100 KCN, and corresponds largely with previous histochemical findings. Optimal **Group I** staining appeared with all substrates except thiaminepyrophosphate and yeast nucleic acid. **Group II** enzymes are demonstrated in cytoplasm of cells lacking **Group I** with muscle adenylic acid and adenosinetriphosphate, but not with yeast adenylic acid or other substrates, suggesting specificity for the purine-ribose-5-phosphate linkage. **Group**

II enzymes occur characteristically in cytoplasm and nuclei of smooth muscle and some connective tissues *Group III* enzymes occur in nuclei Muscle and yeast adenylic acids, creatine and glycerophosphates are more rapidly split than the other substrates, these differences in rates of hydrolysis permit recognition of *Group III* *Group II* and *III* enzymes are less affected by heat, glycine, arginine and KCN than are *Group I* facilitating differentiation Dialysates of autolyzed rat intestine and guinea pig kidney and adrenal enhance staining even with optimal Mg^{++} and inhibitory glycine concentrations

Effect of folic acid, liver extract and vitamin B_{12} on hemoglobin regeneration in chicks C A NICHOL (by invitation), A E HARPER (by invitation), L S DIETRICH (by invitation) and C A ELVEHJEM *Dept of Biochemistry, College of Agriculture, Univ of Wisconsin, Madison, Wis* No change in hemoglobin level accompanied the growth response of chicks to injection of anti-pernicious anemia liver extracts or vitamin B_{12} when a corn-soybean basal ration was fed However, chicks fed a folic acid deficient purified ration for 21 days developed a slight anemia If a single injection of phenylhydrazine hydrochloride (2 mg/100 gm body wt) was given at this time a severe anemia developed within two days Experimental treatments were then begun and hemoglobin levels were determined at 2- to 4-day intervals Injection of liver extract or vitamin B_{12} alone did not cause any significant hemoglobin response Folic acid alone produced a definite increase and pteroyl triglutamate and pteroyl heptaglutamate were equally as effective at equimolar dosage The combined administration of folic acid and liver extract or folic acid and vitamin B_{12} induced a more rapid regeneration of hemoglobin than treatment with folic acid alone The duration of the depletion period influenced this response After 14 days, treatment with folic acid and liver extract did not differ from treatment with folic acid alone After 21 or more days depletion, treatment with folic acid and liver extract consistently caused more rapid regeneration of hemoglobin than treatment with folic acid alone Chicks continued on a folic acid-deficient diet or on a diet containing vitamin B_{12} and low levels of folic acid developed a characteristic paralysis The response of these chicks to treatment with folic acid and related compounds will be reported

Protein and amino acid requirements of the German cockroach J L NOLAN (by invitation) and C A BACMAN *Dept of Biochemistry, College of Agriculture, Univ of Wisconsin, Madison, Wis* Cockroaches were fed semi synthetic diets containing various amounts and kinds of protein, and the rates of growth and maturation were determined The quantitative requirement for casein

was met at a level of 30% casein plus 30% bulk, or by 45% casein in the absence of bulk, indicating a relatively high requirement for dietary protein When various proteins were fed at 15% of the diet, fibrin was superior to casein, followed by lactalbumin, egg albumin, zein, gelatin, oxidized casein and amino acid mixtures, in decreasing order of effectiveness The 'incomplete' proteins zein, gelatin, and oxidized casein supported only slow growth, although some roaches fed these proteins reached maturity after a 30-fold increase in body weight Analyses of cockroaches which had been reared to maturity on diets containing oxidized casein revealed that normal amounts of tryptophane and methionine had nevertheless been formed, indicating the synthesis of these two 'essential' amino acids by the insect or by its associated microorganisms It was concluded from these experiments that the protein and amino acid requirements of the cockroach must be quantitatively and qualitatively different from the corresponding requirements of the rat and chick

Pteridines on hemapoiesis and cell proliferation EARL R NORRIS and JOHN J MAJNARICH (by invitation) *Dept of Biochemistry, Univ of Washington, Seattle, Wash* Using the technique previously described (*Am J Physiol* 152 179, 153 133, 1948), the effect of vitamin B_{12} , xanthopterin, folic acid and products of the action of enzymes, upon xanthopterin and folic acid, upon blood regeneration in rats made anemic on a purified diet containing 1% sulfathiazole, was measured The results obtained with xanthopterin and folic acid checked the results previously obtained Vitamin B_{12} , and the products of the action of enzymes upon xanthopterin and folic acid were much more effective in alleviating the anemia and leukopenia in the rats than were xanthopterin or folic acid The results obtained *in vivo* substantiated the results reported previously for cell culture *in vitro*, where vitamin B_{12} , and the products of the action of enzymes on xanthopterin and folic acid were shown to be more effective than xanthopterin or folic acid The effect of certain minerals upon the anemia will be discussed

Phosphatase activity in homogenates of liver and other organs of the rat ALEX B NOVIKOFF (introduced by HAROLD B PIERCE) *Dept of Pathology, Univ of Vermont College of Medicine, Burlington, Vt* Freshly prepared rat liver homogenates were assayed for phosphatase activity with a variety of substrates in a pH range of 4.0 to 9.1 The activity curves obtained fall clearly into 2 classes *Class I* includes yeast adenylic acid ($A-3P$) and 2 esters widely used in phosphatase determinations, sodium beta glycerophosphate, and disodium phenyl phosphate (ϕP) None of these is present in animal cells Activity is optimum at pH 5.5 low at pH 7.4 and very low at pH 9.1 *Class II*

includes esters of physiological significance adenosine triphosphate (ATP), muscle adenylic acid (A-5-P), glucose-6-phosphate (G-6-P), fructose 1,6 diphosphate (HDP), and glucose-1-phosphate (G-1-P). There is little activity at pH 5.0. At pH 9.1, there is high activity with ATP, A-5-P, and HDP, and low activity with G-6-P and G-1-P. Most significant is the very high activity obtained with all 5 of these esters at pH 7.4, which is presumably close to physiological. When tested with 2 esters from Class I (ϕ -P and A-3-P) and 2 from Class II (A-5-P and ATP), homogenates of spleen and kidney give essentially the same type of activity curves as liver does, with only the Class II esters being actively split at pH 7.4. However, kidney is highly active with all 4 substrates at pH 9.1. The pH activity curves of intestinal mucosa homogenates are strikingly different from those of the other organs tested. All 4 esters yield similar curves, and irrespective of whether they belong to Class I or II they are actively split at pH 7.4.

Enzymatic synthesis of l-malic acid by CO_2 fixation SEVERO OCHOA, J. B. VEIGA SALLES (by invitation), and PRISCILLA J. ORTIZ (by invitation) *Dept. of Pharmacology, New York University College of Medicine, New York City*. As previously reported (Ochoa, Mehler and Kornberg *J. Biol. Chem.*, 174: 979, 1948) the equilibrium of the reaction $\text{l-malate} + \text{TPN}_{\text{red}} \rightleftharpoons \text{pyruvate} + \text{CO}_2 + \text{TPN}_{\text{ox}}$, catalyzed by an enzyme isolated from pigeon liver, can be shifted toward CO_2 fixation by a dismutation with the glucose-6-phosphate dehydrogenase system. The overall reaction $\text{glucose-6-phosphate} + \text{pyruvate} + \text{CO}_2 = \text{6-phosphogluconate} + \text{l-malate}$ thus obtained, has been further studied with highly purified preparations of the pigeon liver enzyme. As with the tricarboxylic system (Ochoa, *J. Biol. Chem.*, 174: 133, 1948) the reaction can be followed manometrically through the liberation of CO_2 from bicarbonate by the phosphogluconic acid formed, since the disappearance of CO_2 by fixation is balanced by the formation of a carboxyl group of malic acid. l-Malate was determined with the enzyme system from *L. arabinosus* which specifically and quantitatively converts it to lactate and CO_2 (Korkes, and Ochoa, *J. Biol. Chem.*, 176: 463, 1948). The isolation and identification of l-malic acid will be described. The disappearance of glucose-6-phosphate and pyruvate was determined enzymatically with 'Zwischenferment' and lactic dehydrogenase respectively. The rate of malate synthesis is proportional to the CO_2 tension. By combination of the isocitric and malic enzyme systems one obtains the reversible reaction $\text{d-isocitrate} + \text{pyruvate} + \text{CO}_2 \rightleftharpoons \alpha\text{-ketoglutarate} + \text{CO}_2 + \text{l-malate}$. The equilibrium position of this reaction favors the formation of malate.

Extraction and estimation of desoxypentose

nucleic acid (DNA) and pentose nucleic acid (PNA) from plant tissues MAURICE OGUR and GLORIA ROSEN (introduced by H. G. ALBAUM) *Botanical Laboratories, Univ. of Pennsylvania, Philadelphia, Penna.* Pentosans and polyuronides in plant tissues invalidate the estimation of PNA by pentose assay. The use of the ultraviolet absorption at 260 m μ , for the estimation of the nucleic acids extracted from tissues, has been studied in this laboratory. Perchloric acid, which does not absorb significantly in the ultraviolet, is used to extract the nucleic acid constituents from the tissue residue. Our data indicate that prolonged contact in the cold with perchloric acid extracts PNA but not DNA from the plant tissue residue. Perchloric acid at elevated temperatures extracts both PNA and DNA. Root tip segments of rye and corn were homogenized in ethanol, centrifuged, and the tissue residue extracted with a mixture of alcohol and ether, and then with cold, dilute perchloric acid. Two alternate procedures were followed after these preliminary extractions: 1) The tissue residue was treated with hot perchloric acid. DNA was determined in the extract by the diphenylamine reaction and total nucleic acid by the ultraviolet absorption at 260 m μ . RNA was estimated by difference. 2) The tissue residue was treated with perchloric acid in the cold and RNA determined in the cold extract. The residue was now treated with hot perchloric acid and the DNA determined. All fractions were examined by photocolometric estimation of: 1) total and inorganic phosphorus 2) total nitrogen 3) pentose 4) desoxypentose 5) ultra-violet absorption at 260 m μ .

Effect of biotin and choline intakes on liver storage of ingested cholesterol RUTH OKEY, ELEANOR RISSER VERNON (by invitation), S. LEFKOVSKI and R. PENCHARZ (by invitation) *Dept. of Home Economics and the Division of Poultry Research, Univ. of California, Berkeley, Calif.* To test the effect on liver cholesterol storage of varying choline and biotin intakes 250 young rats were given biotin deficient diets with and without 1% cholesterol. Protein content of the diets (about 20%) total lipid, salts and vitamin supplements other than choline, biotin, and for one group, inositol, were kept constant and adequate for growth. One diet contained egg white and another whole egg. One third of each group received no biotin, one a borderline supplement and the last an ample amount. Subgroups of the egg white series were given choline in amounts furnishing respectively 0.10 mg, 0.25 mg, 0.50 mg and 1.0 mg/gm of diet. The whole egg diet furnished about 7 mg choline/gr diet. Rats were killed after 7 to 9 weeks on diet. None of them showed kidney damage. In the egg white-fed animals given ample biotin or placed on diet after biotin storage (at 80-90 gm) decreases in liver cholesterol with the increases in

choline intake noted above were slight and inconsistent. Egg white fed rats placed on diet at 45 gm and allowed to develop borderline biotin deficiency stored maximum liver cholesterol (about 1.6%) with 0.5 mg choline and 0.5 mg inositol/gm diet. One group of rats given 0.1 mg choline/gm diet stored less than half this amount. Without added biotin liver cholesterol of rats fed whole egg were as low as 0.3%, with ample biotin supplements they reached 1.7% in the same time.

Acetate metabolism and ergosterol synthesis in *Neurospora* R. C. OTTKE (by invitation), E. L. TATUM and S. SIMMONDS, *Dept. of Microbiology, Yale Univ., New Haven, Conn., and Dept. of Biology, Stanford Univ., Calif.* Ergosterol has been identified as the principal sterol of *Neurospora*. The incorporation of deuterioacetate into mold mycelium, fat, and sterol has been followed using the wild-type strain and a mutant strain (Y-2492), which requires acetate for growth. Since considerably more deuterium is incorporated in the mutant than in wild-type grown under similar conditions, the results support the conclusions obtained from growth experiments (Tatum and Garnjobst, unpublished) and indicate that the mutant, in contrast to wild-type, is unable to form acetate from sugar. The results also show that acetate is incorporated in increasing proportions in *Neurospora* mycelium (about 3%), fat (about 10%), and in ergosterol (about 20%). Thus acetate metabolism in the mold appears to resemble closely that in the rat as shown by Bloch and Rittenberg. In an attempt to determine the location of the incorporated deuterium in the sterol molecule, infra-red absorption spectra have been obtained for the deuterated ergosterol. The spectra of ergosterol isolated from *Neurospora* grown on deuterioacetate and on normal acetate in heavy water show distinct and different absorption peaks, the former sample at 2225 cm^{-1} and the latter at 2190 cm^{-1} . Neither peak is shown by normal ergosterol. Although there is some uncertainty in the interpretation of the infra-red spectra, the location of these absorption peaks suggests that deuterium given as acetate may appear on the more unsaturated carbon atoms in ergosterol and that given as heavy water may appear at more saturated centers, i.e. methyl and methylene carbons.

Electrophoretic studies of the proteins of plasma and ascitic fluid in cirrhosis W. D. PAUL (by invitation), J. D. GORDON (by invitation), ROBERT L. DRYER (by invitation) and JOSEPH I. ROUTH, *Depts. of Medicine and Biochemistry, College of Medicine, State Univ. of Iowa, Iowa City, Iowa*. Marked deviation from the normal plasma protein distribution is an outstanding feature of cirrhosis of the liver. The lowered total protein and inverted A/G ratio is often accompanied by formation of protein-rich ascites. We have determined by

electrophoresis the protein components of simultaneously collected samples of plasma and ascitic fluid taken from several patients over intervals of time ranging from 7 weeks to 6 months. The results show a close qualitative similarity between any plasma sample and the corresponding ascites sample, which is perhaps produced by the dynamic equilibria between the blood and the transudate. Certain quantitative differences are also evident. In every case the ascitic fluid is richer than the plasma in albumin, α_1 -globulin and γ -globulin, while the plasma was richer than the fluid in α_2 -globulin, β -globulin, and fibrinogen. The best available estimates of the molecular weights of the various protein fractions show they increase in the order albumin, γ -globulin, α_1 -globulin, α_2 -globulin, fibrinogen, and β -globulin. These values indicate that possibly the selective protein enrichment of ascitic fluid may be directly related to the molecular weight of the protein components, and that in cirrhosis altered vascular permeability may be as important as altered protein synthesis.

Metabolism of alpha-estradiol in vitro W. H. PEARLMAN and R. H. DEMEIO (by invitation), *Dept. of Biochemistry, Jefferson Medical College, Philadelphia, Penna.* Alpha-estradiol-17-hemisuccinate as dissolved in a Krebs-phosphate medium buffered at pH 7.4 and incubated with rat liver slices at 37° for 4 hours. The ratio of hormone to liver tissue was approximately 1 to 500 parts by weight, in all, 845 mg of alpha-estradiol (as succinate) were used. On extraction and fractionation of the incubation mixture, 54 mg of crude crystalline estrone and 329 mg of crude crystalline alpha-estradiol were obtained. These crystalline products were purified, estrone was further identified by C,H analysis of the acetyl derivative. An unsuccessful search was made for beta-estradiol. Although an appreciable amount of strongly acidic phenols were obtained, this material yielded no crystalline fractions on chromatography. Most of the estradiol succinate had undergone hydrolysis during incubation and very little if any of the hormone had been conjugated (e.g. as sulfate or glucuronide). Evidently alpha-estradiol can be converted to estrone *in vitro*, a process which is also known to occur *in vivo*. Estrone is very likely not the sole metabolite *in vitro* inasmuch as only about half of the estrogenic substance incubated could be recovered. This view is supported by earlier experiments (DeMeio *et al.*) wherein the biological inactivation of hormone was practically complete—the ratio of estradiol to liver tissue was 1 to 5,000 parts by weight.

Electrophoretic and chemical analyses of plasma and its fractions in health and disease H. ROWLAND PEARSON (by invitation) and ALICE CHANUTIN, *Biochemical Institute, Univ. of California, Berkeley, California*. Plasma is a complex

fractions obtained by *Method 6* of Cohn *et al* were analyzed for their electrophoretic distribution and protein nitrogen, total lipid carbon and cholesterol contents. Data are presented for normal individuals and for patients with diseases involving the kidneys, liver, and lungs. General trends are observed without the development of any characteristic pattern for these conditions. The plasma and fractions of multiple myeloma cases present specific electrophoretic patterns. The variations in the electrophoretic and chemical analyses are non-specific and do not yield sufficient information for diagnosis.

Comparative studies in niacin metabolism W A PERLZWEIG, FRED ROSEN (by invitation), I G LEDER (by invitation), S HUNTER (by invitation) and P B PEARSON *Dept of Biochemistry, Duke Univ, Durham, N C, and A and M College of Texas, College Station, Texas*. Data on the urinary excretion of the major known metabolites of niacin in various species after saturation with nicotinamide will be presented. In man approximately 90% of 7 mg/kg/day of nicotinamide was recovered in equal amounts of N¹-methyl-nicotinamide (NMN) and the 6-pyridone of NMN. The major end-product in the dog is NMN which accounts for 90% of 10–20 mg/kg/day. In the rat 50% of 100–200 mg/kg/day was recovered as NMN, 15% as free niacin, and only 3% as the 6-pyridone, leaving 30% unaccounted for. The excretion of NMN in the rabbit (100 mg/kg/day), guinea pig (200 mg/kg/day), goat (80 mg/kg/day), calf (50 mg/kg/day), sheep (50 mg/kg/day), and horse (25 mg/kg/day) is less than 1% of the dose. The rabbit and guinea pig excreted 3% as the 6-pyridone, whereas the goat, calf and sheep excreted less than 1% of this metabolite. After the ingestion of NMN approximately equal amounts of NMN and the 6-pyridone were found in the urine of man and the rabbit. However, in both species the sum of these metabolites accounts for only 50% of the dose. After ingestion of NMN, the rat excretes 50% unchanged and 3% 6-pyridone, leaving 47% unaccounted for. The recovery in the urine of a single oral dose of the 6-pyridone is approximately 70% (of 50 mg) in man, 85% (of 10 mg) in the rat, 90% (of 25 mg) in the rabbit and 40% (of 25 mg) in the guinea pig. These data establish the 6-pyridone as a major metabolite of excretion for man but not for the other species studied. The rabbit, guinea pig and the polygastric herbivora can metabolize large amounts of niacin to unknown end products.

Incorporation of radioactive amino acids into the protein of acellular liver homogenate fractions ELBERT A PETERSON (by invitation), THEODORE WINNICK, and DAVID M GREENBERG *Division of Biochemistry, Univ of California Medical School, Berkeley, Calif*. A homogenate is

employed which consists of equal parts of 1) cytoplasmic granules obtained by fractional centrifugation, and 2) supernatant from a concentrated (1) homogenate centrifuged at 2600 X g. Component (2) is completely inactive in the absence of (1). Component (1) serves as the locus for the amino acid incorporation process. However, radioactive protein appears in higher concentration in (2) during incubations with C¹⁴-labeled amino acids. Accordingly, the mixture of (1) and (2) gives best results. Component (1) is inactivated by freezing, (2) is not. 0.33 ml of the mixture, containing 0.020 gm protein, is incubated with either 0.4 μ M of labeled glycine or 0.8 μ M labeled DL-alanine at 37° in horizontally-rotated centrifuge tubes, flushed with oxygen. The incorporation process is stopped by the addition of trichloroacetic acid. The precipitated protein is centrifuged, washed repeatedly with trichloroacetic acid, and freed of lipid by extraction with hot alcohol-ether. The lipid contains bound radioactive serine, when labeled glycine is used in the incubations. After 1 hour, approximately 0.75 μ M of glycine or 0.35 μ M of alanine are incorporated per gm protein. These values represent uptakes of 3.8% of the glycine and 1.8% of the L-alanine employed in the experiments.

Some factors which influence the synthesis of peptide bonds as catalyzed by papain CORNELIUS W PETTINGA (by invitation) and SIDNEY W FOX *Chemical Laboratory, Iowa State College, Ames, Iowa*. The effects of various experimental conditions upon peptide bond synthesis as catalyzed by papain have been studied. The use of citrate buffer concentrations higher than had previously been reported, was especially effective in increasing the amount of benzoylamino acid anilide formed from the acid and aniline. The pH-yield curves for the reactions were similar in form for both 0.1M and 1.0M buffer concentrations with the optimum pH being near 5.0, pH-activity curves of similar shape have previously been obtained by others for other substrates. With the amounts of substrates, enzyme, and activator held constant, the yield of anilide decreased as the volume of buffer used was increased, for those acylamino acids studied. When benzoylamino acids of different solubility behavior were used, viz, benzoyl-DL-phenylalanine and benzoyl-DL-alanine, the effects of varied experimental conditions were nevertheless qualitatively similar. Six days' incubation gave only slightly greater yields than did 3 days' incubation, commercial papain catalyzed almost as well as a hydrogen sulfide-treated preparation, greater differences resulted when a more concentrated buffer was used, and from use of a higher ratio of aniline to benzoylamino acid. In the case of *N*-benzoyl-phenylalanine another important factor was the type of side-chain substitution. Related behavior

of substrates containing the same amino acid residues has been studied

Oxalacetate decarboxylase of *Azotobacter vinlandii* G W E PLATT (by invitation) and HENRY A LARDY Dept of Biochemistry, Univ of Wisconsin, Madison, Wis The enzyme oxalacetate decarboxylase was isolated from acetone dried cells of *Azotobacter vinlandii* by precipitation of inert proteins at pH 4.8, adsorption on copper hydroxide gel, elution with 0.1M pH 6.8 phosphate buffer and fractionation with ammonium sulfate. An increase in activity of 10-80 fold over that of the original aqueous extract of bacterial acetone powder was achieved. The purified decarboxylase was activated by Mn^{++} , Co^{++} , Zn^{++} and slightly by Mg^{++} . Pyrophosphate, ATP, and orthophosphate acted as inhibitors in the presence of Mn^{++} . When Co^{++} was the activator orthophosphate was not inhibitory. The inhibition by orthophosphate in the presence of Mn^{++} could be competitively reversed by Co^{++} indicating the existence of an inactive phosphate manganese enzyme complex. Co^{++} seems to compete with the phosphate manganese radicle for the same point of attachment on the enzyme. Malate inhibited when either Mn or Co^{++} were used as activators. No fixation of $C^{14}O_2$ into oxalacetate could be demonstrated in the presence of the purified enzyme. Neither was the incorporation of radioactive carbon dioxide stimulated by ATP, fumarate, orthophosphate, or pyruvate either singly or in combination. The lack of fixation indicates that this enzyme merely catalyzes the equilibrium reaction: oxalacetic acid \rightleftharpoons pyruvic acid + CO_2 ($K = 1.9 \times 10^3$) and is free of coupling enzyme systems. The biotin activities for *L. arabinosus* of enzyme fractions in various stages of purity were found to decrease with increasing purity.

Biosynthesis of carotenes in tomatoes JOHN W PORTER and RALPH E LINCOLN (introduced by F W QUACKENBUSH) Depts of Agricultural Chemistry and Botany and Plant Pathology, Purdue Univ, Lafayette, Ind. Several carotenes and 3 colorless polyenes have been found in small quantities in fruit of *Lycopersicon* species and strains. By breeding, it has been possible to obtain selections containing an increased content of 1 or more of these compounds. Chemical analysis of the more prominent colorless polyenes and carotenes has shown the structural similarity between these compounds and the well known carotenes. It is suggested that these compounds are intermediate steps in the synthesis of lycopene and *beta*-carotene. None of these compounds are found in quantities exceeding the quantity of lycopene in commercial tomatoes. Genetic studies have shown that a single gene difference exists between tomatoes containing lycopene and those yellow tomatoes containing little or no lycopene and only 2-3 μg of

carotene per gm of fruit. A single gene difference also exists between tomatoes containing lycopene and those containing predominantly *zeta* carotene and prolycopene. Another single gene difference exists between tomatoes containing lycopene and those containing *beta* carotene. From the chemical and genetic studies, it is postulated that the synthesis of carotenes in tomatoes proceeds from simple compounds through *zeta* carotene to lycopene and then to *beta* carotene. The chemical steps for the postulated scheme involve dehydrogenation to form lycopene, followed by ring formation to yield *beta* carotene. The scheme probably does not apply to carotene formation in green leaves.

Oxidative phosphorylation without deamination in mitochondrial preparations VAN R POTTER and W C SCHMIDT (by invitation) McArdle Memorial Laboratory, Medical School, Univ of Wisconsin, Madison, Wis. In isotonic homogenates phosphorylation could be coupled with the oxidation of mixtures of pyruvate and oxalacetate or fumarate via the Krebs cycle provided the reaction mixture was fortified with magnesium ions, inorganic phosphate and adenosine triphosphate (ATP) or muscle adenylic acid (AMP). During the period of active inorganic phosphate uptake, the rate of NH_3 liberation was very slow, but when the inorganic phosphate concentration began to rise following a decrease in either substrate or enzyme activity, the output of NH_3 began to accelerate and continued until the NH_3 formed was equal to the amount of ATP added. In these experiments the amount of whole homogenate per flask was low, usually 50 mg wet weight of tissue per flask. When homogenates in isotonic sucrose were fractionated by centrifugal means, the mitochondria from liver and kidney possessed considerably less than one-half of the oxidative activity of the whole homogenates, but if amounts of mitochondria were chosen so as to give an oxidative rate equivalent to what was obtained with the whole homogenate, the phosphate balance was maintained much longer with the mitochondria than with the whole homogenate and when phosphate output began, it was not followed by NH_3 output. The results conform with the view that ATP must be dephosphorylated before it can be deaminated. The presence of the deaminase appears to constitute an irreversible leak from the ATP-AMP system, and its separation from the mitochondria by centrifugal means facilitates the study of certain aspects of oxidative phosphorylation.

Water and electrolyte losses from skin of infants EDWARD L PRATT (by invitation), ROBERT E COOKE (by invitation) and DANIEL C DARROW Dept of Pediatrics, Yale Univ School of Medicine, New Haven, Conn. By balance techniques the water loss from the lungs and skin at 80°F was about 48 gm/kg of body weight per day. At 90°F it was

106 gm At 80° skin washings contained about 1 mm of chloride, 0.9 mm of sodium, and 1 mm of potassium per day At 90° skin washings contained about 5 mm of chloride, 4 mm of sodium, and 6 mm of potassium per day Calcium and phosphorus losses were negligible The concentrations of electrolyte in sweat were calculated using 2 assumptions First, all water losses from the skin at 90° were considered due to active sweating with no loss by diffusion Therefore, the volume of sweat equaled the total water loss from the lungs and skin at 90° minus $\frac{1}{3}$ of the insensible water loss at 80° The loss of water via the lungs was considered to be constant in the 2 periods and approximately equal to $\frac{1}{3}$ of the non-sweating insensible water loss Second, skin washings at 80° were considered to arise from desquamation The electrolyte in sweat equaled the skin washings at 90° minus the washings at 80° The concentrations per kg of sweat were about 5 mm of chloride, 4 mm of sodium, and 6 mm of potassium These data indicate a low concentration of sodium and chloride in the sweat of normal babies The concentration of potassium is high

Metabolism of acetone T D PRICE (by invitation) and D RITTENBERG *Dept of Biochemistry, College of Physicians and Surgeons, Columbia Univ, New York City* Acetone labeled with carbon-14 in the methyl groups was administered to rats From 0.2 to 1.1 mg of the acetone was given by stomach tube Little of the acetone is directly excreted, since only 0.6–10% of the acetone radioactivity was expired as carbonyl compounds However, 50–80% of the activity appears in the respiratory carbon dioxide within 24 hours The maximum activity in the expired carbon dioxide occurs about 2 hours after administration of the acetone, the activity of the carbon dioxide drops to a very low value after 12 hours The administration of phenylaminobutyric acid resulted in the urinary excretion of a radioactive acetyl derivative The activity of this product indicates that about half of the acetone was converted to acetate In the normal rat, significant activity appeared in the carbon of the following compounds arranged in order of decreasing specific activity: liver cholesterol, bone carbonate, carcass cholesterol, carcass glutamic acid, liver glycogen, carcass aspartic acid, total carcass amino acids, carcass arginine and carcass fatty acids No activity was found in the carcass leucine or tyrosine

Partial separation of D- and L-peptidases and analogous dehydropeptidase I VINCENT E PRICE (by invitation), ALTON MEISTER (by invitation) and JESSE P GREENSTEIN *National Cancer Institute, National Institutes of Health, Bethesda, Md* The enzymatic hydrolysis of glycyl-L-alanine, glycyl-D-alanine, and glycyldehydroalanine by aqueous homogenates of several normal rat tissues was studied By means of high speed centrifuga-

tion ($18,000 \times g$ for two hours) a partial separation of the enzymatic activities was achieved Between 75 and 95% of the original activity toward glycyl-D-alanine of homogenates of liver, kidney, spleen, brain, pancreas, testis and lung was associated with the sediment, whereas 80–95% of the original activity toward glycyl-L-alanine remained in the clear supernatant About 80% of the original activity toward glycyldehydroalanine was also found in the supernatant in all of the tissues examined except kidney, in which 80–90% of the activity was associated with the sediment Between 85 and 110% of the original activity was recovered in the pellet and supernatant fractions The ratio of D-peptidase, L-peptidase, and dehydropeptidase activities in a preparation derived from kidney sediment was 100:53:122, while the ratio for the original kidney homogenate was 100:193:105 A preparation obtained from lung sediment possessed these activities in a ratio of 100:31:22, compared to 100:230:60 for the original homogenate Thus in a variety of tissues D-peptidase activity may be readily separated from L-peptidase activity These results suggest that hydrolysis of the 3 substrates studied may be catalyzed by different enzymes The ratios obtained for the sediments support this hypothesis

Fate of mercury in the rat following administration as mercuric acetate and phenylmercuric acetate CLAUDIA S PRICKETT (by invitation), EDWIN P LAUG and FRIEDA KUNZE (by invitation) *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D C* When phenylmercuric acetate and mercuric acetate are administered intravenously at a level of 120 μg Hg per rat, marked differences in the excretion and storage of mercury occur Thus, after mercuric acetate, 25% of the dose can be recovered in the kidney within 2 hours, only 4% after phenylmercuric acetate In the liver, the situation is reversed, with phenylmercuric acetate favoring greater storage In line with greater kidney storage after mercuric acetate is a significantly greater urinary excretion in 48 hours this amounts to 17% of the dose for mercuric acetate, but only 3% for phenylmercuric acetate The greater liver storage following phenylmercuric acetate is correlated with a prompt appearance of mercury in the small intestine, and a significantly elevated fecal excretion in 48 hours this amounted to nearly 40% for phenylmercuric acetate, and only 10% for mercuric acetate Oral administration, in contrast to intravenous results in much lower storage and excretion of mercury for both type of salts However, phenylmercuric acetate is absorbed to a much greater extent than mercuric acetate, approximately 5 times as much mercury being stored in the liver and kidney, and twice as much excreted in the urine

Identification of L-ascorbic acid in the urine

of normal and chlorotone-treated rats G W PROBST (by invitation) and M O SCHUITZ Division of Agricultural Biochemistry, Univ of Minnesota, Minneapolis, Minn Preliminary to other studies we have isolated and identified as L ascorbic acid (M P 191°, mixed M P 191°, $[\alpha]_D^{21}$ in methanol + 50.5, 174 mg equivalent to 1 mM iodine, monoacetone derivative M P 220°, 228 mg equivalent to 1 mM iodine) the indophenol reducing substance in urine of rats treated with 1,1,1-trichloro 2-methyl-2-propanol (Longenecker et al *J Biol Chem* 135 497, 1940) Rat urine oxidized with 2,6 dichlorophenolindophenol or norite and treated with 2,4-dinitrophenylhydrazine (DNPH) under conditions prescribed by Roe and Kuether (*J Biol Chem* 117 399, 1943) yields mixtures of DNPH-derivatives from which that of L-dehydroascorbic acid can be isolated in pure form by chromatography on magnesium phosphate tetrahydrate This derivative in 25 N H₂SO₄ has absorption maxima at λ 528 m μ ($E_{1\text{cm}}^{1\%}$ 415) and λ 370 m μ ($E_{1\text{cm}}^{1\%}$ 480) and a minimum at λ 448 m μ ($E_{1\text{cm}}^{1\%}$ 212) The extinction ratios $E_{1\text{cm}}^{1\%} \frac{\lambda 530}{\lambda 450}$ and $E_{1\text{cm}}^{1\%} \frac{\lambda 370}{\lambda 450}$ serve as an excellent criterion of its purity Depending upon the L ascorbic acid content of the urine, the oxidant and the conditions of incubation, compounds other than L-dehydroascorbic acid yield DNPH derivatives which when dissolved in 25 N H₂SO₄ and at λ 530 m μ account for 10-57% of the absorption of crude unchromatographed DNPH derivatives The implications of this to methodology for determination of L ascorbic acid will be discussed From urine of normal rats on a milk diet we have isolated the pure DNPH derivative of L-dehydroascorbic acid The latter, under suitable conditions, can be recovered quantitatively when L-ascorbic acid is added to urine

Studies on lysozyme CHARLES M PROCTOR and SHERMAN R DICKMAN (introduced by EMIL L SMITH) Dept of Biological Chemistry, Univ of Utah Medical School, Salt Lake City, Utah An assay for lysozyme consistent to 5% has been set up The increase in transmission at 4400 Å of *Sarcina lutea* suspensions is measured after incubation with the enzyme, followed by addition of alkali The effect of various agents on rate of lysis has been studied No inactivation resulted from treatment of lysozyme overnight at 22°C with 0.1 M alloxan or 2.0 M hydroxylamine, while 0.1 M sodium hydroxide resulted in about 50% inactivation Galston (1948, private communication) has observed an inactivation of several plant enzymes by incubation with riboflavin in strong light A 40% inactivation of lysozyme has been obtained under similar conditions Meyer and Hahnel (*J Biol Chem* 163

723, 1946) observed a striking increase in lysozyme activity by 0.2 M sodium chloride when estimated viscosimetrically, but no effect on the rate of appearance of reducing sugars This concentration of sodium chloride had no effect on the rate of lysis of *S. lutea*, whereas 0.5 M sodium chloride gave about 50% inhibition and 1.0 M sodium chloride 90% inhibition Treatment of either lysozyme or *S. lutea* cells with high concentrations of sodium chloride had no effect on subsequent lysis

Factors affecting the diazotization of bilirubin BETTY KING PROFFITT (by invitation) and DENNIS B MORRISON Dept of Chemistry, Univ of Tennessee, Memphis, Tenn The theory that serum bilirubin exists in more than one form is largely based on the difference in color developed when diazotization occurs in aqueous (direct reacting) and in alcoholic (total) solutions The difference in color resulting from indirect reacting bilirubin In an attempt to account for this difference we have investigated the diazotization of bilirubin over a wide range of conditions Crystalline bilirubin dissolved in buffers, diluted human bile, a protein-bilirubin complex isolated from human bladder bile, and sera from patients with different types of jaundice and varying degrees of hyperbilirubinemia was used The pH after diazotization of the above varied from 1-9 The concentration of nitrite in the diazo reagent was adjusted to optimum for any series and for a given pH From the data to be presented it will be evident that the concentration of nitrite is highly critical for aqueous solutions By adjustment of nitrite concentration it is possible to produce a biphasic reaction, maximum color and stability, fading colors or to suppress the development of color Methanol solutions are less sensitive since the methanol appears to destroy the excess nitrite Maximum color develops in aqueous solutions for all the materials tested at a pH 7-8 and optimum nitrite concentration All sera so far examined when diazotized with optimum nitrite concentration give a direct/total color ratio of approximately 0.7

Prothrombin consumption test ARMAND J QUICK AND JEAN FAVRE-GILLY (by invitation) Dept of Biochemistry, Marquette Univ School of Medicine, Milwaukee, Wis On adding serum to a mixture of CaCl₂, thromboplastin and fibrinogen, a coagulation time is obtained which can be employed as a measure of the prothrombin remaining unconverted during coagulation in a test tube The test has been standardized as follows As a source of fibrinogen, normal plasma is used which is treated with Ca₃(PO₄)₂ to adsorb prothrombin (component B) To a mixture of 0.1 cc of calcium phosphate-treated plasma, 0.1 cc of 0.02 M CaCl₂, and 0.1 cc of Quick's thromboplastin, 0.1 cc of serum is added and the time required to form a clot accurately measured The serum prothrombin

time (or prothrombin consumption time) for normal blood 1 hour after coagulation generally ranges from 16 to 35 seconds. In hemophilia it varies between 9 and 12 seconds and similar low values are found in severe thrombocytopenic purpura. The thrombin formed is inactivated so rapidly by the normal antithrombin that its effect on the test is not significant. Immediately after coagulation the serum prothrombin time is short (8-10 seconds) and remains constant for approximately 20 minutes or more before it begins to increase and finally reaches a maximum value. The increase in serum prothrombin time occurs sooner if the coagulated blood is centrifuged. The reason for this has not been determined.

Growth-promotion of yeasts by neopyrithiamin
JESSE C. RABINOWITZ (by invitation) and **ESMOND E. SNELL**, *Dept. of Biochemistry, College of Agriculture, Univ. of Wisconsin, Madison, Wis.* Several yeasts are inhibited by thiamin when grown in the absence of vitamin B₆ (Schultz and Atkin, *Arch. Biochem.* 14: 369, 1947). *Saccharomyces carlsbergensis* 4228, commonly used for determination of vitamin B₆, shows a similar behavior. In the absence of vitamin B₆ or thiamin, 3.0 mg. of dry cells were obtained per cc. of culture medium. The addition of only 0.017 mγ of thiamin per cc. of culture medium caused 50% growth inhibition, and the addition of 1.7 mγ of thiamin per cc. completely prevented growth. This inhibition of growth by thiamin is alleviated by either vitamin B₆ or by neopyrithiamin, a growth-inhibiting analogue of thiamin. Thus, in the absence of other data, neopyrithiamin might erroneously appear to act as an essential growth-factor for the yeast cells under these conditions. Inhibition of the organism by the addition of thiamin may result from increased synthesis of intermediate products, which may be toxic unless further metabolized. If vitamin B₆ were the limiting factor in this metabolic process, the addition of this vitamin would be expected to overcome the inhibition. Addition of neopyrithiamin would similarly overcome the inhibition since by acting as a competitive inhibitor of the added thiamin it could block formation of an excess of the toxic product. Thus, the antimetabolite would appear to be an essential growth factor, even though not involved in the normal metabolism of the cell. Reported instances in which organisms have come to require streptomycin or sulfonamides for growth may have a similar explanation.

Biosynthesis of heme **NORMAN S. RADIN** (by invitation), **D. RITTENBERG** and **DAVID SHEMIN**, *Dept. of Biochemistry, College of Physicians and Surgeons, Columbia Univ., New York City*. In an attempt to determine the precursors of heme we have incubated duck blood cells, both in plasma and buffer, with various compounds labeled with C¹⁴ and N¹⁵. The mixture of preformed and newly

synthesized heme was isolated as hemin and analyzed. Although the α-carbon and nitrogen atoms of glycine are utilized for porphyrin synthesis the carboxyl carbon atom is not. Comparison of C¹⁴ and N¹⁵ concentrations in hemin arising from glycine labeled in the α-carbon and nitrogen atoms suggests that more than 4 carbon atoms per heme molecule are derived from the α-carbon atom. The carboxyl carbon atom of acetic acid participates in the synthesis of heme. Degradation of this hemin reveals that about half of the activity is in the carboxyl group. Some of the activity is in the rings containing the vinyl side chains (rings I and II). The hemin obtained from methyl labeled acetic acid is 6 times as radioactive as that obtained from carboxyl labeled acetate. The α-carbon atom of pyruvic acid is also utilized. The implications of these findings in relation to the biosynthesis of porphyrins will be discussed.

Effect of Theiler's GD VII virus on P32 uptake of one-day-old-mouse brain cultures **MAX E. RAFELSON, JR.** (by invitation), **HAROLD E. PEARSON** (by invitation), and **RICHARD J. WINZLER**, *Depts. of Biochemistry and Bacteriology, Univ. of Southern California School of Medicine and Laboratory Division, Los Angeles County Hospital, Los Angeles, Calif.* The influence of Theiler's GD VII strain of mouse encephalomyelitis virus on the incorporation of P32-labelled phosphate into the phospholipid, total protein bound and organic acid-soluble fractions of minced one-day-old-mouse brain cultures incubated in Simm's solution at 35°C has been studied. The total protein bound phosphate was fractionated by combining the methods of Schmidt and Thannhauser (*J. Biol. Chem.* 161: 83, 1945) and Schneider (*J. Biol. Chem.* 161: 293, 1945) yielding four fractions: ribonucleic acid, deoxyribonucleic acid, phosphoprotein and inorganic phosphate liberated from phosphoprotein. The virus-infected cultures, in comparison with non-infected cultures, had a markedly higher rate of P32 incorporation into the phospholipid, total protein bound and ribonucleic acid fractions in the first 6-24 hours of incubation. This is the period of maximal virus propagation. Phosphate turnovers in the organic acid-soluble, phosphoprotein, deoxyribonucleic acid and protein-liberated inorganic phosphate fractions were no different in the infected than in noninfected cultures. Both infected and noninfected cultures had P32 uptakes increasing up to 48 hours of incubation after which time the relative specific activities of the fractions decreased.

A new ganglionic blocking agent **LOWELL O. RANDALL**, **WILLIAM G. PETERSON** (by invitation) and **G. LEHMANN**, *Pharmacology Dept., Hoffmann-La Roche, Inc., Nutley, N. J.* The d-3,4-(1',3'-Dibenzyl-2'-keto-imidazolido)-1,2-trimethylene-thiophanium d-camphor sulfonate (Nu-2222) is a

ganglionic blocking and vasodepressor agent which has about 30 times the potency and twice the duration of action of tetraethylammonium bromide. The vasodepressor effect is attributed to the blocking action on ganglia. Nu-2222 blocks transmission through the superior cervical ganglia since the reaction of the nictitating membrane to preganglionic stimulation is inhibited while the response to postganglionic stimulation is not affected. It blocks the vasodepressor response to vagus stimulation and the vasopressor response to carotid occlusion. The vasodepressor and ganglionic effects are counteracted by large doses of acetylcholine and Prostigmin. Ephedrine is an effective antidote for the vasodepressor action. Relatively large doses inhibit gastric and intestinal motility. There is little or no demonstrable curare-like action. Nu-2222 is 2 to 4 times as toxic as tetraethylammonium bromide by the intravenous route in mice, rats and rabbits but about 75 times as toxic in dogs. Lethal doses produce a hemorrhagic disorder in dogs which is accompanied by a prolonged clotting time. Such effects are not apparent in rabbits or cats. Nu-2222 has an approximate safety margin of 25 in dogs and 700 in rabbits.

Enzymatic mechanism of arginine synthesis
S. RATNER and ANNE PAPPAS (by invitation)
Dept. of Pharmacology, New York Univ. College of Medicine, New York City. It has been reported previously (S. Ratner, *J. Biol. Chem.* 170: 760, 1947) that rapid synthesis of arginine from citrulline occurs with partially purified enzyme preparations from extracts of beef liver acetone powder. The isolated system catalyzes the overall reaction: $L\text{-citrulline} + L\text{-aspartate} + \text{adenosinetriphosphate (ATP)} \rightarrow L\text{-arginine} + L\text{-malate} + \text{ADP} + \text{H}_2\text{PO}_4$. The reaction has been found to proceed in several steps. Two distinct enzymes have now been obtained by fractionation with ammonium sulfate. One of these catalyzes a reaction between citrulline and aspartic acid to form an intermediate condensation product. ATP participates in the condensation reaction and inorganic phosphate is formed as citrulline is removed. A portion of the endergonic requirement of urea synthesis may therefore be associated with the formation of this condensation product, the necessary energy being derived from the high energy phosphate group of ATP. Magnesium ion is required and 0.01 M fluoride causes a 60% inhibition. The second enzyme catalyzes the hydrolytic cleavage of the intermediate accumulated in the first step, with the simultaneous appearance of arginine and malic acid. Experimental details relating to the enzymatic mechanisms and to the fractionation procedure will be discussed. Experiments on homogenates demonstrate that the above mechanism represents the main pathway of the conversion of citrulline to arginine in the biosynthesis of urea.

Studies on prolamines treated with nitrogen trichloride I Fractionation of hydrolysates
L. REINER, PHILIP WEISS (by invitation), FERNANDA MISANI (by invitation), M. G. CORDASCO (by invitation), and T. W. FAIR (by invitation) *Research Laboratories, Wallace & Tiernan Products, Inc., Belleville, N. J.* Finely divided zein and gliadin were reacted with NCl_3 vapors or by suspending them in a solution of NCl_3 in carbon tetrachloride. About 20 gm. of NCl_3 are used up per kg. of prolamine. The reacted prolamine produces typical running fits in rabbits in a minimum dose corresponding to 500 mg. N orally. Zein was hydrolyzed with pancreatin and with Protease 16 to an $-\text{NH}_2/\text{N}$ value (formol titration) of 45-65% without impairment of its toxicity. It was then adsorbed to a cation exchange resin from which it was eluted with 2% pyridine. The eluate was toxic in a dose of 220 mg. N/kg. in rabbits, orally. Repeating this procedure with the first eluate yielded a second eluate which was toxic in a dose of 70 mg. N/kg. The second eluate was passed through charcoal previously conditioned with dilute acetic acid. The adsorbed material was eluted with large volumes of water. The combined eluate was toxic at a dose of 20 mg. N/kg. and some fractions of the eluate were toxic at a dose of 10 mg. N/kg. Part of the dried residue of the eluate was crystallized from EtOH. This was also toxic at 10 mg. N/kg. Its paper chromatogram (butanol-acetic acid) contained 5 spots suggesting that the product was not pure.

Studies on prolamines treated with nitrogen trichloride II Acetylated zein
L. REINER, FERNANDA MISANI (by invitation), PHILIP WEISS (by invitation), M. G. CORDASCO (by invitation), and T. W. FAIR (by invitation) *Research Laboratories, Wallace & Tiernan Products, Inc., Belleville, N. J.* Fractions which were isolated from zein treated with NCl_3 and which were toxic to rabbits in a dose corresponding to 36-220 mg. N/kg. were acetylated with acetic anhydride. The acetylated products did not produce running fits in dogs or rabbits when fed in doses 4 times as great as the minimum toxic dose of the unacetylated parent compound. Hydrolysis with 5N HCl reestablished their toxicity. Differences in the ability to acetylate *in vivo* may account for the great differences in the sensitivity of various species towards NCl_3 treated prolamines. Unhydrolyzed toxic zein was also detoxified by acetylation. Toxic zein lost its ability to produce fits when it was deaminated by diazotization. When zein was first acetylated, then treated with NCl_3 , a product resulted which was as a rule less toxic, but it became more toxic upon deacetylation. These results suggest that a free $-\text{NH}_2$ group which is at the end of a peptide chain is essential for the elicitation of the toxicity but that blocking this group by acetylation does not interfere with the

formation of the toxic residue through reaction with NCl_2 .

Determination of serum protein Studies of agents for separation of albumin and globulin JOHN G REINHOLD, L W BLUEMLE, JR (by invitation), FRANCISCO CAMPOY (by invitation), LOGAN GILMAN (by invitation), EDWARD F GOULD (by invitation), VERNON L MARTENS (by invitation) and RICHARD SHUMAN (by invitation) *Pepper Laboratory of Clinical Medicine and Nutritional Service, Hospital of the Univ of Pennsylvania, Philadelphia, Penna* Need exists for a rapid procedure for determination of serum albumin and globulin incorporating advances made recently in this field. A survey of agents proposed for separation of serum albumin and globulin was made by comparing the results with those of electrophoretic measurements at pH 8.6 and ionic strength 0.1. Sodium sulfate at a concentration of 26% and an approximate pH of 7.0 gave results approximating albumin and globulin values found by electrophoresis. Values obtained by use of sodium sulfite at 26.9% concentration were in fair agreement, while results obtained using 0.5 saturated ammonium sulfate (at a pH of 5.4 or 7.0), or methanol at 42.5% concentration pH of 6.8 and temperature of 1°C were less satisfactory. None of the salting-out methods were consistently in agreement with the electrophoretic separations. Attention to details of technique in the salting-out procedures appears important. The rapid separation method employing ether and centrifugation devised by Kingsley gave results similar to those obtained by filtration when 26% sodium sulfate was used. The applicability of the biuret reaction has been reevaluated. Determinations made by the biuret methods of Kingsley or Weichselbaum were compared with those of Kjeldahl or gravimetric methods. The means of all methods agreed closely, but occasional individual variations were marked. Discrepancies were least frequent between Kjeldahl and Weichselbaum biuret methods and the two may be used interchangeably for the usual serum analyses.

Influence of high levels of fat with suboptimal levels of riboflavin on the growth of chicks RAYMOND REISER (by invitation) and P B PEARSON *Agricultural and Mechanical College of Texas, College Station, Tex* Chicks receiving moderately high levels of refined cottonseed oil (Wesson Oil) in diets low in biotin ceased to grow much sooner than those on a similar diet without added fat. The higher the cottonseed oil content of the diet the greater the inhibitory effect on the rate of gain. The addition of riboflavin to the diets at a level of 4 $\mu\text{g}/\text{gm}$ of feed prevented or corrected the phenomenon. Lard did not retard the growth rate on low riboflavin diets and a commercial hydrogenated vegetable oil to only a slight degree. The effect is not due to the development of rancidity in the high fat diets. The efficiency of utilization of the

feed was lower on the cottonseed oil diets than on the low fat diets at low levels of riboflavin, but no differences were found in the riboflavin content of the livers.

Liver respiration as related to its xanthine oxidase activity DAN A. RICHERT (by invitation) and W W WESTERFELD *Dept of Biochemistry, Syracuse Univ College of Medicine, Syracuse, N Y* The determination of xanthine oxidase activity (Axelrod and Elvehjem) in a series of rat livers whose xanthine oxidase activity varied from 0-1800 units ($\text{Cmm O}_2/\text{gm}/\text{dry liver}/\text{hour}$) has demonstrated the following: 1) The initial rate of the endogenous respiration of liver decreased 50% as the xanthine oxidase activity fell from 1600 units to zero. 2) Tipping in xanthine to a respiring liver brei of low xanthine oxidase activity inhibited the endogenous respiration instead of increasing the oxygen consumption. 3) Adding hypoxanthine to the brei at the start intensified the xanthine inhibition effect and prevented the detection of xanthine oxidase in livers with normal activity. 4) Adding purified milk xanthine oxidase to livers of low activity restored the endogenous respiration to normal and prevented the inhibition otherwise caused by tipping in xanthine. 5) Dialysis of the liver brei removed the substrates otherwise detected by adding purified xanthine oxidase, and also removed the xanthine inhibition effect. The results show that about 50% of the initial endogenous respiration in a normal rat liver homogenate is due to the oxidation of one or more substrates similar to hypoxanthine, and the relative accumulation of such substrates in a brei with low xanthine oxidase activity is responsible for the inhibition produced by added xanthine, in accordance with the known enzyme-substrate relationships for this system.

Utilization of human serum albumin injected intravenously CECILIA RIEGEL and NICHOLAS GIMBEL (by invitation) *Harrison Dept of Surgical Research, Schools of Medicine, Univ of Pennsylvania, Philadelphia, Penna* Human serum albumin was administered both orally and intravenously as the sole source of protein to 3 human volunteer subjects. Control periods using either milk or casein orally were also studied. Nitrogen, calcium, potassium, phosphorus and sulfur balances, changes in albumin-globulin ratio in the blood, blood and plasma volumes, thiocyanate space, nitrogen content of leg lymph and urea excretion were determined. Albumin administered orally was utilized in comparable fashion to milk and casein. On an oral intake of albumin of 12.0 gm nitrogen daily 1 of 2 subjects was in nitrogen equilibrium, 1 had a daily negative balance of 1.6 gm. A 3rd subject was in negative balance on an oral intake of 8.0 gm nitrogen daily. All 3 were in marked positive balance on an intravenous intake of either 8.0 or 12.0 gm of albumin nitrogen daily.

From 1) increase in total circulating plasma protein, 2) excess circulating interstitial fluid albumin, as determined from thiocyanate space determinations and from analysis of lymph obtained from the leg, and 3) amount of albumin excreted in the urine as albumin, we can, in *Patient S*, account for 774 of the 1100 gm injected over a period of 22 days, leaving 326 gm to be accounted for. If the difference between an assumed endogenous nitrogen excretion and the actually determined nitrogen excretion represents metabolic breakdown of albumin, the excretion of this amount of nitrogen in the urine is more than sufficient to account for the injected albumin not present in the body fluids. It is possible that this amount of albumin may have been metabolized during the 22 days of injection. That such breakdown is slow is indicated by the steady accumulation of albumin in plasma the longer the albumin injections were continued, by the low level of nitrogen excretion during the period when albumin was administered intravenously, and by a slowly increasing excretion of sulfur, which fell to a very low level when the subject was first changed from oral albumin to intravenous albumin.

Studies on the origin of serum proteins SIDNEY ROBERTS (by invitation) and ABRAHAM WHITE, *Dept of Physiological Chemistry, School of Medicine, Univ of California at Los Angeles, Los Angeles, Calif*. Serum protein levels have been determined electrophoretically in the rat after either partial hepatectomy or abdominal evisceration. After partial hepatectomy, the earliest change was a decline in α_1 -globulin, followed by later decreases in albumin and in the other globulins. Subsequently, the globulins, especially α_1 -globulin, were rapidly restored. Albumin levels, however, remained low over the 48 hour experimental period. A maximum reduction in total serum protein concentration of 1.2 gm/100 ml occurred 24 hours after partial hepatectomy. Following abdominal evisceration, the serum protein level dropped rapidly, falling 2.0 gm/100 ml within the 23-hour experimental period. In this instance, the decline was due almost entirely to a diminution of albumin. Incubation of surviving liver mince in rat serum in an atmosphere of 95% oxygen, 5% carbon dioxide resulted in the liberation of large amounts of a protein with the electric mobility of serum

α globulin. The rate of liberation of this liver protein was initially as high as 30 mg/gm of tissue per hour, but declined rapidly over the 3 hour incubation period. Similar experiments in a nitrogen atmosphere resulted in an increased rate of liberation of protein. In all experiments, a disappearance of albumin from the serum medium accompanied the release of ' α globulin'. The results provide further evidence that the formation of blood proteins involves the integrated action of the liver and extra-hepatic tissues.

Tissue collagen in experimental scurvy WILLIAM V B ROBERTSON (introduced by H B PIERCE), *Division of Experimental Medicine and Dept of Biochemistry, College of Medicine, Univ of Vermont, Burlington, Vt*. The collagen concentrations in the skin and liver of normal guinea pigs, of those on a scorbutogenic diet and moribund with acute scurvy, and those on a scorbutogenic diet but maintained deficient for about 10 weeks with 0.35-0.15 mg of ascorbic acid per day were determined. No changes in the collagen concentration in skin were found, whereas that in the liver was higher than normal in both scorbutic groups. The implications of these findings will be discussed.

Chemical changes in isotonic saline solutions placed in successively lower levels of human small intestine C S ROBINSON, GLADYS R BUCHER (by invitation) and D DZIEWIATKOWSKI (by invitation), *Dept of Biochemistry, Vanderbilt Univ, Nashville, Tenn*. The survey of the gradient in the small intestine altering saline solutions introduced at successive levels spaced 60 minutes apart, through a Miller-Abbott tube, has been continued using 50 cc volumes per instillation of approximately isotonic solutions of Na_2SO_4 , NaH_2PO_4 or Na_2HPO_4 . Samples withdrawn 20 minutes after instillations were analyzed for total base, total CO_2 , pH and the anions. The differences between successive pairs of samples (lower minus upper) were examined statistically. The average differences, with standard error, which represent the rate of change at intestinal levels 60 minutes apart as the tube advanced from pylorus to terminal ileum, are tabulated. A = number of samples, B = number of subjects, C = hours pylorus-terminal ileum, D = volume recovered in 60 minutes, E = mean difference in pH.

Saline used	A	B	C	D	E
NaCl , 50 cc 150 mM	88	14	7-8	52.5 \pm 3 cc	+0.186 \pm 0.03*
Na_2SO_4 , 50 cc 112 mM	28	8	4-5	63.7 \pm 3 cc*	+0.290 \pm 0.05*
NaH_2PO_4 , 50 cc 104 mM	48	11	6-7	74.4 \pm 3 cc*	+0.036 \pm 0.02
Na_2HPO_4 , 50 cc 101-143 mM	52	11	5-6	62.3 \pm 4 cc*	+0.109 \pm 0.02*
Total Base	mm CO_2		Cl mEq/l		SO_4 mEq/l PO_4 mEq/l
NaCl , +0.70 \pm 0.28*	+3.80 \pm 0.63*		-3.32 \pm 0.80*		+9.57 \pm 4.8*
Na_2SO_4 , +1.02 \pm 0.11	+4.54 \pm 1.09*		-7.1 \pm 2.99*		+9.3 \pm 6.9
NaH_2PO_4 , +0.67 \pm 0.17	+0.82 \pm 0.24*		-9.9 \pm 2.80*		+9.6 \pm 6.2
Na_2HPO_4 , +4.80 \pm 0.13*	+0.97 \pm 0.40*		-5.6 \pm 2.24*		

The differences marked * are statistically significant. Sulfate and phosphate ions suppress the entrance of chloride and this suppression is more pronounced as the ileocecal valve is approached.

Analysis of certain components of skeletal muscle during vitamin E deficiency CHARLOTTE E RODERUCK (introduced by H A MATTILL) *Dept of Biochemistry, State Univ of Iowa, Iowa City, Iowa* In nutritional muscular dystrophy resulting from a deficiency of vitamin E, the free and total biotin content of skeletal muscle of hamsters, rabbits and guinea pigs did not vary from that of control animals. Reduction of the biotin intake (hamsters) decreased muscle biotin irrespective of the adequacy of the vitamin E intake. It is therefore unlikely that vitamin E acts as an antioxidant in tissue to preserve biotin, although this relationship has been observed in the autooxidative destruction of biotin by unsaturated fats *in vitro*. A sparing action of vitamin E on DPN was not demonstrated by mere addition of coenzyme I to Ringer phosphate medium in which dystrophic muscle strips were respiring, Q_{O_2} was not affected. Q_{O_2} values were also not influenced by addition of glucose, glutamic acid or phenylalanine. Muscle strips from hamsters on vitamin E-deficient diets but not grossly dystrophic had Q_{O_2} values 33% higher than those from control animals. Differences of the same order were found in control and dystrophic guinea pig muscle. The glutamine content of skeletal muscles of dystrophic guinea pigs was about one-third that of muscle from control animals, the average content of non-glutamine amino acids was unchanged. In vitamin E deficient rabbits, the difference was less marked. The possible significance of decreased glutamine levels is discussed briefly with reference to energy relationships. These results may be further evidence of an inefficient expenditure of energy possibly due to impairment in the process of phosphorylation.

Effect of a series of flavanoids on hyaluronidase activity GERTRUDE RODNEY, L M WHEELER and ANN SWANSON (introduced by J J Pfiffner) *Research Laboratories, Parke, Davis & Co, Detroit, Mich.* The action of a series of flavanoids related to rutin on bovine hyaluronidase was studied *in vitro* by both the viscosity-reduction test and the turbidity-reduction method. Crystalline rutin was found to have no effect in concentrations of $5 \times 10^{-4}M$ to $1 \times 10^{-3}M$ on 10 units of enzyme but a product formed by pancreatic digestions showed some inhibition of the enzyme activity. Investigation of a series of 15 related flavanoids showed inhibitory activities which could be correlated with structural differences in the compounds. The action of these compounds *in vivo* on the spreading effect of hyaluronidase injected with hemoglobin in guinea pig skin was compared to the activity shown *in vitro*.

Studies in the metabolism of d-arabinose JOSEPH H ROE, EUGENE W RICE (by invitation) and BENJAMIN W SMITH (by invitation) *Dept of Biochemistry, School of Medicine, George Washing-*

ton Univ, Washington, D C Following oral administration of D-Arabinose to rabbits, in doses of 2 gm/kg of body weight, this sugar appears in the blood within $\frac{1}{2}$ hour, reaches a peak level in 1-2 hours and disappears from the blood stream in 6-8 hours. The rate of absorption of D-Arabinose in the rabbit, determined by the Cori technique, was found to be 0.036 gm/100 gms of body weight/hour. Balance studies after the oral administration of D-Arabinose, in which analyses of the alimentary tract, tissues, feces and urine were made, showed the following average total recoveries of this sugar: after 1 hour, 80%, after 2 hours, 67%, after 6 hours, 15%, after 12 hours, 9.5%, after 24 hours, 5.9%. In the 24-hour analyses the pentose was found only in the urine. Analyses were made with the colorimetric method of Roe and Rice (*J Biol Chem* 183: 507, 1948) which is based on furfural formation. These data show that D-Arabinose undergoes a metabolic transformation in the rabbit. *In vitro* studies of D-Arabinose added to homogenates of liver, kidney, muscle, blood and brain have shown a disappearance of this sugar after 13 to 24 hours' incubation at 37°C, under aerobic and anaerobic conditions. This disappearance is prevented by heating, iodoacetic acid and fluoride.

Hemolytic action of alloxan and alloxan derivatives CATHARINE S ROSE (by invitation) and PAUL GIBRGY *Nutritional Service of the Dept of Pediatrics and the Gastro-Intestinal Section of the Medical Clinic, School of Medicine, Univ of Pennsylvania, Philadelphia, Penna.* In the course of studies on the effect of dietary factors in alloxan diabetes it was found that alloxan caused marked hemolysis in rats receiving a tocopherol-deficient diet (*Science* 108: 716, 1948). The nature of this reaction has been investigated. Dialuric acid, alloxantin and ninhydrin caused hemolysis in the same manner as alloxan, ninhydrin being the most active of the substances tested. The increased fragility of the red blood cells towards these compounds could be demonstrated after the rats had received the tocopherol-deficient diet for only 1 week. Alloxanic acid did not cause hemolysis. Studies were made *in vitro* using a suspension of red blood cells, all substances tested for hemolytic activity were prepared in phosphate buffer at pH 7.4. With dialuric acid the cells of tocopherol deficient animals were hemolyzed. Alloxan was without effect, but treated with an equivalent amount of cysteine it behaved as dialuric acid. Larger amounts of cysteine inhibited hemolysis with either alloxan or dialuric acid. Various alloxan-related compounds and reducing agents have been studied. The red blood cells of animals receiving tocopherol were resistant to the action of dialuric acid and addition of tocopherol in about 1/50th the amount of dialuric acid protected the cells of tocopherol-deficient animals. These observations are in accord with an as

sumption that tocopherol intereferes with the formation of a free radical, a first oxidation product of dialuric acid, which is the actual hemolytic agent.

In vitro action of gentisic acid on hyaluronidase
SAUL ROSEMAN (by invitation), FAITH PEARSON (by invitation), and ALBERT DORFMAN *Dept of Pediatrics, Univ of Chicago, Chicago, Ill* The administration of sodium salicylate to rabbits inhibits the spreading effect of hyaluronidase (Guerra, F, *J Pharmacol & Exper Therap* 87: 193, 1946) It has been demonstrated that salicylates do not inhibit hyaluronidase *in vitro* in concentrations that are achieved *in vivo* (Dorfman, Reimers, and Ott *Proc Soc Exper Biol & Med*, 64: 357, 1947, Pike *Science*, 105: 391, 1947, Meyer *Physiol Rev* 27: 337, 1947) Meyer and Ragin (*Science* 108: 281, 1948) have recently claimed that this is due to the fact that salicylates are converted to gentisic acid which acts as the true inhibitor Analytically pure gentisic acid was prepared by the oxidation of salicylic acid Purification was achieved through lead salt fractionation and repeated recrystallization from water and ethyl acetate Gentisic acid so prepared did not inhibit bovine testicular hyaluronidase This confirms the findings of Lowenthal and Gagnon (*Canad J Research* 26: 200, 1948) Crude fractions were found to be inhibitory as was pure gentisic acid treated with alkali in the presence of air Similar results were obtained with homogentisic acid Thus the apparent inhibitory effect of crude gentisic acid is due to impurity which is probably an oxidation product The possible significance of these results in the explanation of the *in vivo* effects of salicylates will be discussed

Arginase, rhodanese ATPase activity of regenerating livers in protein-depleted rats
OTTO ROSENTHAL, CHARLES S ROGERS (by invitation) and COLIN C FERGUSON (by invitation) *Harrison Dept of Surgical Research, Schools of Medicine, Univ of Pennsylvania, Philadelphia, Penna* Arginase, rhodanese and ATPase activities of regenerating liver following 70% hepatectomy have been studied in male 250-gm Wistar rats maintained on a protein-free diet Enzyme assays and determinations of the protein nitrogen were done upon aliquots of glass-homogenized tissue specimens During 2 weeks of preoperative protein starvation the animals lost about 44% of the protein, 70% of the arginase, 65% of the rhodanese, and 50% of the ATPase of their livers Thus while arginase and rhodanese activities of the liver protein were markedly diminished ATPase activity decreased but little Following partial hepatectomy the concentrations of rhodanese and ATPase in the regenerating remnant continued to decrease slightly whereas the arginase concentration rose strikingly The rise was apparent as early as 12 hours after hepatectomy At 24 to 48 hours, i e, at a time when

only about 36% of the operatively removed protein was regenerated, the liver remnant contained as much or even more arginase than the entire pre-operative liver Smaller changes in arginase were found in livers examined 4 to 8 days after partial hepatectomy The fluctuations of the liver arginase concentrations are probably a reflection of the changes in the endogenous protein metabolism which temporarily increases as a consequence of traumatic tissue breakdown and inadequate food consumption No similar changes were observed in regenerating livers of rats kept on an 18% casein diet during the entire experimental period

Hydrolysis of esters by rabbit polymorphonuclear leucocytes
R J ROSSITER and ESTHER WONG (by invitation) *Dept of Biochemistry, Univ of Western Ontario, London, Canada* It has been found that washed suspensions of rabbit polymorphonuclear leucocytes, besides hydrolysing phosphate esters (*Biochem J* 43: 111, 1948), also hydrolysed simple methyl esters of the aliphatic acids That polymorphonuclear leucocytes hydrolyse tributyrin has been reported previously by Fleischmann (*Biochem Ztschr* 200: 25, 1928) and by Barnes (*Brit J Exper Path* 21: 264, 1940) These workers, however, referred to the enzyme as a lipase It has now been shown that the enzyme is capable of hydrolysing simple triglycerides and simple methyl esters (up to C_8 have been tested), but not higher glycerides such as triolein The enzyme should, therefore, be referred to as an esterase rather than a lipase The rabbit polymorphonuclear leucocyte can hydrolyse neither acetylcholine, acetyl- β -methylcholine nor benzoylcholine and, therefore, contains neither 'true' cholinesterase, 'pseudo'-cholinesterase nor specific benzoylcholinesterase

Studies on copper-containing oxidases in higher plants
M L ROTHCHILD and ROBERT MACVICAR (introduced by V G HELLER) *Depts of Chemistry, Oklahoma A and M College and Agricultural Chemistry Research, Oklahoma Agricultural Experiment Station, Stillwater, Okla* The distribution of polyphenol oxidase and ascorbic acid oxidase in leaf-blade tissue of several species of higher plants has been determined Rapid oxidation of both catechol and 3,4-dihydroxy-DL-phenylalanine occurred in the presence of leaf tissue homogenates of several species of *Dicotyledonaceae* Of the plants studied tomato and sweet potato contained the most highly active enzyme system Polyphenoloxidase activity could not be demonstrated in wheat leaf-blade homogenate Ascorbic acid oxidase was present in sweet potato, soybean, and tomato leaf tissue, Swiss chard and wheat were less active Sodium diethyldithiocarbamate in concentration of 0.01 and 0.01M did not appreciably inhibit O_2 consumption by leaf tissue homogenates in the presence of added DOPA, ascorbic acid oxidation was

markedly inhibited by both concentrations of this substance. The endogenous rate of O_2 consumption was not significantly affected by this inhibitor. Copper deficient tomato plants showed a marked decrease in polyphenoloxidase and ascorbic acid oxidase activity. The administration of copper ions resulted in rapid restoration of oxidative activity of these two enzyme systems.

Relationship of cell surface phosphatases to sugar metabolism in yeast. ASER ROTHSTEIN and REBECCA MEIER (introduced by H. C. HODGE). *Atomic Energy Project of the Univ. of Rochester, Rochester, N. Y.* It has been shown that the surface of the yeast cell contains phosphatases, which can dephosphorylate a large variety of compounds including phosphorylated sugars. The surface phosphatases can be completely inhibited by very low concentrations of molybdate or tungstate without any apparent injury to the cell. The inhibition is reversible and competitive in nature. It seems to be specific for the phosphatases. In the absence of molybdate, phosphorylated sugars can be metabolized at a slow rate which is limited by the rate of dephosphorylation. In the presence of molybdate, the phosphorylated sugars are not metabolized at all although glucose and fructose are consumed at a normal rate. It can be concluded, therefore, that phosphorylated sugars cannot be metabolized directly as such, but that the sugar residue can be metabolized after the phosphate group is split off. The function of the cell-surface phosphatases seems to be limited to the splitting of phosphate containing compound in the medium into utilizable residues, with no direct role in carbohydrate metabolism involved.

Inhibition of oxidation and phosphorylation in kidney homogenates by yeast adenylic acid. HENRY Z. SABLE (introduced by CARL F. CORI). *Dept. of Biological Chemistry, Washington Univ. School of Medicine, St. Louis, Mo.* Various agents are known to inhibit aerobic phosphorylation in tissue homogenates. It was found that the oxygen consumption of washed kidney particles was increased to a maximum value, when, in addition to glutamate, Mg^{++} and fluoride, about $1 \mu M$ of muscle adenylic acid (adenosine 5' phosphoric acid) was added per 2 ml of reaction mixture. Increasing amounts of this nucleotide up to $12 \mu M$ caused a progressive increase in the uptake of inorganic phosphate from the medium, and correspondingly increased formation of acid labile phosphate compounds. When $6 \mu M$ of yeast adenylic acid (adenosine-3'-phosphoric acid) was added along with $12 \mu M$ of muscle adenylic acid, oxygen consumption was decreased, in a particular experiment, from 34 to $10 \mu M$, uptake of inorganic phosphate was decreased from 775 to $107 \mu g$ and formation of acid labile phosphate was decreased from 553 to $130 \mu g$. In a similar experiment muscle inosinic acid

(inosine-5'-phosphoric acid) in place of yeast adenylic acid had no inhibitory effect. The inhibitory effect of yeast adenylic acid on phosphorylation is probably due to competition with muscle adenylic acid for the enzyme which catalyzes the transfer of phosphate from intermediates formed during oxidation.

Formation of serine from glycine in the intact rat. WARWICK SAKAMI (introduced by HARLAND G. WOOD). *Dept. of Biochemistry, Western Reserve Univ. School of Medicine, Cleveland, Ohio.* Glycine has been shown to condense with formate or a formate derivative to form serine. When C^{13} carboxyl-labeled glycine and C^{14} -labeled formate were administered to rats the liver serine was found to contain the C^{13} in the carboxy group and C^{14} largely in the β -position. In the present investigation we have studied the physiological formation of 'formate'. A possible mechanism is the deamination of glycine (Ratner, Nozito and Green) and oxidative decarboxylation of the resulting glyoxylic acid: glycine \rightarrow glyoxylic acid \rightarrow 'formate' + CO_2 . To test the possible significance of this pathway in providing 'formate' for the conversion of glycine to serine we have administered glycine labeled with C^{14} in the methyl carbon to rats by stomach tube and determined the distribution of isotope in the liver serine and glycogen. The β -carbon of serine was almost (80%) as active as the α -carbon while the C^{14} was equally distributed between the 1, 6 and 2, 5 carbons of the glucose units of the glycogen. These results indicate that glycine itself may provide the formate or formate derivative involved in its conversion to serine. The data of Winnick, Moring-Claesson and Greenberg are in accord with this view. The β -carbon of their serine isolated from liver homogenate after equilibration with C^{14} methyl-labeled glycine contained a small amount of the isotope.

Tryptophane requirement of the rat as related to dietary level of other amino acids. W. D. SALMON. *Laboratory of Animal Nutrition, Alabama Polytechnic Institute, Auburn, Ala.* The addition of 12% of acid hydrolysate of casein or an equivalent amount of gelatine to basal diets containing 7% of casein, 0.02% of niacin, and either 40% of corn grits or 7% of corn gluten meal depressed growth of rats. The growth depression was corrected by DL-tryptophane but not by additional niacin, 0.5% of DL-tryptophane added to the diet prevented growth depression or even supported growth at a higher rate than was obtained on the original basal diet. When the casein content of the basal diet was increased to 20%, the addition of gelatine depressed growth only if niacin was omitted from the diet. With niacin in the diet, this level of casein provided sufficient surplus tryptophane to balance the added gelatine. When 5% of glycine was added to the diets containing 7% of casein, growth was de-

Pressed and the effect was not nullified by either niacin or tryptophane, the further addition of casein hydrolysate restored growth. Moreover, 5% of glycine added to a 21% casein basal diet, containing adequate niacin, did not depress growth significantly. It is apparent that, as the dietary level of other amino acids is increased, the essential requirement for tryptophane is also increased. The results with glycine suggest that the requirement for other essential amino acids may be similarly increased.

Effect of x-ray radiation and of starvation on hemoglobin synthesis in the rat. K. SALOMON, K. I. ALTMAN, G. W. CASARETT, and T. R. NOONAN (introduced by F. L. HAVEN). *Dept. of Radiation Biology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.* After whole body radiation and in acute starvation erythropoiesis is depressed. Information concerning the anabolism of the circulating hemoglobin under these conditions seems desirable. Since the alpha-carbon atom of glycine has been shown to be incorporated in hemin and globin, methylene labeled glycine was used in this investigation. Glycine (one or two microcuries) was administered to radiated and to starved rats. All animals were killed 24 hours later. Hemoglobin, hemin and globin were isolated from their blood and the C^{14} content of these compounds determined. The C^{14} activity ratio of hemin to globin on a gram to gram basis was found to range from 4-13 in all control animals. The ratio of C^{14} activity of hemin to globin isolated from rats injected with glycine immediately after radiation with 600 r varied from 25 to 32. Hemin synthesis was increased in all these rats. On the other hand, when glycine was injected six days after exposure to 600 r no measurable hemin synthesis occurred, and the activity ratio was found to be zero. The activity ratio after five days starvation was 64, hemin synthesis in these rats was increased whereas globin formation was depressed. In seven days starvation the activity ratio was zero, hemin synthesis was not measurable, globin formation was depressed. In both radiated and starved animals the ratio of C^{14} activity of hemin to that of globin deviates considerably from the control values.

Effects of india ink injections in avitaminosis A. STELIOS C. SAMARAS (by invitation), NICHOLAS DIETZ, JR. (by invitation) and VICTOR E. LEVINE. *Dept. of Biological Chemistry and Nutrition, Creighton Univ. School of Medicine, Omaha, Nebr.* One hundred male rats were divided into several groups, some of which served as controls while others were allowed to develop the classical signs of vitamin A deficiency. Rats on the adequate stock diet and those on the vitamin A-deficient diet plus carotene tolerated injections of India ink. Rats on the vitamin A-deficient diet presented a sudden aggravation of the usual deficiency symp-

toms when injected subcutaneously with India ink. The rats injected in the afternoon, when examined the next morning, showed startling symptoms referable to the visual apparatus. They seemed nearly blind or totally blind, and could not find their food and water. Enophthalmos was marked, the decrease in the size of the eyeball being apparently due to loss of fluid. In vitamin A-deficiency blindness has a slow onset and is the end result of dryness, bacterial invasion and infection, bacterial damage or ulceration, and repair with formation of opacities. In vitamin A deficiency aggravated by blocking or irritating the reticulo endothelial system blindness occurs suddenly due to loss of physiological function, and is probably a depletion phenomenon. The phagocytosis induced by the introduction of the foreign body, India ink, may completely exhaust the vitamin reserve or may increase the metabolic demand for vitamin A to such an extent that hardly any of the small quantity yet available can be allocated to the retina for the rhodopsin-retinene cycle. Histological studies and analyses of tissues for carotene and vitamin A are in progress.

Relationship of purines, folic acid, and other principles in the nutrition of *Leuconostoc citrovorum* 8081. H. E. SAUBERLICH (introduced by W. D. SALAMON). *Laboratory of Animal Nutrition, Alabama Polytechnic Institute, Auburn, Ala.* Further investigations on the nutrition of *Leuconostoc citrovorum* 8081 has revealed that the unknown factor appears to be involved in purine metabolism. The organism was found to require for growth either xanthine, guanine, or hypoxanthine. Adenine, thymine, and uracil had no apparent activity when tested alone, but did have a sparing action on xanthine, guanine, or hypoxanthine. Increased amounts of anti-pernicious anemia preparations, liver concentrates, rat urine preparations, or vitamin B₁₂ concentrates could replace (in part at least) the requirements for guanine, xanthine, or hypoxanthine. High amounts of folic acid could, in the presence of purines, replace the unknown factor required by the organism. The omission of folic acid had no effect upon the response of the organism to the various concentrates of the growth principle. However, aminopterin at a level of 10 gamma/10 ml assay tube inhibited growth completely. The inhibition could be reversed by anti-pernicious anemia preparations, liver concentrates, rat urine preparations, or thymidine. Folic acid was effective only when extremely high amounts were used. Rats injected with folic acid or fed diets containing 5 or 10 mg of folic acid/kg excreted in the urine a compound(s) active for the growth of *Leuconostoc citrovorum*. Sulfaguanidine fed to rats along with the folic acid did not decrease the activity of the urine. The feeding of vitamin B₁₂ concentrates, ascorbic acid, or thymine in the place of folic acid in the

diets to rats did not cause an increase in the activity of the urine

Effect of low environmental temperature on thyroid function as studied with radioactive iodine H G SCHACHNER, Z S GIERLACH and A T KREBS (introduced by H JENSEN) *Medical Dept, Field Research Laboratory, Fort Knox, Ky* From studies with radioactive iodine, it was concluded by Leblond *et al* (Leblond, Gross, Peacock and Evans *Am J Physiol* 140 671-676, 1943-44), that "exposure of rats to cold (0° - 2° C) for various periods of time produces a thyroid stimulation which is doubtful after 1-3 days, definite after 7 days, maximal at 26 days, but absent after exposure for forty days" A reinvestigation of the problem seemed indicated to show how early stimulation occurs and compares with the long time interval Rats were exposed in individual cages to temperatures of 1° - 4° C and the uptake and organic binding of tracer and carrier doses of inorganic iodide after 1, 3, 7, 9, 26 and 60 days exposure were determined A definite increased uptake of iodine was shown in the 1-, 3-, 7- and 9 day group The results of these early time intervals will be compared with 26-, 40- and 60-day groups

A study of tryptophane metabolism using isotope tracer technique R W SCHAYER (by invitation), G L FOSTER, and DAVID SHEMIN *Dept of Biochemistry, College of Physicians and Surgeons, Columbia Univ, New York City* Tryptophane containing N^{15} in the indole nucleus has been synthesized and resolved into the D- and L- isomers Tryptophane containing C^{14} in the β -position of the side chain has also been synthesized After the intraperitoneal injection of N^{15} L-tryptophane into rats and rabbits, the isolated kynurenine, kyurenic acid and xanthurenic acid contained N^{15} having approximately the same concentration as the ring nitrogen of the tryptophane Similarly, N^{16} -labeled kynurenine produced xanthurenic acid in rats without dilution The 6-pyridone of N-methyl nicotinamide, isolated from rat urine with the aid of carrier after intraperitoneal injection of N^{15} DL-tryptophane, was found to have a small but significant N^{15} concentration In contrast to this finding, the 6-pyridone isolated after incubation of N^{16} DL tryptophane with rat liver slices, and after intraperitoneal injection of the C^{14} DL-tryptophane in rats, contained no excess isotope Hemin isolated from rats after injection of large quantities of N^{15} DL-tryptophane had an insignificant N^{15} concentration Studies are in progress on the fate of N^{15} indole, N^{15} L-tryptophane and N^{15} D-tryptophane in rats

Determination of uric acid in whole blood and plasma ARNOLD H SCHEIN and EMILY F RICE (introduced by HAROLD B PIERCE) *Dept of Biochemistry, College of Medicine, Univ of Vermont, Burlington, Vt* The determination of uric acid by

the Folin method (or by its various modifications) is beset with many difficulties which render uncertain the values obtained thereby Lack of specificity, inhibition of color development by various substances and low recoveries of added uric acid have proved to be a great hindrance to accurate estimation of uric acid in biological material Specificity can be increased by employing uricase (Blauch-Koch, Buchanan) Starting at this point we have investigated the conditions necessary for the accurate and precise determination of uric acid in blood and plasma It was found that pH control (pH 9.2—borate buffer) during color development is a requisite for good recoveries of uric acid Folin-Wu filtrates of many substances, notably proteins and amino acids which cause color inhibition do so partially by virtue of their depressing effect upon the pH of the color reaction True inhibitions of color development of the order of 10-20% caused by many amino acids in their expected normal (and abnormal) plasma concentrations, can be completely compensated by 'swamping' both the standards and the solutions containing inhibitors with adequate amounts of glycine Experiments upon blood and plasma in which these new modifications of the Folin method were employed revealed 90-95% recovery of added uric acid Recoveries of the same order of magnitude were obtained upon addition of known amounts of uric acid to uricase incubated blood and plasma (uric acid free) These results were checked by ultra violet enzyme spectrophotometry

Intermediary metabolism of phenylalanine labeled with C^{14} BERNARD SCHEPARTZ (by invitation) and SAMUEL GURIN *Dept of Physiological Chemistry, School of Medicine Univ of Pennsylvania, Philadelphia, Penna* Administration of DL-phenylalanine (labeled in the carboxyl and alpha positions) to phlorhizinized rats results in the appearance of C^{14} in the urinary ketone bodies as well as in the respiratory CO_2 Upon incubation of this type of phenylalanine with liver slices, acetoacetate labeled solely in the carboxyl group is obtained When ring-labeled phenylalanine (carbons 1, 3, 5) is administered to phlorhizinized rats, C^{14} appears in the respiratory CO_2 as well as in the acetone moiety of the urinary acetoacetate Incubation of ring-labeled phenylalanine with liver slices produces acetoacetate labeled chiefly in the terminal methyl group The aromatic ring of phenylalanine readily undergoes cleavage in the course of metabolism, the immediate precursor of acetoacetate being a four-carbon unit derived from the ring and side-chain Apparently no 2 carbon units are involved in this pathway It is apparent that the alpha carbon of phenylalanine becomes the carboxyl carbon of acetoacetate while either carbon atom 1 or 3 of the ring forms the terminal carbon of acetoacetate This furnishes direct proof that

the side chain of phenylalanine shifts to an adjacent ring carbon during the formation of ketone bodies

The quantitative determination of glycerylphosphorylcholine (GPC) in tissues GERHARD SCHMIDT, L HECHT (by invitation), N STRICKLER (by invitation) and S J THANNHAUSER *Tufts College Medical School, Boston, Mass* Trichloroacetic acid filtrates of tissues are freed from interfering substances by precipitation with mercuric acetate at pH 5 (GPC and choline remain quantitatively in the supernatant) Aliquots of the Hg free supernatant are precipitated with Reinecke salt before (A) and after (B) acid hydrolysis (N HCl, 20', 100°) The reineckates obtained from the majority of tissues can be analyzed colorimetrically without further purification, but in certain tissues (intestinal mucosa) a reprecipitation of the concentrated acetone solution of the reineckate with aqueous Reinecke salt is necessary in order to obtain the choline reineckate in well crystallized form The hydrolyzable choline (B-A) represents the choline group of GPC The following amounts of GPC (mg P/100 gm fresh tissue) were found in fresh beef organs liver 25, pancreas 15, submaxillaries 4, muscle, heart, brain, intestines negligible Free choline negligible in all fresh tissues After 3 hours' incubation, a strong increase in the amounts of GPC occurs in pancreas (40), and strong increases of free choline, but not of GPC, occur in kidney and intestines In incubated liver the amount of GPC remains practically unchanged, and no free choline appears in appreciable quantities

Factors concerned in the liberation of pteroylglutamic (folic) acid from its heptaglutamate by chick liver M L SCOTT (by invitation), C H HILL (by invitation), and L C NORRIS *Cornell Univ, Ithaca, N Y* Previous results from this laboratory have shown that under certain conditions the activity of the vitamin B₉ conjugase system in the livers from young chicks is increased by the addition of β -pyracin (4-pyridoxic acid) (*J Biol Chem* 158 291, 1945, *ibid* 160 265, 1945) In a continuation of these studies, it has been found that livers from day-old chicks, produced by hens whose diet contained no animal protein, were nearly devoid of vitamin B₉ conjugase activity Under these conditions the effect of added β -pyracin was variable On the other hand, the addition of certain concentrates derived from the alcohol-soluble material obtained in the preparation of a commercial liver extract produced marked increases in the liberation of folic acid from pteroyl-heptaglutamate This effect was further enhanced when both the concentrates and β -pyracin were combined In view of the suspected presence of small amounts of the antipernicious anemia factor in the liver concentrates, it was thought that

vitamin B₁₂ might be one of the factors required in the conjugase system Accordingly, experiments were conducted in which concentrates of this vitamin were added to the liver incubation system with and without β -pyracin Synergistic effects were also obtained between these two factors at a level of the vitamin B₁₂ concentrate which provided approximately 4 μ M of vitamin B₁₂ Preliminary work using crystalline vitamin B₁₂ have yielded similar results The results obtained in these studies indicate, therefore, that this vitamin is required in addition to β -pyracin in the enzymatic release of folic acid

Activation of purified prothrombin with sodium citrate WALTER H SEEGER and ARNOLD G WARE (by invitation) *Dept of Physiology and Pharmacology, Wayne Univ, Detroit, Mich* Purified prothrombin prepared by the method of Ware and Seegers (*J Biol Chem*, 174 565, 1948) is free of thromboplastin and Ae-globulin The protein, when dissolved in 30% sodium citrate solution, is transformed to thrombin At room temperature thrombin first begins to appear slowly after 16 hours, then rapidly for about 3 hours, and thereafter again more slowly The form of the activation curve suggests autocatalysis The prothrombin molecule apparently contains all of the necessary structural material for the formation of thrombin The activation with sodium citrate can be completely blocked with small amounts of 3,4,4'-triaminodiphenyl sulfone or with 2-hydroxy-4,4'-diaminodiphenyl sulfone All of the protein of the prothrombin preparation can be adsorbed on BaCO₃ The thrombin activity is not adsorbed on BaCO₃ Consequently purified prothrombin can be activated with sodium citrate, and any protein which is then not adsorbed on BaCO₃ must be thrombin alone or thrombin together with other parts of the original prothrombin This provides an easy method to prepare thrombin Moreover, all of the above facts are unusually valuable for gaining insight into the nature of prothrombin, its activation, and the nature of thrombin

The estimation of glycogen in liver and muscle SAM SEIFTER and SEYMOUR DAYTON (by invitation) *Dept of Biochemistry, Long Island College of Medicine, Brooklyn, N Y* Dieywood (*Ind Eng Chem, Anal Ed*, 18 499, 1946) described the use of anthrone for detection of carbohydrates, and Morris (*Science*, 107 254, 1948) suggested its use for the estimation of glycogen The present authors have employed the reagent in a simple direct method for glycogen in liver In this method 4 to 8 gm of liver (less may be used) are delivered into a weighed, graduated tube containing approximately 25 cc of 30% KOH, and the tube reweighed The tube contents are digested for 30 minutes in a boiling water bath, cooled, and made up to 35 cc with KOH Appropriate dilutions of the digest are

made with water (1 50, 1 250, etc.), and 5 cc aliquots are transferred to Evelyn tubes. While submerged in cold water, the tubes then receive 10 cc of 0.2% anthrone made up in 95% H_2SO_4 . 5 cc of standard containing 80 γ of glucose is similarly treated. All tubes are then heated for 10 minutes in boiling water, cooled, and read in the Evelyn photoelectric colorimeter at wavelength 620. The glycogen content of the liver is then calculated. When the glycogen content of liver or muscle is less than 0.6% this method is inapplicable due to interference of color produced by anthrone with the relatively increased protein contents of adequate aliquots. In these cases the glycogen may be first precipitated with alcohol, redissolved, and determined as above. The results agree with those obtained by the method of Good, Kramer and Somogyi.

Effects of ionizing radiation on the anaerobic CO_2 production and colony production in *saccharomyces cerevisiae* FREDERICK G. SHERMAN and HERMAN B. CHASE (introduced by P. H. MITCHELL) *Brown Univ., Providence, R. I.* Yeast was grown (48 hours at 30°C) in Reader's medium to which was added glucose 20 gm/liter and biotin, 2 micrograms/liter. Cells were washed twice with $\text{m}/15 \text{ KH}_2\text{PO}_4$ or supplemented Reader's medium, diluted to standard density and dispensed into lusteroid centrifuge tubes. One half the tubes were irradiated 30 minutes (90,000r at 3000r/min). A 200 kv therapy tube operated at 20 ma was used as the source. After irradiation cell suspensions were transferred to small flasks and incubated at 30°C for 2 to 24 hours. Aliquots were taken for plate counts and manometric estimation of anaerobic CO_2 production at stated intervals. Two to 4 hours after the beginning of irradiation the anaerobic CO_2 production (glucose or supplemented Reader's as substrate) is inhibited approximately 30% and about 85% of the irradiated cells are unable to form visible colonies. No inhibition of anaerobic CO_2 production was observed immediately after irradiation. Between 12 and 24 hours anaerobic CO_2 production on a per cell basis progressively declines suggesting that some 'injured' cells have further reduction in ability to ferment glucose, or have lost it entirely. Investigations by earlier workers on the effect of γ -radiation on enzyme systems *in vivo* indicate that fairly large doses have practically no effect in diminishing their activity (Wels and Osann, *Pflügers Arch.* 207: 156, 1925). Only with yeast grown on Reader's medium plus biotin have we been able to obtain consistent and reproducible inhibition of fermentation after irradiation.

The biological synthesis of serine from glycine PHILIP SIEKEVITZ (by invitation), THEODORE WINNICK and DAVID M. GREENBERG *Div of Biochemistry, Univ of California Medical School, Berkeley, Calif.* The *in vitro* synthesis of serine

from glycine has been demonstrated both in rat liver slices and homogenates by means of tracer experiments with C^{14} -labeled glycine. The reaction proceeds anaerobically. Employing-carboxyl-labeled and methylene-labeled glycine, it has been shown that the carboxyl-carbon of glycine appears in the carboxyl-group of serine, while the methylene-carbon of glycine appears in both the alpha and beta carbons of serine. Aerobically, the C^{14} concentration of the beta-carbon was approximately 40% that of the alpha-carbon. Anaerobically, it was only 5%. Sakami (*J Biol Chem* 176: 995, 1948) has shown that formate reacts with glycine to form serine, the formate carbon appearing in the beta carbon of serine. We have now shown that this formate, at least in part, may be derived from glycine itself. Rat liver slices were incubated in nitrogen with radioactive glycine and non-radioactive formate, the latter at twice the concentration of the former. One flask (I) contained methylene-labeled glycine, the other (II) had carboxyl-labeled glycine. Carrier formate was added, steam distilled, and oxidized to CO_2 (Osborn, Wood, and Werkman, *Anal Ed, Ind Eng Chem* 5: 47, 1933). Glyoxylic acid is not oxidized to CO_2 by this method. The CO_2 obtained from flask (I) was radioactive, that from flask (II) was not, showing that formate is derived from the alpha carbon of glycine. The dilution of radioactivity in the beta-carbon indicates a non glycine source of formate, which is more active anaerobically than aerobically.

Oxygen consumption of 'Killer' and 'Sensitive' stocks of *paramecium aurelia*, variety 4 D. H. SIMONSEN (by invitation) and W. J. VAN WAGEN-DONK *Dept of Zoology, Indiana Univ., Bloomington, Ind.* Two genetically diverse stocks occur within variety 4 of *Paramecium aurelia*. The 'killer' stocks produce a toxic substance, 'paramecin', which kills 'sensitive' animals in a typical manner. The two types are determined genetically. The killer stocks possess the killer gene K which can support the multiplication of the self-duplicating cytoplasmic factor, 'kappa', but cannot initiate its production *per se*. Kappa in turn controls the production of paramecin. Sensitive animals may be those having the genotype KKK without kappa, or those with the recessive allele kk. Sensitive animals are killed in a typical fashion when mixed with killer animals, when placed in culture fluid in which killers have grown, or when exposed to a dilute brew prepared by crushing killer animals. Measurements of the rates of oxygen consumption of these stocks were made using the Cartesian diver micro-respiration technique. The stocks used for comparison belonged to the same mating type and were grown under the same conditions of feeding. The live animals were suspended in sterile 'exhausted' lettuce infusion in the divers. Rates of oxygen consumption were determined over a period of 8-10 hours at 27° .

Killer animals were found to respire at a higher rate than sensitive animals. The respiration of sensitive animals while under the influence of paramycin was followed. Pertinent data will be presented.

Serum lipides of Eskimos. Effect of a high fat diet (pemmican) and of fasting. R. G. SINCLAIR, G. M. BROWN (by invitation) and L. B. CRONK (by invitation). *Depts. of Biochemistry and Medicine, Queen's Univ., Kingston, Canada.* As part of a clinical and biochemical study of the Eskimos on Southampton Island in the Eastern Canadian Arctic, lipides were determined in the serum of 126 persons. Regardless of age, sex and absorptive state, the average values, in mg/100 ml, were: Total fatty acids, 329 ± 103 (S.D.), lipide P, 9.9 ± 2.0 , total cholesterol, 173 ± 29 , free cholesterol, 47.5 ± 9.4 . Free cholesterol amounted to $27.7 \pm 3.0\%$ of total. The molar ratio of phospholipide to total cholesterol was 0.72 ± 0.09 . A palpable hepatomegaly was observed in about $\frac{1}{3}$ of the Eskimos. The average values for the serum lipides in this group were the same as in those without enlarged livers. In a small group of 2 men and 2 women who subsisted for 6 days exclusively on pemmican (75% of the calories from beef fat), the levels of all lipides increased considerably. A subsequent 4 day fast by 3 subjects caused a further appreciable increase in all lipides—total fatty acids, 55% increase, lipide P, 30%, total cholesterol, 15%. A similar fast by a man who had been on his customary diet of meat and bannock caused an increase in total fatty acids but not in lipide P, total or free cholesterol. Little or no ketonuria developed after 6 days on pemmican, the subsequent fast caused a definite ketonuria.

The lipotropic action of threonine. S. A. SINGAL, V. P. SYDENSTRICKER (by invitation) and JULIA M. LITTLEJOHN (by invitation). *Univ. of Georgia School of Medicine, Augusta, Ga.* While studying the nicotinic acid-tryptophan relationship in rats on 9% casein ration containing 5% fat and 0.2% cystine and choline, yellowish livers were observed at autopsy. Upon analysis the liver lipids were 16.0%. Supplementary nicotinic acid, tryptophan, valine, histidine, phenylalanine or serine were without effect. The addition of threonine reduced the liver lipids to 5.1%. Because of the growth depressing action of threonine under these experimental conditions (*J. Biol. Chem.* 176: 1063, 1948), nicotinic acid or tryptophan was added to this diet under which conditions growth was satisfactory and the liver lipids were 6.6%. When choline was increased to 1.0%, a similar reduction of liver lipids was observed. That the lipotropic action of threonine was not the result of increased food intake was demonstrable in paired-feeding experiments. Here the liver lipids of rats on the casein ration supplemented with tryptophan was 14.4% as compared to 5.9% when threonine was also included. When the dietary fat was increased to 40%, the liver lipids

were 21.8 and 10.1% respectively. The livers of rats on an amino acid diet simulating the 9% casein ration supplemented with tryptophan contained 17.0% lipids. Supplementary threonine reduced the lipids to 5.3%. When threonine was omitted from the entire ration, the rats lost weight and the liver lipids were only slightly above normal.

Unlikelihood of specific long range forces in immunologic and enzymatic reactions. S. J. SINGER (introduced by L. ZECHMEISTER). *Gates & Crellin Laboratories of Chemistry, California Inst. of Technology, Pasadena, Calif.* Rothen has performed experiments (*J. Biol. Chem.* 168: 75, 1947) in which antibody is adsorbed on antigen films which are apparently covered with inert barriers, and other experiments (*J. Am. Chem. Soc.* 70: 2732, 1948) in which enzymatic reactions occurred despite apparent separations of enzyme and substrate proteins. From these he concludes that specific long range forces are involved in immunologic and enzymatic reactions. By 3 approaches, we have obtained evidence that holes in the barrier films are responsible for the effects observed. 1) Certain cellulose derivatives used as inert barriers reduce the adsorption of antibody on films of bovine serum albumin considerably. Ethyl cellulose films cast from ethylene dichloride solution are particularly effective, but those cast from acetone solution permit increased antibody adsorption. This demonstrates the importance of the physical state of the barrier films. 2) With the electron microscope, we find that antibody is concentrated in clumps when adsorbed on formvar films above bovine serum albumin layers, although antibody is evenly distributed when adsorbed directly on the antigen, and the surfaces of the formvar films without antibody are uniform. This suggests that reaction occurs through holes in the formvar films. 3) Intervening formvar films permit antibody directed against the p-azophenylarsonic acid group to be adsorbed on p-azophenylarsonic acid conjugated proteins. Since the work of Landsteiner, Pauling and collaborators, among others, makes it extremely likely that these conjugated protein systems react by a short range mechanism, these experiments indicate that the antigen and antibody molecules actually come into contact through holes in the polymer films.

Inhibitors of the L-amino acid oxidase of snake venoms. THOMAS P. SINGER and EDNA B. KEARNEY (by invitation). *Dept. of Biochemistry, Western Reserve Univ. School of Medicine, Cleveland, Ohio.* The L-amino acid oxidase of snake venoms, apparently a yellow enzyme, is inhibited by flavin analogues. The analogue, isoriboflavin, which has proved inhibitory to every yellow enzyme tested in this laboratory, inhibits the L-amino acid oxidase of moccasin venom 50% at 0.0004M concentration. Riboflavin itself inhibits appreciably even at

0.00004M concentration 0.002M atabrine decreases the activity by 50%. A group of polyvalent anions are even more inhibitory to this enzyme. Notable among these are inorganic phosphate and arsenate, which completely inactivate the enzyme at 0.001M or lower concentrations, under appropriate conditions. An extensive study of this enzyme in various venoms revealed that the oxidases of pit vipers are highly sensitive to phosphate, those of true vipers less sensitive, and those of colubrine venoms insensitive. While the action of riboflavin analogues on the enzyme is instantaneous, the effect of phosphate and arsenate increases with time and also with temperature, pH, and the molarity of phosphate, it is inversely related to the protein concentration. Monovalent anions and organic bases protect the enzyme from the action of phosphate, while substrates and riboflavin analogues protect in catalytic amounts. The inhibition is not of the competitive type. Metaphosphate, pyrophosphate, organic phosphates, arsenite, molybdate, sulfate, and thiosulfate are less inhibitory, but ferricyanide is as effective as phosphate. The kinetics of the reaction have been studied in detail, and a combination with the enzyme protein is suggested as a probable mechanism.

Phosphomannose isomerase MILTON W. SLEIN (introduced by GERTY T. CORI) *Dept. of Biological Chemistry, Washington Univ. School of Medicine, St. Louis, Mo.* Lohmann's phosphohexose isomerase catalyzes the reaction, glucose-6-phosphate \rightleftharpoons fructose-6-phosphate. A purified isomerase which can be prepared from rabbit muscle or from yeast does not act on mannose-6-phosphate. The latter was prepared by allowing crystalline yeast hexokinase to act on mannose in the presence of ATP. It was found that rabbit muscle contains a separate enzyme which together with Lohmann's isomerase establishes an equilibrium between the three phosphorylated hexoses in the approximate ratio of glucose 56, fructose 29, and mannose 15%.

Isolation of the staphylococcus stimulatory factor(s) (SSF) NATHAN H. SLOANE (by invitation) and RALPH W. MCKEL *Dept. of Biological Chemistry, Harvard Medical School, Boston, Mass.* *Staphylococcus albus* grows maximally on a synthetic medium but with a lag phase of 12-16 hours. Growth is stimulated with extracts of vegetable, animal and bacterial products. Antipernicious anemia liver extract is inactive. The active factor(s) in deproteinized plasma and urine are non-dialyzable, heat stable, moderately stable to acid and alkali, stable to proteolytic enzymes, soluble in methanol (pH 3), insoluble in common organic solvents, precipitable with silver (pH 6) and inactivated by ninhydrin. Combining dialysis and silver precipitation the activity in human urine is concentrated 200- to 400-fold (nitrogen) with almost 100% recovery. The material is quite unstable upon

standing but activity can be restored by reaction with glutathione. The electrophoretic pattern (pH 8.6) shows five peaks, the mobilities of two being within the range of α_1 and α_2 globulins, the other peaks had mobilities greater than the normal plasma constituents. Using bovine plasma, deproteinizing with heat (about pH 5), dialyzing and precipitating with silver, the activity is concentrated 140- to 400-fold (0.06-0.021 mg N/μ) with 50-100% recovery. The electrophoretic pattern (pH 8.6) shows 4 peaks, the mobilities of 2 being within the range of α_1 and α_2 globulins. This concentrate gave a positive orcinol hexose test. Treatment of this concentrate with chloroform-isoamyl alcohol (pH 6.6) causes a 90-100% loss of activity, which is restored with glutathione or cysteine. The degree of purification is 850- to 1700-fold (nitrogen). The purified material is non-dialyzable. Human plasma and its protein fractions (Cohn *et al.*) were studied and fraction 1V-4 contained the greatest concentration of SSF.

Studies on transaminase in germinating seeds and its relation to protein synthesis BETSY PATTERSON SMITH (by invitation) and HAROLD H. WILLIAMS *Dept. of Biochemistry and Nutrition, Cornell Univ., Ithaca, N. Y.* The relative changes in the amounts of glutamic-alanine and glutamic-aspartic transaminase and protein were determined during the first 120 hours of germination of seeds of *Graminae* (barley and corn), *Leguminosae* (pea and wax bean), and *Cucurbitaceae* (pumpkin and squash). All the seeds tested began germination with the same low level of transaminase, but the amount synthesized during growth ranged from 0.3 units in the pea to 1.33 units in barley for the glutamic-aspartic transaminase and from 0.20 units in squash to 0.70 units in barley for the glutamic-alanine enzyme. In almost every case the glutamic-aspartic transaminase increases more rapidly than the glutamic-alanine enzyme. In general, the rate of transaminase formation during germination was greater than that of protein as judged by the increased number of units of the enzyme per milligram of protein nitrogen. Supplying the germinating seeds with a nutrient solution caused an increased rate of synthesis of both transaminase and protein, but the change is greater for the enzyme than for protein. Thus it is indicated that transamination is limited in its role in the plant to the interconversion of amino acids and keto acids, and that the new amino acids formed may have several possible fates, only one of which is the synthesis into protein.

Optical and side-chain specificity of leucine aminopeptidase EMIL L. SMITH and W. J. POLGLASE (by invitation) *Laby for the Study of Hereditary and Metabolic Disorders and the Depts. of Biochemistry and Medicine, Univ. of Utah School of Medicine, Salt Lake City, Utah.* The side chain

specificity of this enzyme is not absolute, a number of aliphatic amino acid amides are attacked at different rates. These rates were estimated from the first-order coefficients obtained with two different preparations of the enzyme, one having 20 times the activity of the other. Nevertheless, the relative rates for the 2 preparations for the following compounds were identical: L-leucinamide (100), DL-norleucinamide (100), DL-norvalinamide (80), DL- α -n-aminobutyrylamide (44), L-alaninamide (6.3), and glycylamide (0.34). Only the L forms are hydrolyzed, D-leucinamide, D-leucylglycine, and D-leucyl-L-tyrosine are completely resistant to the enzyme. L-Leucyl-L-alanine is split about 25 times faster than L-Leucyl-D-alanine. Both bonds of L-alanyl-L-leucinamide are rapidly hydrolyzed, and the rate is about 850 times greater than the action on D-alanyl-L-leucinamide where only the terminal amide linkage is split. β -Alanyl-L-leucinamide is hydrolyzed even more slowly than is the latter compound. An interpretation of the action of this enzyme has been made in terms of the poly-affinity theory. The substrate combines with the protein through the metal (Mn^{++} or Mg^{++}) at the free amino group and at the nitrogen of the sensitive amide or peptide linkage. The residue which bears the carbonyl of the sensitive bond has its side-chain bound directly to the protein, the affinity of the protein for the side-chain is a function of the mass of the aliphatic group. The enzymatic specificity thus depends on correct steric relationships of the side-chain and of the groups which are bound to the metal.

Interrelationship between chloramphenicol (chloromycetin) and enzymatic systems. GRANT N. SMITH and CECILIA WORRELL (introduced by O. D. BIRD). *Research Laboratories, Parke, Davis & Co., Detroit, Mich.* Studies of the action of chloramphenicol on enzymatic systems have been conducted to determine if the action of the new antibiotic can be explained on the basis of an inhibitory effect on enzymes and enzyme systems. Members of the following classes of enzymes: esterases, carbohydrases, amidases, proteolytic enzymes, iron oxidases, copper oxidases, dehydrogenases and miscellaneous enzymes were subjected to the action of chloramphenicol. Three methods were used to evaluate these actions. The first method used was to test the action of the antibiotic on highly purified preparations of the isolated enzymes. The other 2 methods consisted of studies on the intact enzymes present in bacterial cells using the plate technique developed by Dufrenoy *et al.* and the broth culture technique. No indications of inhibitory action of chloramphenicol could be detected in any of the systems studied. The effect of various enzymes and enzymatic systems on chloramphenicol have also been studied to determine if the antibiotic can be destroyed by enzymatic conversion to an inactive

form. Both isolated enzymatic systems and enzymes liberated into the culture media by intact organisms have been tested on the antibiotic. Results have shown that a partial breakdown of chloramphenicol is possible.

Phosphorus metabolism of molds. I. Uptake and transformation of orthophosphate by penicillium chrysogenum Q176. J. F. SNELL (by invitation) and W. H. PETERSON. *Univ. of Wisconsin, Madison, Wis.* Uptake of orthophosphate by *P. chrysogenum* Q176 in shaken flask and stirred jar fermentations on various media has been determined. Although there was considerable variation, the most rapid and complete uptake occurred in stirred jar fermentations on corn steep media. Mycelia varied in total phosphorus content (0.7%–5%, dry basis) depending upon aeration, age, media, etc. In a given fermentation, for example, the dried mycelium contained 3.1% at 3 days, reached a maximum of 4.7% at 6 days and fell to 2.1% at 8 days. On media containing a large excess of orthophosphate, higher amounts accumulated in the mycelium. From 30–60% of the total phosphorus was extracted by 4% trichloroacetic acid in 2 hours at 0°. The proportion of labile phosphorus to total phosphorus in the extract (0–6%) was affected by medium age of mycelium, method of extraction, etc. A typical extract of mycelium showed a $P_0:P_1:P_{>1}$ ratio of 25:6:69. Inorganic pyrophosphate and metaphosphate have been isolated from the extract, in confirmation of the work of Mann. Further components of this extract have been studied. No adenosine triphosphate has been obtained from the mycelium by procedures which gave good preparations from rabbit muscle in parallel experiments. Studies on the rate of turnover of the forms of phosphorus have been limited by inadequate fractionation procedures. Fractionation has not been successful by schemes proposed to date in the literature. Some fractionation has been achieved, however, and turnover studies utilizing P^{32} are in progress.

Aqueous dispersions of fat-soluble vitamins. ALBERT E. SOBEL, ABRAHAM ROSENBERG (by invitation), ROY GEDULDIG (by invitation), ELI ENGEL (by invitation), MICHAEL WEST (by invitation) and BENJAMIN KRAMER. *Dept. of Chemistry and Pediatrics, Jewish Hospital of Brooklyn, Brooklyn, N. Y.* Post-partum mother rats were fed vitamin A in aqueous dispersion or the same A in oily solution. The suckling rats of the aqueous group showed higher vitamin A stores. The same preparations had been previously fed nursing women. Distinctly higher rises of A were found in the milk of the aqueous group. Thus, one might conclude that with aqueous dispersions (without and with other vitamins, Vifort) there is an increased total secretion of milk vitamin A. The higher blood levels obtained with aqueous dispersions fulfill the theoretical requisites for greater diffusion to the site of

milk production To produce controlled high levels and overcome absorption problems, conditions were developed for intravenous injection of aqueous dispersions of fat-soluble vitamins No hemolysis occurs in whole blood when concentration of the dispersing agent is 0.1% or less Bearing this in mind, aqueous dispersions of vitamins A and E were intravenously injected into rabbits with no pathologic effects That aqueous dispersions may help in the absorption of other fat-soluble vitamins besides A was shown by studies with E Higher absorption in normal adults occurred when aqueous dispersions of vitamin E were given orally as measured by vitamin E absorption curves

Cystine and methionine content of portal blood plasma JAMES D SOLOMON (by invitation), and HAROLD D WEST *Dept of Biochemistry, Meharry Medical College, Nashville, Tenn* The cystine and methionine levels in the plasma of portal and peripheral blood were determined before and after administration of these amino acids to the dog Since failure of cystine to rise in the peripheral blood plasma after feeding this amino acid has been demonstrated, it appeared that a study of its content in the portal blood plasma might throw further light on the absorption of this amino acid Cystine together with methionine was fed by gavage to the tracheal-cannulated dog under ether anesthesia Blood samples were taken from portal and femoral veins before feeding and at intervals of one and one-half and three hours Cystine and methionine were determined in the plasma tungstate filtrates before and after hydrolysis by use of microbiological methods The control samples from the portal and femoral (peripheral) vessels showed no increase in the microbiologically available methionine or cystine following hydrolysis of the tungstate filtrates In samples taken after administration of these acids to the animal there was no significant rise in the level of the cystine in either case, but there was significant rise in the methionine level in portal blood accompanied by a much greater increase in its content in peripheral (femoral) blood When these filtrates were hydrolyzed there was a considerable increase in cystine but the increase in methionine content of the portal blood plasma was not significant, in the peripheral blood filtrate hydrolysates there was no increase in the content of cystine or methionine

Electrophoretic studies of the soluble proteins of rabbit liver S SOROF (by invitation) and P P COHEN *Laby of Physiological Chemistry, Univ of Wisconsin, Madison, Wis* Reproducible electrophoretic patterns have been obtained using high speed supernates of homogenates of perfused rabbit liver All experiments were carried out in a cold room maintained at 4°C Aliquots of whole liver, or homogenate, or supernate from the same rabbit liver were used for comparative electrophoretic ex-

periments The following factors were investigated with reference to their effects on the electrophoretic patterns homogenization medium, pH stability, time stability, effect of freezing and thawing, and ultracentrifugation From the large number of studies carried out, the following emerged as significant findings 1) the electrophoretic pattern of rabbit liver supernate consists of four principal components The component in liver supernate with the mobility of rabbit serum albumin electrophoretically represents 4% of the soluble liver proteins 2) The soluble proteins of rabbit liver are unstable, unless special measures are taken Certain of these alterations are reversible 3) Exposure to air causes changes principally in two components Cysteine, thioglycollate and glutathione are each capable of reversing the aerobic changes in one of these two components However, in order to reverse the aerobic changes in the other component, glutathione is required Ascorbate is ineffective toward both components 4) In the case of first mentioned component, present evidence indicates that protein sulfhydryl groups are involved in a reversible reaction

Composition of brain lipids in male and female rats WARREN M SPERRY, FLORENCE C BRAND (by invitation), and MERRILL WEBB (by invitation) *Dept of Biochemistry, New York State Psychiatric Inst, New York City* Weil reported (*Growth*, 7: 257, 1943) that brains of female rats, 90-180 days old, contained more 'ether-soluble phospholipids (cephalin)' and less 'alcohol-soluble (lecithins), pyridine-soluble (galactolipids), and -insoluble lipids (sphingomyelins)' than male brains Such a sex difference should manifest itself in the P:choline ratio of the brain lipids In a few preliminary measurements, reported previously (*Proc Div Biol Chem, Am Chem Soc*, 50C Sept, 1948), a small, but not statistically significant difference in this ratio was observed However, further studies under more carefully controlled conditions the same average P:choline ratio was obtained in rats of both sexes Following are the average values, with standard deviations, for various lipid constituents, determined in this investigation, expressed as percentage of fresh brain, from 125-156 days-old male and female rats, respectively Choline, 0.462 ± 0.019 , 0.469 ± 0.027 , P, 0.245 ± 0.018 , 0.248 ± 0.011 , cholesterol, 1.89 ± 0.14 , 1.89 ± 0.11 , glycosphingosides, 2.47 ± 0.13 , 2.45 ± 0.11 Average stoichiometric ratios calculated from these data are P:choline, 2.07 ± 0.15 , 2.07 ± 0.19 , cholesterol:choline, 1.28 ± 0.10 , 1.27 ± 0.13 , P:cholesterol, 1.62 ± 0.08 , 1.64 ± 0.07 , P:glycosphingosides, 2.63 ± 0.13 , 2.68 ± 0.22 No support for the hypothesis that there is a sex difference in the lipid composition of the brain may be derived from these data The findings will be com-

pared with values determined in the same way in rats of other ages

Combined ultraspectrographic and chemical studies in cerebrospinal fluids (CSFs) in normal and pathologic conditions M SPIEGEL-ADOLF and H T WYCKS (by invitation) *Depts of Colloid Chemistry and Neurosurgery, Temple Univ Medical School and Hospital, Philadelphia, Penna* Systematic ascorbic acid determinations in CSFs (*Federation Proc* 7 No 1) were made according to Robinson and Stotz In 20 cases 2 ascorbic acid standards were used The average error was $0.17 \pm 1.83\%$ Ascorbic acid was determined simultaneously with the ultraspectrographic examinations The extinction coefficients E_{CSF} at $265 \text{ m}\mu$ were corrected for the corresponding values of ascorbic acid E_A and proteins E_P $D = E_{\text{CSF}} - (E_A + E_P)$ was determined in 60 cases of functional and organic neurological conditions The lowest D values ($0.0-1.4$) were observed in psychoneurotics, old brain injuries, initial stages of brain affections The highest D values (from $0.61-2.16$) occurred in brain tumors, frequent grand mal attacks, spinal block, compression of the cord, recent cerebral concussion Intermediate values were found in milder convulsive states, herniated discs, head traumata without unconsciousness and brain tumors of specified histological and/or anatomical properties In an attempt to analyze the substances producing the D values micro-Kjeldahl determinations of the non-protein-nitrogen content were carried out in CSFs showing low and on samples showing high D values The NPN values of the latter exceeded the supposedly normal values by an average of 50% The above results seem to indicate that the selective absorption of pathologic CSFs at $265 \text{ m}\mu$ is not explained by the ascorbic acid and protein content alone Presence of nucleic acids and/or their cleavage products is suggested by the optical behavior NPN determinations as well as the clinical findings support this interpretation

Relative importance of plasmatic and vascular factors of hemostasis in the hemorrhagic diathesis of liver dysfunction MARIO STEFANINI (introduced by A J QUICK) *Dept of Internal Medicine, Univ of Roma, Italy, and Dept of Biochemistry, Marquette Univ School of Medicine, Milwaukee, Wis* Of 200 patients with evident liver dysfunction, 30 (15%) presented spontaneous hemorrhages The effectiveness of the hemostatic mechanism was studied in all, in an attempt to evaluate the relative importance of vascular and plasmatic factors of hemostasis in the pathogenesis of the hemorrhagic manifestations of these cases Capillary fragility was variably increased in all patients Plasma prothrombin activity was below 50% of normal in 53.3% of the cases, and below 20% in 23.3% Plasma fibrinogen level varied from 90-356 mgr % being, therefore, constantly above the critical level

of 60 mgr % established by Pinniger and Prunty Changes in antithrombin activity of plasma were too inconsistent for definite conclusions Therefore the diminution of capillary resistance was predominantly important in the pathogenesis of spontaneous hemorrhages in this series of liver patients Furthermore, among the remaining 170 patients not presenting hemorrhages, capillary resistance was found diminished in 2 cases only, and prothrombin activity below 20% of normal in 9, so it appears that even a serious defect of the coagulation mechanism did not determine spontaneous hemorrhages when capillary resistance was normal Hypoprothrombinemia and diminution of the capillary resistance found in liver disorders may present a common pathogenetic factor because of their association in about 50% of cases As vitamin K normalizes the diminished capillary resistance of reversible liver dysfunction, this common factor might be represented by a defect of utilization of the vitamin by a diseased liver

Effect of various adsorbants on the components of the prothrombin complex MARIO STEFANINI (by invitation) and ARMAND J QUICK *Dept of Biochemistry, Marquette Univ School of Medicine, Milwaukee, Wis* Oxalated plasma when treated with $\text{Ca}_3(\text{PO}_4)_2$, $\text{Al}(\text{OH})_3$, BaSO_4 , or $\text{Mg}(\text{OH})_2$ will not clot on recalcification even in the presence of excess thromboplastin These agents adsorb varying quantities of the components of the prothrombin complex and also some fibrinogen By carefully regulating the quantity of adsorbant, a fairly quantitative separation especially of component B, the factor which diminishes in dicumarol poisoning, can be achieved The best agent is $\text{Ca}_3(\text{PO}_4)_2$ since in the proper concentration (0.005 M) it adsorbs component B completely, but removes only a trace of the labile factor and fibrinogen The action of BaSO_4 is similar Both of these adsorbants are unable to remove component B from citrated plasma, therefore no decrease in prothrombin activity occurs on mixing citrated plasma with either of these adsorbants The prothrombin time of citrated plasma is prolonged to infinity by $\text{Al}(\text{OH})_3$ and $\text{Mg}(\text{OH})_2$ Citrate therefore does not block the adsorption of component B by these agents Neither $\text{Al}(\text{OH})_3$ nor $\text{Mg}(\text{OH})_2$ are as selective as $\text{Ca}_3(\text{PO}_4)_2$ for they adsorb appreciable amounts of the labile factor, some fibrinogen, and probably other factors that are associated with coagulation This explains why the prothrombin time curve constructed by diluting plasma with alumina treated plasma is similar to the curve using saline as diluent, whereas the curves obtained with BaSO_4 and $\text{Ca}_3(\text{PO}_4)_2$ treated plasmas as diluents are not

Combination of wool protein with strong bases JACINTO STEINHARDT and ETHEL M ZWISER (by invitation) *Dept of Chemistry, Massachusetts*

Inst of Technology, Cambridge, Mass Earlier work with 2 proteins, wool and egg albumen, has shown that their titration curves, obtained with different strong acids, differ widely in position on the pH axis. This effect is due to combination of the proteins with anions as well as hydrogen ions, and the differences between titration curves permit calculation of the relative affinities for protein of the anions. The effect of added salt on the titration is, in part, another manifestation of combination with anions. Since the effect of salt on the acid titration is exactly paralleled by its effect on titration with bases, these proteins must bind cations also. It is now shown that titration curves of wool at 0°C with 10 strong monovalent bases also exhibit wide differences. As with anions, the affinity of cations for protein increases with increasing molecular dimensions. Among organic cations of the same weight, asymmetric ions produce much the larger shift. Unlike the effects of anions, which are uniform over the entire acid curve, the effect of cations is more marked on the same portion of the titration curve which is affected by formaldehyde, i.e., the part due to lysine. The maximum shift in position of this part of the titration curve, obtained with tetradecyltrimethyl ammonium hydroxide, exceeds two pH units. Marked effects on other portions of the curve are shown only by asymmetric ions larger than the hexyltrimethyl ammonium ion. Explanations of this distinction are suggested, and effects on the stability of the protein will be described.

Conversion of S³⁵ homolanthionine to S³⁵-cystine in the rat JACOB A. STEKOL and KATHRYN WEISS (by invitation) *Lankenau Hospital Research Inst and Inst for Cancer Research, Philadelphia, Penna*. We reported that a mixture of isomers of homolanthionine is available to rats for growth purposes on a diet free of cystine but with minimal amounts of methionine. These results were interpreted as indicating the conversion of one or more isomers of homolanthionine to cystine *in vivo* (*J Biol Chem* 175:405, 1948). To secure a proof for the hypothesis we synthesized S³⁵-homolanthionine and fed it to young rats mixed with the diet (amino acid mixture diet) which was free of cystine but with 0.2% of methionine. After the rats grew for some time on the S³⁵ homolanthionine containing diet, the hair was clipped off, hydrolysed, and pure cystine was isolated and its radioactivity was measured. The product had a high radioactivity. These data establish beyond doubt the availability of the sulfur of homolanthionine for the synthesis of cystine *in vivo*.

Metabolism of hydroxyproline MARJORIE R. STETTIN *Dept of Biological Chemistry, Harvard Medical School, and Division of Nutrition and Physiology, Public Health Research Inst of The City of New York, New York City*. The amino acid hydroxyproline was synthesized in such a way as

to contain about 30 atom % excess N¹⁵. The synthetic mixture of 4 isomers was fractionated into the two DL forms. The DL(a) form, containing the naturally occurring l(-) hydroxyproline, was fed to 2 rats for 3 days and the distribution of N¹⁵ in the carcass, excreta, and a number of isolated amino acids was studied. The isotope analysis of the hydroxyproline isolated indicated that the dietary hydroxyproline was incorporated into the body proteins of the rat. The proline isolated contained only a trace of N¹⁵, indicating that hydroxyproline is not converted to proline to any appreciable extent in the rat. This is in contrast to the previously observed extensive biological oxidation of proline to hydroxyproline. These findings are in accord with other indications that proline and hydroxyproline are not metabolically equivalent.

d-Aspartic oxidase of rabbit kidney and liver J. L. STILL and M. V. BUELL (introduced by D. E. GREEN) *Inst for Enzyme Research, Univ of Wisconsin, Madison, Wis*. Rabbit kidney and liver contain an oxidase which catalyzes specifically the oxidation by molecular oxygen of d-aspartate to oxaloacetate according to the equation: d-Aspartate + O₂ → oxaloacetate + H₂O₂. A stable preparation of this enzyme can be prepared by homogenizing the tissue, centrifuging off the insoluble residue, then removing the precipitate formed at pH 5.4 and finally making an acetone powder of the clear supernatant fluid. This preparation contains little if any of Krebs' d-amino acid oxidase. Unlike the d-amino acid oxidase the aspartic oxidase is insensitive to benzoic acid in M/300 concentration. The liver and kidney cyclophorase preparations at the 3rd residue stage contain significant amounts of the d-aspartic oxidase. Under appropriate conditions the complete oxidation of d-aspartate to CO₂ and H₂O can be accomplished. Fluoride and the presence of some member of the citric acid cycle are essential for the complete oxidation. Aspartate can be synthesized by incubating the cyclophorase system with oxaloacetate and NH₃ under aerobic conditions. The basic reaction appears to be: 3 oxaloacetate + NH₃ → citrate + aspartate + CO₂. Under the aerobic conditions of the experiment citrate undergoes further oxidation. The oxidase does not oxidize l-aspartate, d-glutamate or other d-amino acids.

Metabolism of 16-ketoestrone and 16-keto-α-estradiol in man BENJAMIN F. STIMMEL, ARTHUR GROLLMAN, and MAX N. HUFFMAN *Recs-Stealy Medical Research Fund, Ltd, San Diego, Calif, and the Depts of Pharmacology and Experimental Medicine and of Biochemistry, Southwestern Medical College, Dallas, Texas*. Colorimetric urinary estrogen excretion data on men after injection of 16-ketoestrone and 16-keto-α-estradiol indicate that man is capable of converting these compounds in part to estrinol, but not to estrone and estradiol.

Zinc-hydrochloric acid hydrolysis of aliquots of the post-injection urine yields greatly augmented estrogen titers in the estradiol and estriol fractions of our (Stimmel *J Biol Chem* 162 99, 1946) liquid chromatogram. There is evidence that 16-ketoestrone appears in the urine following injection with either of these compounds and that reduction of it to 16-ketoestradiol by zinc-hydrochloric acid hydrolysis of its conjugate in urine, accounts for the augmented estrogen titers obtained by this method of hydrolysis. These studies with exogenous estrogens therefore provide a working model in support of the hypothesis of Smith and Smith (*Endocrinology* 28 740, 1941) that more highly oxidized estrogens than those presently known are excreted in the urine and that these may be converted to more active forms during hydrolysis by the addition of zinc dust.

Microbiological assay of vitamin B₁₂ by *Lactobacillus leichmannii* E. L. R. STOKSTAD, A. C. DORNBUSH (by invitation), A. L. FRANKLIN (by invitation), C. E. HOFFMANN (by invitation), B. L. HUTCHINGS and T. N. JUKES, *Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y.* *Lactobacillus leichmannii* 313 (ATCC 7830) was reported to respond to the crystalline anti-pernicious-anemia factor (vitamin B₁₂). Further studies showed that thioglycolic acid increased the growth response produced by vitamin B₁₂. This was shown to be primarily due to protection of vitamin B₁₂ against destruction during autoclaving of the samples with the medium. Protection was also accomplished by the addition to the medium of other reducing agents such as ascorbic acid or certain natural supplements that had been freed such as ascorbic acid or certain natural supplements that had been freed of thymidine and vitamin B₁₂. Similar results were obtained by adding the sample aseptically. Thioglycolic acid did not increase the response due to thymidine, presumably because thymidine is more stable and is not destroyed during autoclaving. *L. leichmannii* 313 was found to require a growth stimulant produced during autoclaving of the medium, as very little growth was obtained with a sterile-filtered or steamed medium even in the presence of vitamin B₁₂. This growth stimulation could alternatively be supplied by methyl glyoxal or thioglycolic acid, suggesting an effect due to a reduction in oxygen tension. Similar results were obtained with another strain, ATCC 4797, of *L. leichmannii*, which was found to grow more slowly and to have a lower requirement of vitamin B₁₂ for half-maximum growth.

Non-participation of acetyl phosphate in the fixation of formate into pyruvate HAROLD STRECKER (by invitation) and HARLAND G. WOOD, *Dept. of Biochemistry, Western Reserve Univ. School of Medicine, Cleveland, Ohio*. It has been

previously demonstrated with *E. coli* extracts that synthetic acetyl phosphate was not an active intermediate in the dissimilation of pyruvate. The possibility that biologically produced acetyl phosphate might differ from the synthetic material was investigated in the following manner. C¹⁴ carbonyl labeled pyruvate was synthesized and incubated with cell free extracts of *E. coli*. Fermentation was allowed to proceed until 30-50% of the original pyruvate was determinable as acetyl phosphate by the Lipmann hydroxylamine method. The residual pyruvate was then diluted approximately tenfold with non-isotopic pyruvate, and C¹⁴ formate equivalent to the acetyl phosphate formed was also added for measurement of the extent of reversibility. An aliquot was withdrawn for analysis and degradation and the remainder was allowed to ferment. If the biologically formed high count acetyl phosphate was in dynamic equilibrium with pyruvate in accordance with the reaction $\text{CH}_3\text{COCOOH} \rightleftharpoons \text{CH}_3\text{COOP}(\text{OH})_2 + \text{HCOOH}$, the final residual pyruvate should show an increase of specific activity in the carbonyl group comparable to that of the carboxyl group as introduced from the formate. No such increase was obtained although C¹⁴ was introduced into the carboxyl group. It is therefore concluded that acetyl phosphate is not an intermediate in the formate exchange reaction. That exchange of formate may not occur through the phosphoroclastic reaction is indicated by experiments with aged enzyme. Pyruvate was not broken down as measured by acid production in bicarbonate yet exchange with formate occurred at the same rate as with a fresh preparation.

Effect of dihydroxyphenylalanine on enzymatic digestion M. X. SULLIVAN and S. STEPHEN SCHIAFFINO (by invitation), *Chemo-Medical Research Institute, Georgetown Univ., Washington, D. C.* In 1913, Torquato Torquati isolated a nitrogenous pyrocatechol complex from the germ of the broad bean (*Vicia faba*) and Guggenheim extracted a similar material from the broad bean pods and identified it as dihydroxyphenylalanine. Since then this compound has been found in a number of legumes. Some years ago we had occasion to study the lentil which had been found of poor nutritive value and we found that the lentil contained dihydroxyphenylalanine as determined colorimetrically. The same color reaction was positive with other legumes such as the Georgia velvet bean known to contain dihydroxyphenylalanine. Accordingly a study was made of the effect of dihydroxyphenylalanine on peptic and tryptic digestion of egg albumin. By the procedure described by Callvary (*J Biol Chem* 102 73, 1933) no inhibition of peptic digestion was found when dihydroxyphenylalanine was added at various levels to the digestion mixture, albumin, pepsin held at pH 2. In tryptic

digestion, however, dihydroxyphenylalanine has an inhibitory action. This was proved by estimating the amount of dye released from a dyed protein during 4-16 hours of digestion by trypsin and also by the amount of methionine released and estimated by the McCarthy, Sullivan colorimetric procedure. On estimating at different levels, it was found that 25 mg of dihydroxyphenylalanine to 1 gm of albumin had the same effect as 50 mg, a very marked inhibition. Even with 10 mg and 5 mg of 'dopa' there was 59 and 52% inhibition respectively in the amount of methionine liberated.

Reactions of hemoglobin with aliphatic and inorganic nitrate and nitrite esters F WILLIAM SUNDERMAN and HAROLD WOOSTER (by invitation) *Mellon Institute, Pittsburgh, Penna., and Cleveland Clinic, Cleveland, Ohio*. The quantitative aspects of methemoglobin formation have been studied following incubation at 38°C of washed, laked human erythrocytes with solutions of potassium nitrate, butyl nitrate, diethyleneglycol dinitrate, nitroglycerine, potassium nitrite, and amyl nitrite. All of the nitrate compounds studied produce 2 molecules of methemoglobin per molecule of nitrate in accordance with the equation $\text{NO}_3^- + 2\text{Fe}^{++} (\text{hemoglobin}) \rightarrow \text{NO}_2^- + 2\text{Fe}^{+++} (\text{methemoglobin})$. Our analyses reveal a disappearance of nitrate without a corresponding increase in nitrite. Hemoglobin solutions incubated with potassium nitrite for 5 hours at 38°C yield 2 molecules of methemoglobin per molecule of nitrite, when incubated for 24 hours they yield more than 3 molecules of methemoglobin. If nitrites were to act directly as oxidizing agents, they would theoretically produce only 1 methemoglobin molecule per molecule of nitrite. The finding of higher ratios, therefore, suggests that nitrite probably first forms nitrate, which then reacts with hemoglobin to produce methemoglobin. Our studies indicate that amyl nitrite differs from other compounds studied in that it may either oxidize hemoglobin or reduce methemoglobin.

Activation of phosphoglucumutase E SUTHERLAND (by invitation), T POSTERNAK (by invitation), and C F CORI *Washington Univ., School of Medicine, Biological Chemistry Dept., St. Louis, Mo*. Various samples of glucose-1-phosphate (G-1-P), prepared enzymatically or chemically, were found to differ greatly in the rate of conversion to glucose-6-phosphate (G-6-P) by yeast or crystalline muscle phosphoglucumutase. Addition of active to inactive G-1-P raised the rate of conversion of the latter to that of the former. A possible explanation was the suggestion of Leloir and coworkers (*Arch. Biochem.* 19: 339, 1948) that glucose-1,6-diphosphate (G-1,6-P) was an activator in catalytic amounts according to the equation $\text{G-1-P} + \text{G-1-P} \rightleftharpoons \text{G-6-P} + \text{G-1,6-P}$. G-1,6-P was synthesized by interaction of crystalline α -1-bromo-2,3,4-triacetyl-6-diphenylphosphonoglucose with silver

phosphate, followed by catalytic hydrogenation to remove phenyl groups and partial hydrolysis with hydrochloric acid in methanol to remove acetyl and two hexosephosphate groups. The substance was isolated as the barium salt, more soluble in cold than in hot water, and gave correct analytical values for labile and stable phosphate, and for reducing power after hydrolysis. Cysteine (0.01M) was used to insure full activity of the enzyme. Cysteine could be replaced by heavy metal reagents such as diphenylthiocarbazon, 8-hydroxyquinoline, and histidine. Both the yeast and muscle enzyme were strongly activated by these reagents or protected by them when traces of zinc were added. The cysteine-activated enzyme did not convert G-1-P to G-6-P unless G-1,6-P was added in catalytic amounts, confirming Leloir's scheme. Half maximal activity of the enzyme was obtained when the concentration of the G-1,6-P was about 2×10^{-6} molar. A sample of G-1-P containing only 0.1% G-1,6-P will therefore be fully active in the usual reaction mixture containing 6×10^{-3} molar G-1-P.

Specificity of liver glucose-6-phosphatase MARJORIE A SWANSON (introduced by CAMILLO ARTOPI) *Dept. of Biochemistry, Bowman Gray School of Medicine, Winston-Salem, N. C.* It has been known for some time that liver extracts hydrolyze hexosemonophosphates, but the number of enzymes concerned and their degree of specificity are uncertain. In the present investigation, extracts variously prepared from rat liver were found to liberate inorganic phosphate from glucose-1-phosphate, fructose-1,6-diphosphate, ATP, and glycerophosphate at a low rate, and from glucose-6-phosphate and fructose-6-phosphate at a much higher rate. Isoelectric precipitation at pH 5.5 increased the glucose-6-phosphatase activity/mg of protein about threefold. Simultaneously the ability to split glucose-1-phosphate and fructose-1,6-diphosphate was lost, and the activity toward fructose-6-phosphate was diminished. The decrease in the rate of splitting fructose-6-phosphate paralleled the decrease in hexose isomerase activity, suggesting that its presence is necessary for the liberation of phosphate from fructose-6-phosphate. Thus it might logically be concluded that there is in the liver a specific glucose-6-phosphatase which, in the absence of phosphoglucumutase and hexose isomerase, hydrolyzes neither glucose-1-phosphate nor fructose-6-phosphate. Glycerophosphatase activity accompanied glucose-6-phosphatase (in the ratio of 1.5 to 1.6) through several attempts at purification. Presumptive evidence for the non-identity of these two enzymes was obtained on the basis of differential fluoride inhibition, lack of competitive inhibition, and different pH-activity curves.

Changes in the cholesterol concentration of rat liver homogenates during incubation LEON SWELL (by invitation) and C R TREADWELL *Dept. of*

Biochemistry, George Washington Univ, School of Medicine, Washington, D C Normal rat livers were homogenized with 0.154M phosphate buffer of pH 7.2 and diluted to definite volume with buffer. All manipulations were performed at 0–3°C. Fifteen cc aliquots were transferred to test tubes, mixed with 100 mg of sodium glycocholate and placed in a shaker at 38°C. Duplicate 1 cc samples were removed at zero hours and after various periods of incubation. The free and ester cholesterol were determined by a modification of the method of Hess (*Federation Proc* 6:260, 1947). During the first 2 to 4 hours the total cholesterol concentration decreased markedly, then decreased slowly until 6 hours had elapsed. With continued incubation the total cholesterol increased over that observed at 6 hours. After 8 to 12 hours the concentration approximated the zero hour value. Still longer incubation up to 36 hours usually gave values above the original level. The trend was toward a decrease in both free and ester cholesterol from 0 to 6 hours and an increase from 6 to 24. Studies of the influence of pH on these changes indicated that while the initial phase of decrease occurs over a range of 5.8 to 8.0, the increase in concentration occurs only from pH 6.8 to 7.2. Other experiments show that the observed changes in cholesterol concentration are not caused by microorganisms or volume changes. The effects of different temperatures and of the addition of possible precursors of cholesterol on these changes have been studied.

Isolation of a crystalline heat stable trypsin inhibitor from lima beans HENRY TAUBER V D. *Research Laboratory, U S Public Health Service, U S Marine Hospital, Staten Island, N Y* A crude concentrate, containing at least two proteinase inhibitors, was prepared by a modification of Kunitz' (*J Gen Physiol* 29:149, 1946) procedure for the initial purification of crystalline soybean trypsin inhibitor. Ground lima beans were extracted with 0.25N H₂SO₄. The proteinase inhibitors were adsorbed on Bentonite, eluted with 10% aqueous solution of pyridine, precipitated by (NH₄)₂SO₄, dialyzed salt-free, and 3 volumes of acetone added for precipitation. From this precipitate a crystalline heat stable trypsin inhibitor was isolated as follows: the precipitate was dissolved in 1% NaCl solution. In order to remove impurities, a small amount of Bentonite was added and the mixture was shaken for 15 minutes. The clear filtrate was dialyzed for about 20 minutes against ethanol, applying occasional stirring. Small microscopic crystals formed. The solution was allowed to crystallize overnight at room temperature. The crystals were dissolved in 1% NaCl. Acetone was added till the mixture was slightly turbid. The solution was allowed to crystallize overnight at room temperature. Two recrystallizations of this material resulted in colorless octahedra of much larger size.

This crystalline material is a globulin. It is precipitated by acetone, ethanol and trichloroacetic acid. It does not dialyze through cellophane. It powerfully inhibits crystalline trypsin. Proteolysis and milk clotting by chymotrypsin are moderately effected. The proteolytic and milk-clotting action of pepsin are not inhibited. This trypsin inhibitor somewhat resembles Kunitz' crystalline soybean trypsin inhibitor. In contrast to his inhibitor, however, the crystalline lima bean inhibitor is exceptionally heat stable. The soybean inhibitor is destroyed on short boiling.

Molecular weight of human fetal hemoglobin JOHN FULLER TAYLOR and RICHARD L SWARM (by invitation) *Washington Univ School of Medicine, Dept of Biological Chemistry, St Louis, Mo* We have measured the sedimentation and diffusion constants of fetal oxyhemoglobin (HbO₂) prepared from cord blood taken from normal full-term infants. The erythrocytes were washed with saline solution and laked with toluene. Adult HbO₂ solutions were similarly prepared from bank blood. Sedimentation velocity was measured in a 'Spinco' analytical ultracentrifuge at 20 to 30°. Diffusion was followed in a Claesson cell at 2°. Results have been corrected to water at 20°. We observed for fetal HbO₂ an S_{20,w} of 3.98 at 0.9% protein, and 4.01 at 0.5%, and for adult HbO₂ an S_{20,w} of 4.00 and 3.99 respectively. Essentially identical S values were obtained for fetal HbO₂ and HbCO in dilute phosphate buffer. M. A. Andersch, D. A. Wilson, and M. L. Menten (*J Biol Chem* 153:301, 1944) had reported values of 2.6 to 3.0 for 0.5% fetal hemoglobin. An average diffusion constant, D_{20,w}, of 6.6 cm² sec⁻¹ was obtained for 1% fetal HbO₂ in 1% NaCl. D values of 6.3 to 6.9 for adult hemoglobin have been reported in the literature. The identity in the sedimentation constants and the agreement in the diffusion constants of fetal and adult HbO₂ indicate that the hemoglobins from either source have the same molecular weight under the conditions of our experiments.

Bound coenzymes of the cyclophorase complex of rabbit kidney and liver L. J. TEPLY (introduced by D. E. GREEN) *Institute for Enzyme Research, Univ of Wisconsin, Madison, Wis* The cyclophorase gel of rabbit kidney or liver at the third residue stage (exhaustively washed with dilute salt solution) contains considerable amounts of the following coenzymes in bound form: adenine nucleotides, pyridine nucleotides, acetylation coenzymes, flavin dinucleotide, pyridoxal phosphate and diphosphothiamine. It also contains appreciable amounts of bound biotin and folic acid. The adenine nucleotides exist in the form of AMP, ADP, and ATP. By various devices AMP can be converted largely to ATP and vice versa. During storage of the enzyme at 0° for several days or at 35° for 24 hours the adenine nucleotide level declines. The pyridine

nucleotides are a mixture of CO_I and CO_{II} in approximately equal amount. The relative proportion of these two coenzymes remains fairly constant under a variety of conditions. With aging of the enzyme there is a steady decline of the level of pyridine nucleotides. The bulk of the acetylation coenzyme of rabbit liver and kidney appears to be bound up with the protein of the cyclophorase complex. All the above coenzymes are released from the cyclophorase gel in varying degrees by treatment with boiling water or 5% trichloroacetic acid. A crude barium salt fraction prepared from the neutralized trichloroacetic acid extract contains mainly pyridine nucleotides, flavin dinucleotide, the acetylation coenzyme, adenine nucleotides and inorganic phosphate.

Studies of growth factors in liver extract using paper partition chromatography GARSON H TISHKOFF, H T PEELER, ALEJANDRO ZAFFARONI, HENRY TESLUK, and L C NORRIS (introduced by ELMER H STOTZ) *Depts of Pathology and Biochemistry, School of Medicine and Dentistry, Univ of Rochester, Rochester, N Y, and School of Nutrition, Cornell Univ, Ithaca, N Y* Recent investigations by Daniel, Peeler, Norris, and Scott (*J Biol Chem*, in press) have demonstrated the presence of two unidentified factors in purified liver extract which are distinct from streptogenin but required by *Lactobacillus casei* for growth. These investigators have also demonstrated that a correlation exists between the antipernicious anemia activity of the extract and the growth response of *Lactobacillus casei* to one of these factors (APA factor) in the presence of an excess of the second factor ('whey' factor). In order to establish the nature of these factors, and as a possible method for their isolation in pure form, liver extract was fractionated using paper partition chromatographic technique and the individual fractions assayed for the APA factor and 'whey' factor.

Preparation of hyaluronate from some mammalian sources SIBYLLE TOLKSDORF (by invitation), JAMES W CASSIDY (by invitation), CAROL BONSAI (by invitation) and D ROY McCULLAGH *Dept of Biochemistry, Schering Corporation, Bloomfield, N J* Human umbilical cords, bovine synovial fluid, bovine vitreous humor and hog belly skin have been processed by various methods in investigating the production of hyaluronic acid. Modifications of the Rogers method (*Biochem J* 39 1945) have been employed and in general his technique has been used successfully except in the case of hog belly skin. However, it has been found that certain substances which interfere with the assay of testicular hyaluronidase are frequently present in such hyaluronate preparations. We have found that these impurities can be removed by washing the crude mucin clot with 0.2% acetic acid before separating the proteins by precipitation with chloroform. A

simpler method has been employed for the production of hyaluronate from umbilical cords for assay purposes. The macerated cords were heated with water to 80°C to precipitate proteins. Impurities were removed by adsorption on bentonite or other agents. The hyaluronates form a white powder when precipitated with acetone and dried with acetone and ether. No success has been attained in applying this procedure to other starting materials. Elementary chemical analysis would suggest that the polysaccharide from all these sources is essentially the same. The characteristics of the hyaluronate molecule which govern its viscosity must be due to different aspects of the molecular structure than those which control its capacity to cause turbidity with acidified proteins. This is attested to by the fact that preparations of similar purity show no quantitative relationship between the two phenomena.

Biochemical studies on the livers of chicks receiving graded levels of pteroylglutamic acid JOHN R TOTTER, WILLIAM MARTINDALE (by invitation), MARION MCKEE (by invitation), CECILIA K KEITH (by invitation), and PAUL L DAY *Dept of Biochemistry, School of Medicine, Univ of Arkansas, Little Rock, Ark* Groups of day-old White Leghorn chicks were given a purified diet (KEITH ET AL *J Biol Chem* 176 1095, 1948) supplemented with 0, 5, 10, 20, 40, 80, 200, and 1000 μg of pteroylglutamic acid (PGA) per 100 grams. An additional control group received a commercial starter diet. At the end of 4 weeks the birds were killed. The livers were analyzed for 1) PGA, using *Streptococcus faecalis* and conjugases from hog kidney and chicken pancreas, 2) conjugase, 3) desoxyribose nucleic acid and 4) ribose nucleic acid. The PGA levels in the livers indicated that storage of excess vitamin did not occur until the dietary level exceeded 40 $\mu\text{g}/100\text{ gm}$. The liver PGA freed by autolysis was approximately the same as that determined with conjugases from hog kidney or chicken pancreas. The conjugase levels of the livers appeared to be unrelated to either the dietary level of PGA or the nucleic acid content of the livers. Desoxyribose nucleic acid was found to be somewhat low in the chicks receiving no dietary PGA, but the differences among all other groups were of doubtful significance.

Cholesterol esterase of pancreatin C R TREADWELL, R S YAMAMOTO (by invitation), and NORMAN P GOLDSTEIN (by invitation) *Dept of Biochemistry, George Washington Univ School of Medicine, Washington, D C* Preliminary to an investigation of the significance of cholesterol and its esters we have attempted to evaluate the optimum conditions for demonstrating the activity of the enzyme. Commercial pancreatin was used as a source of cholesterol esterase. In most of the previous work natural materials, such as serum, have

been used to supply the substrate. In these experiments synthetic cholesterol oleate has been used as the substrate. Technical difficulties were encountered in the preparation of a stable emulsion of the ester. When various emulsifying agents were tested, singly and in combination, the most efficient found was phosphate buffer containing albumin and bile salts. When cholesterol oleate was emulsified in this mixture and the emulsion incubated at 38°C with shaking, the solution was homogeneous in respect to the ester for periods up to 48 hours as shown by analysis of duplicate aliquots. When 1 cc of a 20% suspension of pancreatin in 50% glycerol and 14 cc of the substrate mixture were incubated for 24 hours, 45% hydrolysis was obtained. The rate of hydrolysis was constant for 24 hours. The optimum pH was found to be approximately 7. Heat inactivation experiments indicated that the enzyme was completely inactivated by heating at 65°C for 1 hour. The degree of hydrolysis was proportional to the concentration of the enzyme.

Quantitative aspects of coprecipitation in the purification of substances isolated in tracer studies. SIDNEY UDENFRIEND (by invitation), ALBERT S. KESTON (by invitation), NORA LEVITAS (by invitation) and MILTON LEVY, *Dept. of Chemistry, New York Univ. College of Medicine, New York City*. In the isotopic derivative method we encountered, in several instances, considerable difficulties in the elimination of impurities by crystallization. The basis of the difficulty was a distribution of the impurity between crystals and solution as though between immiscible solvents and may be formulated $K = I_s/C_s/I_c/C_c$ where I and C are the amounts of impurity and carrier in supernatant (subscript s) and crystals (subscript c). The amount of purification achieved in a single crystallization is greater the larger is K and the greater the loss of carrier in the supernatant. Addition of the non-isotopic analogue of the impurity to dilute its activity has no advantage as long as the above formulation holds and it has been found to hold in instances from an impurity level of 0.0001% to 5%. Crystallization of DL leucine containing S-35 methionine gave $K = 2.5$. For alanine with glycine as the impurity $K = 1.5$. Practically the same constants are found for the same amino acids as pipsyl derivatives. Thus it is not necessarily advantageous to prepare different derivatives in the course of purification. As an example of the import of this phenomenon only by 200 recrystallizations, sacrificing 5% of the remainder at each, would a system with $K = 1.5$ reach a level of 1% of the original impurity. Coprecipitation must be considered in evaluating any 'constant isotope concentration' attained by crystallization. Chromatographic and countercurrent solvent extractions techniques are more dependable in this respect.

Action of streptomycin in tissue homogenates

W. W. UMBREIT and N. E. TONHAZY (by invitation) *Merck Institute for Therapeutic Research, Rahway, N. J.* Previous reports have demonstrated that one of the actions of streptomycin is to inhibit the condensation between oxalacetate and pyruvate in susceptible strains of *Escherichia coli*. Inasmuch as this is an important reaction in animal cells, the question arises as to why streptomycin is able to enter the animal body, kill or inhibit the susceptible bacteria contained in it, without comparable harm to the animal. The condensation of oxalacetate and pyruvate is largely localized in the mitochondrial fractions, whose activity may be preserved by homogenizing in isotonic KCl or isotonic sucrose. Using isotonic homogenates from rat liver and rat kidney, it was demonstrated that streptomycin in concentrations of 30–60 γ /ml had no effect upon the oxalacetate-pyruvate reaction. However, when the concentration was increased to 300 γ /ml inhibition was frequently obtained. Inhibition of this reaction could be demonstrated for low levels of streptomycin if the homogenate and the streptomycin were incubated in the cold for two hours. The circumstances of these experiments are such as to suggest that streptomycin will inhibit the oxalacetate-pyruvate condensation in the animal as well as in the bacteria but that in the animal there is a permeability barrier at the cell wall and at the mitochondria which prevents the streptomycin from reaching the enzymes in the animal cell concerned with catalyzing this reaction.

Conversion of isotopic pyruvate to oxalacetate by pigeon liver. M. F. UTTER and MARY T. CHENOWETH (by invitation) *Dept. of Biochemistry, School of Medicine, Western Reserve Univ., Cleveland, Ohio*. Although it has been assumed that the formation of oxalacetate in the Wood-Werkman reaction is accomplished by the carboxylation of pyruvate, previous studies have dealt only with CO_2 without identification of the other component. In the present work, the incorporation of labeled pyruvate into oxalacetate has been compared with the incorporation of C^*O_2 to determine the nature of the immediate precursor of oxalacetate. Carboxyl labeled pyruvate was synthesized from acetyl-bromide and $Cu_2(C^*N)_2$ via pyruvonitrile and pyruvamide. Oxalacetate was decarboxylated by a pigeon liver preparation in the presence of pyruvate and C^*N_2 and the residual oxalacetate separated and purified as the mercurous salt. The oxalacetate was analyzed for isotope content in the two carboxyl groups since one group originates from CO_2 and the other from the pyruvate carbonyl. Results indicate that the fixation of pyruvate in oxalacetate occurs with certain enzymatic preparations but is not correlated with the fixation of CO_2 . Some enzymatic preparations that fix CO_2 very rapidly are inactive on pyruvate. In contrast to the marked stimulation of CO_2 fixation caused by ATP, a 1

pyruvate fixation occurs it is relatively unaffected by ATP. The foregoing findings lead to the tentative conclusion that pyruvate is not the immediate precursor of oxalacetate. Preliminary results show that labeled pyruvamide can be substituted for pyruvic acid in the reaction mixture and is also incorporated in a compound resembling oxalacetate. The mechanism is under investigation.

Isotopic studies of the biosynthesis of nucleic acid components II allantoin JEROME D VALENTINE (by invitation), SAMUEL GURIN and D WRIGHT WILSON *Dept of Physiological Chemistry, School of Medicine, Univ of Pennsylvania, Philadelphia, Pa*. It has been established that the carboxyl carbon of glycine is a source of carbon 4 in the uric acid excreted by the pigeon. In order to determine what role glycine plays in the biosynthesis of purines in mammals, glycine labeled in the carboxyl position with C^{14} was mixed with a stock diet and fed to young albino rats for a period of 10 days. From pooled 24-hour collections of urine, allantoin was isolated, purified and analyzed. All samples of allantoin were found to be radioactive, the specific radioactivity increasing gradually to a maximum on the 10th day. By degradation of allantoin to glyoxylic acid and urea the C^{14} was found in the glyoxylic acid fraction, corresponding to carbons 4 and 5 of the allantoin molecule. Allantoin isolated after giving carboxyl labeled sodium acetate intraperitoneally to 5 rats over a 5-day period was found to be very weakly radioactive. It would appear that this compound is either not directly utilized as such for purine synthesis or that it is directed into other metabolic pathways. These results are of interest in view of the fact that much of the carboxyl carbon of acetate is incorporated into carbons 2 and 8 in the uric acid excreted by the pigeon.

Inactivation of stilbestrol in alkali RAYMOND VANDERLINDE (by invitation) and W W WESTERFELD *Dept of Biochemistry, Syracuse Univ College of Medicine, Syracuse, N Y*. The inactivation of stilbestrol that occurs on standing in an alkaline solution results from a dehydrogenation of the stilbestrol to isodienestrol and a subsequent ring closure to indenestrol. Both of these products have been isolated from reaction mixtures. A nearly quantitative conversion to indenestrol was obtained when 0.8 mg of stilbestrol per cc of 0.8N NaOH stood at room temperature for 30 days. Isodienestrol and small amounts of unchanged stilbestrol were the principal products obtained when 1 mg of stilbestrol per cc of 0.02N NaOH stood at room temperature for 14 days. Isodienestrol is approximately 1/700 as active estrogenically as stilbestrol, indenestrol is at least 1/10 as active as stilbestrol. The relative decrease in estrogenic potency of the original stilbestrol solution will obviously depend upon the proportions of the three

main constituents in the final solution, and this varies with the conditions of inactivation.

Antigenic transformation in *paramecium aurelia* under the influence of proteolytic enzymes W J VAN WAGTENDONK *Dept of Zoology, Indiana Univ, Bloomington, Ind*. Within stock 51 of variety 4 of *Paramecium aurelia* 6 antigenically diverse strains have arisen (strains A, B, C, D, E and G). Each of these 6 types is inherited under standard conditions of culture. Differences in antigenic type do not depend on differences in genes but on cytoplasmic differences. These cytoplasmic factors are subject to environmental influences. Exposure to dilute antiserum, high or low temperature, brings about a transformation in antigenic type to any one of the possible types in this stock. The new antigenic type is inherited true to type, as long as the cultures are grown under standard conditions (Sonneborn *Scientific Monthly* 67:154, 1948). Five strains of *P. aurelia* (stock 51, variety 4) have been exposed to the action of trypsin and chymotrypsin at 27° for 12 and 24 hours. After exposure single isolations were grown for 8 fissions at 20°, 27° and 32°, and the antigenic type of the resulting clones determined. Under these conditions, strains 51A, 51B and 51G very rarely transformed into another type. Types 51C, 51D and 51E gave rise to a large variety in types. The susceptibility to trypsin and chymotrypsin of the latter three types is correlated with their instability under other external conditions.

Influence of biliary obstruction upon liver protein regeneration HARRY M VARS, COLIN C FERGUSON (by invitation) and CHARLES S ROGERS (by invitation) *Harrison Dept of Surgical Research, Schools of Medicine, Univ of Pennsylvania, Philadelphia, Pa*. Groups of male Wistar rats, previously protein-depleted for a period of 14 days, were subjected to partial hepatectomy (70%) and to simultaneous high ligation and division of the common bile duct. Postoperatively these animals were fed a 10% protein, low fat diet and were then killed on the 2d, 4th, eighth and 14th days. Control groups, only partially hepatectomized, were paired with the above rats. Total nitrogen balances were determined. Following death the amounts of new liver protein were measured, together with liver mass (wet and dry), glycogen and total lipid. Histological examination of all livers was made. At all periods postoperatively it was found that the biliary-obstructed rats not only had livers of greater mass but livers containing greater amounts of new liver protein than did the controls. Undoubtedly a portion of this protein increment is ductal in origin, and some may be present in the increased fluid content of the obstructed livers as plasma or lymph. However, estimation of the ductal proliferation as seen on histological examination and estimation of the protein associated with the

increased fluid indicate that liver protein regeneration occurred in the biliary-obstructed rat to a degree almost equal to that in the unobstructed pair-fed animal. Counts of parenchymal cell mitoses in both groups were approximately equal.

Rat liver fructokinase. CARL S. VESTLING, URSULA IRISH (by invitation), AUGUSTA K. HIRSCH (by invitation) and NORMAN H. GRANT (by invitation). *Division of Biochemistry, W. A. Noyes Laboratory of Chemistry, Univ. of Illinois, Urbana, Ill.* A study of the phosphorylation of glucose and fructose by rat liver homogenates has been carried out. It has been found that systems which phosphorylate fructose very effectively are quite inert to glucose. Addition of numerous materials (DPN, HDP, phosphocreatine, insulin, various phosphatase inhibitors and others) fails to allow phosphorylation of glucose at a rate at all comparable to that encountered with fructose. The Nelson procedure (*J. Biol. Chem.* 153: 375, 1944) and that of Roe (Browne and Zerban, *Sugar Analysis*, p. 961, 1941) have been employed in studying sugar disappearance. Slightly hypertonic phosphate buffer homogenates of livers from fasted rats have been used in this work. The complete systems contain ATP, $MgCl_2$, α -ketoglutarate or glutamate, substrate and homogenate. The effect of NaF has been studied. In certain experiments liver slices have been substituted for homogenates with essentially similar results. The oxidation of α -ketoglutarate or glutamate by these systems greatly improves the phosphorylating capacity. When glucose-1-phosphate, glucose-6-phosphate, or fructose-6-phosphate is incubated in rat liver systems, about one-fifth reappears as 'apparent glucose' and none as fructose. When a beef brain hexokinase preparation, which will phosphorylate both glucose and fructose, is added to a rat liver system, the combined system phosphorylates both glucose and fructose effectively. The combined system phosphorylates about twice as much glucose as the brain hexokinase alone. Boiling the brain hexokinase extract abolishes this effect.

In vitro utilization of C^{14} labeled acetate and pyruvate by rat diaphragm muscle. CLAUDE A. VILLEE, OLOF H. PEARSON (by invitation) and A. BAIRD HASTINGS. *Dept. of Biological Chemistry, Harvard Medical School, Boston, Mass.* Diaphragm muscle from normal, diabetic and adrenalectomized rats was incubated, using the technique of Gemmell, in a medium containing 0.04M sodium phosphate, 0.005M $MgCl_2$, 0.08M NaCl, and either carboxyl labeled C^{14} acetate or carbonyl labeled C^{14} pyruvate, 10 mM/l, initial pH, 6.8. A half diaphragm was placed in each Warburg vessel, to one of each pair of vessels 0.5 U insulin/ml medium was added. The diaphragms were incubated for 2 hours in an atmosphere of oxygen. The CO_2 collected in the center well was precipitated as $BaCO_3$, plated

and counted with an end window Geiger tube. Samples of the media before and after incubation were analyzed for pyruvate and lactate, and the amount of glycogen in the diaphragm before and after incubation was estimated. The metabolism of acetate to CO_2 in diabetic diaphragm was decreased to 35% of normal and this decrease was unaffected by the addition of insulin. Insulin had no effect on the metabolism of acetate to CO_2 in normal diaphragm. Experiments on the effect of adrenalectomy and on the metabolism of labeled pyruvate are underway and will also be reported.

Anesthesia with methylal in mice and dogs. ROBERT W. VIRTUE. *Division of Anesthesiology, Dept. of Surgery, State Univ. of Iowa, Iowa City, Iowa.* Exposure of mice to concentrations of 7% methylal ($CH_3-O-CH_2-O-CH_3$) in air was followed within 10 minutes by loss of reflexes producing a condition compatible with surgical anesthesia. Increasing the concentration to 17.5% methylal produced respiratory arrest. The anesthetic index was therefore 2.5. The corresponding index for ethyl ether was 2.0. Twenty-five% methylal in 0.9% sodium chloride solution was injected intravenously into dogs. Surgical anesthesia was produced with blood concentrations of methylal between 109 and 235 mg %. After injection of sufficient methylal to produce respiratory arrest the blood methylal values varied between 265 and 453 mg %, and one hour later the blood methylal values ranged between 99 and 295 mg %. As has been consistently observed during ether anesthesia, concentrations of blood glucose rose considerably during methylal anesthesia, the carbon dioxide combining power diminished, and the blood urea values increased slightly. Recovery of both mice and dogs from anesthesia with methylal was much slower than from anesthesia with ether, probably due to slower elimination of methylal because of its greater solubility in water.

Chromatographic separation of ribonucleotides and its application to the study of ribonucleic acid structure. ERNST VISCHER (by invitation), BORIS MAGASANIK (by invitation), and ERWIN CHARGAFF. *Dept. of Biochemistry, College of Physicians and Surgeons, Columbia Univ., New York.* The ribonucleotides derived from yeast ribonucleic acid, viz. adenylic, guanylic and cytidylic acids, can be separated from each other and also from muscle adenylic acid by chromatography on filter paper with isobutyric acid (saturated with water) as the solvent in a suitably maintained NH_3 atmosphere. The separated components are fixed on the paper as the uranyl salts and demonstrated by the conversion of the latter to brown uranyl ferrocyanide. Parallel undeveloped chromatograms serve for the extraction of the adsorbates with M phosphate buffer of pH 7.1 and the spectrophotometric estimation of the separated nucleotides by proce-

dures similar to the recently described method for purines and pyrimidines (Visseher and Chargaff *J Biol Chem* 176 703, 1948) The recoveries ranged from 90 to 100% If the distance of the yeast adenylic acid adsorbate from the starting point is taken as 100, muscle adenylic acid is 75, cytidylic acid 60, and guanylic acid 40 Alkaline hydrolysates of yeast ribonucleic acid exhibited 3 zones of adsorption which in their positions on the chromatogram and in their absorption spectra corresponded to guanylic, cytidylic and adenylic acids, respectively, accounting for a total of 75-85% of the nucleic acid used The quantitative distribution of the nucleotides thus determined following the degradation by various means of ribonucleic acids of yeast and of mammalian tissue will be discussed with particular emphasis on the bearing of these studies on the problem of nucleic acid structure

Function of the system, glutamic acid-glutamine, in brain metabolism HEINRICH WAELSCH, PAULA SCHWERIN (by invitation) and S P BESSMAN (by invitation) *Depts of Biochemistry, N Y State Psychiatric Institute and Columbia Univ, New York City* The protein-free filtrate of brain (rat, mouse, rabbit, calf) contains about 160 mg glutamic acid and 60 mg glutamine/100 gm tissue The concentration of free glutamic acid in brain is higher than in any other organ analyzed (muscle, liver, kidney, blood) After intravenous injection of large amounts of glutamic acid or of glutamine into rats and mice no increase in the glutamic acid content of the brain was found while the entrance of glutamine into the brain could not be excluded with certainty Glutamine appears to enter the liver with greater ease than does the parent amino acid The data suggest that brain glutamic acid may be derived from glucose through the tricarboxylic acid cycle At present, however, the possibility still exists that this amino acid originates at least in part from circulating glutamine The function of the system glutamic acid-glutamine is discussed as one of the metabolic buffers of the tricarboxylic acid cycle Under physiological conditions the large concentration of glutamic acid in the brain assures an optimal functional rate of the cycle at the ketoglutaric acid step Glutamic acid may furthermore act as a respiratory substrate under conditions of impaired glucose supply

Influence of lipotropic compounds on the lipid and protein fractions of blood plasma from children with nephrotic syndrome CHI CHE WANG, H H BOYLE and S FREEMAN *Children's Memorial Hospital & Northwestern Univ Medical School, Chicago, Ill* A total of 11 children with nephrotic syndrome and lipemia were treated during varying periods with one or more of the following compounds Methionine, inositol, choline In some cases an effect was demonstrated in the blood lipid values In one case all fractions but phospholipids

definitely decreased following the ingestion of 3 gm of inositol for 11 weeks and 5 days On the other hand 4 of the 5 patients receiving inositol alone showed no consistency in the lipid values during inositol intake One out of 5 patients showed a definite decrease in lipid values during the administration of choline alone Two children who received a combination of 3 gm of inositol and 2 gm of choline for 9 days in the case of one patient and for 14 weeks 4 days in another patient showed a definite lowering of all the lipid fractions Four out of 5 children receiving 5 gm of methionine showed a lowering of the lipid fractions with some fluctuations In the fifth child there was a slight increase in neutral fat The lipotropic compounds did not produce a definite change in the protein fractions of blood plasma of these patients

Determination of pyrimidines by the isotope derivative method ROBERT C WARNER *Dept of Chemistry, New York Univ College of Medicine, New York City* Conditions have been established for the formation of derivatives of uracil and thymine with pipsyl (para-iodo-phenyl sulfonyl) chloride The reaction proceeds at room temperature in 75% acetone solution buffered with bicarbonate to give a 94% yield of the mono pipsyl ester With I^{131} in the derivatizing reagent, this reaction has been made the basis of a quantitative determination of uracil and thymine by the same method that has been applied to amino acids by Keston, Udenfriend and Cannan (*J Am Chem Soc*, in press) The pyrimidine solution is derivatized with isotopic reagent of known specific activity A large excess of the unlabelled derivative of the desired constituent is added as carrier A sample is purified to constant specific activity from which the quantity of the constituent originally present may be calculated Purification is accomplished by means of repeated recrystallization with nortite treatment and countercurrent distribution The latter method makes it possible to analyze for both uracil and thymine on the same reaction mixture after adding carriers for both Recoveries from mixtures and nucleic acid hydrolysates indicate an overall accuracy of 1-2% on quantities of 10 μ g Cytosine can be determined after conversion to uracil Data on the pyrimidine content and rate of hydrolysis of nucleic acids have been obtained by this method The indicator technique (Keston, Udenfriend and Levy *J Am Chem Soc* 69 3151, 1941) using both isotopes I^{131} and S^{35} and employing paper strip chromatography can also be applied to 0.1 μ g quantities of pyrimidine

In vitro metabolism of testosterone and its metabolites by kidney tissue CHARLES DONALD WEST (by invitation) and LEO T SAMUELS *Dept of Biochemistry, Univ of Utah, Salt Lake City, Utah* This investigation was undertaken to determine whether or not the kidney is involved in the

metabolism of testosterone When testosterone was incubated with dog or rabbit kidney mince, the testosterone was destroyed enzymatically and 17-ketosteroids were formed This destruction was accomplished by at least two enzyme systems One has DPN for its coenzyme and is concerned in the oxidation of the hydroxyl group on C-17 to the ketone The other enzyme system is much less active and is concerned in the saturation of the α - β unsaturated bond in the A ring When androstenedione was incubated with rabbit kidney mince, the 17-ketone group was destroyed while the α - β unsaturation in the A ring was left intact The addition of citrate greatly accelerated this reaction Similarly, the 17-ketone group of androsterone was destroyed when it was incubated with rabbit kidney mince This destruction was also catalyzed by the addition of citrate No α - β unsaturated steroids were formed from the androsterone The relation of these enzymes to those in the liver will be discussed

Dietary effects on liver xanthine oxidase W W WESTERFELD and DAN A RICHERT (by invitation) *Dept of Biochemistry, Syracuse Univ College of Medicine, Syracuse, N Y* Rats were born without detectable liver xanthine oxidase activity At 21 days of age the average activity was 720 units (Cmm O_2 /gm dry liver/hr) Adult rats on dog chow averaged 1550 μ Weanling rats were brought to 1535 and 1385 μ respectively by feeding dog chow (21% protein) or mineralized whole milk for 2 weeks Feeding purified diets containing 21% protein (casein, egg albumin, peanut protein) maintained the liver xanthine oxidase activity at approximately the weanling level (600-900 μ) for about 6 weeks, but gave values of 1000 to 1200 μ in 8 to 10 weeks Increasing the protein content of the diet to 25 and 30% by the addition of peanut meal gave only 900 to 1000 μ of liver xanthine oxidase after 6 weeks Supplementing the 21% purified casein diet with 10% dry liver gave 1290 μ of activity in 2 weeks, supplementing the diet with raw cream was similarly effective with some samples of cream but not with all A diet containing 21% crude casein gave 1260 μ of activity in 4 weeks Supplementing the purified casein diet for 2 weeks with a mixture of Cu, Zn, Mn, Co, and I or with a mixture of flavine, adenine, and ribose or with a mixture of biotin, inositol, p-aminobenzoic acid, pteroylglutamic acid, rutin and ergostanyl acetate did not significantly increase the liver xanthine oxidase above the control levels A concentrate of vitamin B_{12} was also ineffective

Progesterone and testosterone reversal of diethylstilbestrol inhibition of succinoxidase of tumor mitochondrial elements KENT WIGHT (by invitation) and DEAN BURK *National Cancer Institute, National Institutes of Health, Bethesda, Md* It has been found that certain hormones act

competitively on the succinoxidase system of the mitochondrial elements of the S91 and Harding-Passey melanomas grown in dba and C mice respectively The mitochondrial elements (microscopically visible cytoplasmic particulates mostly melanized, see Woods *et al J Nat Cancer Inst* Feb 1949) were obtained by centrifugation at 10°C of saline, glass wool-filtered tumor homogenates at 10,000 g for 30 minutes, after preliminary centrifugation at 25 g for 10 minutes to eliminate nuclei and heavy phagocytized cell material Oxygen consumption was measured manometrically after addition of 0.05M phosphate (pH 7), 0.00001M cytochrome C, and 0.02M succinate Diethylstilbestrol, at a concentration of $\sim 135 \gamma/\text{ml}$ ($\approx 0.0005M$), inhibited particulate oxygen consumption 60 to 75% This inhibition was markedly reduced or entirely eliminated by the simultaneous addition of $\sim 125 \gamma/\text{ml}$ ($\approx 0.0002M$) progesterone or testosterone Progesterone alone had no effect on oxygen consumption The S91 and Harding-Passey tumor particulates behaved similarly except that the normal QO_2 values ($\text{ml}^3 O_2$ consumed/mg/dry wt/hour) were about 5 times as great in the case of the former (QO_2 of 10-20 compared to 2-5), and that testosterone alone sometimes inhibited the latter Essentially no succinoxidase activity was shown by the initial nuclei fractions or the final supernatants Diethylstilbestrol also markedly inhibited or eliminated oxygen consumption by mitochondrial elements from non-melanoma tumors such as C3HBA breast carcinoma and sarcoma 37 grown in C3H and C mice respectively The foregoing observations on sex hormone antagonisms at the mitochondrial enzyme level may offer some basis of mechanism for the numerous similar antagonisms noted at organ and body levels

Paper chromatography of vitamin B_{12} and related bacterial growth factors WALTER A WINSTEN and EDWARD EIGEN (introduced by PHILIP B HAWK) *Food Research Laboratories, Inc, Long Island City, N Y* In a preliminary note (letter to the *Journal of Biological Chemistry*), *Lactobacillus leichmannii* 313 has been reported to utilize six alternate growth factors Two of these were found in vitamin B_{12} concentrates and presumably represent two forms of the vitamin Hoffmann, *et al (J Biol Chem* 176 1465, 1948) previously reported that crystalline APA factor (vitamin B_{12}) supports the growth of this organism A third growth factor may be identified as thymidine, reported by Snell, *et al (J Biol Chem* 175 474, 1948) to support the growth of *L leichmannii* 313 The three other growth factors are of unknown composition In the present report, the paper chromatographic procedure for separating the 6 alternate growth factors is described as well as the method of recognizing the positions of the several growth factors on a chromatogram by the use of *L leichmannii* 313 as

a microbiological indicator. This technique allows one to interpret assay values for apparent vitamin B₁₂ activity as measured in the tube microbiological assay procedure. Due to the very slow movement of the two forms of vitamin B₁₂ on a paper chromatogram using n-butanol as the mobile phase, it is possible to separate these entities from the faster moving substitute growth factors. The isolated vitamin B₁₂ entities may then be assayed by the usual tube assay procedure. Illustrative data are presented.

Metabolism of tissue cultures of mouse-brain in relation to propagation of Theiler's GD VII virus

RICHARD J. WINZLER and HAROLD E. PEARSON (by invitation) *Depts. of Biochemistry and Bacteriology, Univ. of Southern California School of Medicine and the Laboratory Division of the Los Angeles County Hospital, Los Angeles, Calif.* Sterile cultures of minced newborn mouse brain incubated at 35°C are capable of supporting the multiplication of Theiler's GD VII strain of mouse encephalomyelitis virus. Studies on the oxygen consumption in Warburg vessels of such cultures with or without virus reveal no significant difference in the QO₂ values in the presence or absence of the virus over the period of most rapid virus growth (0-24 hours) and up to 72 hours. Similarly with cultures grown in Erlenmeyer flasks the presence or absence of virus had no influence on the rate of glucose utilization or of lactic acid production by the tissues. The rates of oxygen consumption, glucose utilization and lactic acid production fall rapidly with time and reach levels of about 10% of the original rates after 48 hours. In spite of this marked diminution of metabolism, the tissue retains its ability to support the growth of the virus for as long as 10 days of incubation before virus is added. It thus appears that the virus has little effect on the overall metabolism of the host tissue and that the rate of metabolism of the host tissue is not of paramount significance in governing the multiplication of the virus.

Acetoacetate production from hexanoic and other six carbon acids by rat liver preparations

ROBERT F. WITTER (by invitation) and ELMER STOTZ *Dept. of Biochemistry, School of Medicine and Dentistry, The Univ. of Rochester, Rochester, N. Y.* Quantitative measurements of acetoacetate production from 6 carbon acids were made with rat liver homogenates and washed liver particulate matter. With the latter at pH 7.4-8.0, 1 mole of hexanoate yielded 1½ moles of acetoacetate, proving a recondensation mechanism. At pH 6-7 lower yields of acetoacetate were obtained at a slower rate, and a heat stable factor from liver was necessary. The rate of acetoacetate formation from several possible intermediates of hexanoate oxidation was measured with both types of liver preparations at pH 8.0. Only α-hexanoate yielded acetoacetate

at a rate comparable with hexanoate under the same conditions. In the washed liver system, sorbate, β-hydroxyhexanoate, β-hydroxy-γ-hexanoate, and β-ketohexanoate gave low rates of acetoacetate production. Upon addition of the heat stable factor, these rates were all increased but only with sorbate did the rate approach that of hexanoate or α-hexanoate. The washed liver system, unlike the homogenate or the soluble extract (Witter and Stotz *J. Biol. Chem.* 176: 501, 1948), did not metabolize triacetic (β,δ-diketohexanoic) acid. By use of a micro-bromination procedure for α-hexanoate, it has been found that its disappearance in liver homogenates is dependent on the presence of oxygen. Aerobically, β-hydroxyhexanoate did not inhibit the metabolism of α-hexanoate. The collected facts cast some doubt on a simple hydration of α-hexanoate and on β-hydroxyhexanoate as an intermediate. A study of various inhibitors is being made in an attempt to differentiate the steps in the production of acetoacetate from hexanoic acid.

Sulfur-free protein hydrolysates for cystine-

and methionine-free diets JOHN L. WOOD, SHIRLEY LUCILLE COOLEY (by invitation) and NELSON KINGSLAND (by invitation) *Dept. of Chemistry, School of Biological Sciences, Univ. of Tennessee, Memphis, Tenn.*

In order to prepare a diet entirely devoid of organic sulfur, excepting for vitamins, a method for removal of all sulfur from protein hydrolysates has been devised. The protein hydrolysates were treated with freshly prepared Raney Nickel which removed all organic sulfur as nickel sulfide. Model experiments indicate that the cystine in the mixture was converted to alanine and the methionine to α-aminobutyric acid. A 15% solution of the hydrolysate was shaken with Raney Nickel for 3 hours at room temperature and pH 8.2. The solution was separated from the nickel and neutralized before concentration to dryness. The nickel adsorbed considerable of the nitrogenous products of the solution, but much of this was eluted by treatment with hydrogen sulfide. The ratio of amino nitrogen to total nitrogen in the final product was approximately the same as in the untreated hydrolysate. Qualitative tests for cystine and methionine and sulfhydryl were negative. Threonine analyses indicated that this amino acid was not destroyed by the alkaline treatment. A small amount of sulfate was found in the hydrolysate. In one instance this was removed with barium hydroxide to produce an entirely sulfur-free hydrolysate. Growth response in young rats demonstrated that essential amino acids other than methionine were retained in the desulfurized preparation. Both an acid hydrolysate and an enzyme hydrolysate have been desulfurized. When supplemented with methionine, or with cystine and methionine, each preparation supported

growth at approximately the same rate as the untreated hydrolysate

Prolongation of pentobarbital sodium anesthesia by nitrates and nitrites HAROLD WOOSTER (by invitation) and F WILLIAM SUNDERMAN *Mellon Institute, Pittsburgh, Penna., and Cleveland Clinic, Cleveland, Ohio* The recovery time of white mice anesthetized with pentobarbital sodium is significantly prolonged by the intraperitoneal injection of nitroglycerine, butyl nitrate, diethyleneglycol dinitrate and sodium nitrite Nitroglycerine appears to be the most effective of the compounds studied The dosages required to demonstrate this phenomenon are of the order of 20-50 mg/kg of body weight Significant prolongation of recovery time may also be produced by the inhalation for 20 minutes of butyl nitrate in concentrations of 30-45 mg/l

Interference of gentisic acid in determination of urinary uric acid after administration of salicylates T F YÜ (by invitation) and ALEXANDER B GUTMAN *Research Service, First (Columbia Univ) Division, Goldwater Memorial Hospital, and Dept of Medicine, College of Physicians and Surgeons, Columbia Univ, New York City* The chromogen removed by treatment of urine or serum with uricase is generally considered to be true uric acid We find that in the urine of normal and gouty subjects receiving 5 gm acetylsalicylic acid daily, as much as 25% of the total chromogens (as measured by urea-cyanide-carbonate and arsenophosphotungstic acid) may consist of a chromogen which is not uric acid but is destroyed by digestion with uricase, due to instability in the alkaline buffer This chromogen was identified by counter-current techniques as largely gentisic acid, showing essentially the same distribution between dilute HCl and a mixture of isopropyl ether and petroleum ether as an authentic sample of gentisic acid When 16 gm of sodium gentisate was fed by mouth, the chromogenicity of the urine was tremendously increased, of this over 90% was gentisic acid In determining uric acid in the urine of patients receiving salicylates, gentisic acid should first be removed This can be accomplished by shaking an aliquot of acidified urine (pH 4.5-5.0) 2 or 3 times with 4 to 5 times the volume of ethyl ether The gentisic acid is quantitatively removed in the ether extract

Metabolism of isovaleric acid studied with isotopic carbon IRVING ZABIN (by invitation) and KONRAD BLOCH *Dept of Biochemistry and Institute of Radiobiology and Biophysics, Univ of Chicago, Chicago, Ill* In continuation of earlier studies on the degradation of branched chain compounds and their possible role in sterol synthesis (*J Biol Chem* 155 255, 1944), isovaleric acid containing C^{13} in the two end methyl groups and C^{14} in the carboxyl group has been synthesized

After incubation of labeled isovaleric acid in rat liver slices ketone bodies were isolated according to Van Slyke and analyzed for C^{13} and C^{14} The acetone fraction which includes acetone formed by decarboxylation of acetoacetate contained the C^{13} and C^{14} isotopes in a ratio of about 8 to 1 Two possible pathways for the formation of ketone bodies from isovaleric acid are a) loss of one of the end methyl groups with formation of a 4 carbon acid followed by oxidation of the latter to acetoacetate, b) oxidation of isovaleric acid at the tertiary carbon atom with production of acetone and a 2 carbon fragment The 2 carbon fragment might condense to acetoacetate The high absolute C^{13} concentration and the high C^{13}/C^{14} ratio found in the acetone fraction in the present experiment favors a pathway which involves splitting of isovaleric acid into three carbon and two carbon fragments Cholesterol isolated from the same incubation experiment contained both carbon isotopes, the incorporation of C^{13} being significantly greater than that of C^{14}

Distribution of radioisotopes of carbon and calcium administered to rats by continuous intraperitoneal infusion S H ZBARSKY (by invitation), ROGER LIENKE (by invitation) and W D ARMSTRONG *Dept of Physiological Chemistry, Univ of Minnesota Medical School, Minneapolis, Minn* Studies were made of tissue uptake and excretion of C^{14} and Ca^{45} by rats receiving these isotopes by intraperitoneal infusion The C^{14} was given as a solution of $Na_2C^{14}O_3$ in saline and the C^{14} in the expired air and excreta determined at intervals over a period of 5 days The C^{14} content of the expired air rapidly reached a high value which was maintained throughout the injection Over 90% of the injected C^{14} was excreted in the expired air and the C^{14} contained in the urine was present chiefly as urea The specific activities of the tissues and tissue components were found to be higher than in earlier experiments in which rats received intermittent injections of $Na_2C^{14}O_3$ or intraperitoneal implants of $CaC^{14}O_2$ The Ca^{45} was given over a period of 3 days as a solution of $Ca^{45}Cl_2$ in saline Feces and urine were collected daily during this time and for 5 days after the infusion was stopped, Ca^{45} appeared in all samples of feces and the total fecal excretion of Ca^{45} was nearly equal to the amount in the urine The Ca^{45} present in the skeleton, teeth and other tissues was determined The results and conclusions derived from these and other similar experiments will be presented

Effect of antimalarial agents on adenosine triphosphatase of snake venoms E ALBERT ZELLER (introduced by MARSCHELLE H POWER) *Division of Biochemistry, Mayo Foundation, Rochester, Minn* Many snake venoms are able to catalyze the hydrolysis of one phosphoric acid from ATP

This reaction is activated by some bivalent ions like magnesium, calcium, manganese and cobalt, and is inactivated by some other ions such as zinc, copper and mercury (E A Zeller, 1948) Hydrogen sulfide, cysteine and ascorbic acid inhibit the reaction while sodium cyanide activates it, possibly by removing inhibiting heavy metals Histamine inactivates the enzyme considerably in 0.001M solutions Other diamines, like pyridoxamine, have the same effect (E A Zeller *Proc Soc Exper Biol & Med*, 1949) It has been observed recently that still other drugs which are derivatives of diamines also influence ATPase in a similar way Thus, stilbamidine in 0.004M solution produces 63% inhibition of ATPase from *Butis gabonica*, regardless of the degree of activation of the enzyme by magnesium ions An approximately similar degree of inhibition of this enzyme is also produced by 0.003M quinine and 0.0015M atabrine in the presence of various concentrations of magnesium ion It is of interest to note that dephosphorylation of ATP by homogenates of guinea-pig adrenal glands was likewise inhibited to a similar extent by stilbamidine and atabrine

Non-choline esters as substrates of e-cholinesterase ('true' cholinesterase) E ALBERT ZELLER, GERARD A FLEISHER and ROBERT A McNAUGHTON (introduced by MARSCHELLE H POWER) *Division of Biochemistry, Mayo Foundation, Rochester, Minn* It has been shown that cholinesterase, which is present in certain snake venoms, is of the 'e' type (Zeller *Helvet chim acta* 33 1949) and that it is able to hydrolyze ethyl haloacetates (Zeller and Utz, *Helvet chim acta* 33 1949) The same result has been obtained with ethyl chloro acetate and e-cholinesterase of human erythrocytes (McNaughton and Zeller *Proc Soc Exper Biol & Med*, 1949) The use of the above-mentioned esters has two disadvantages 1) the enzymatic reaction is rather slow as compared to that obtained with acetylcholine and 2) the substrate blanks are relatively high We looked, therefore, for better non-choline substrates of cholinesterase β -Chlorethyl acetate was hydrolyzed by different snake venoms (*Naja naja*, *Naja melanoleuca* and *Pseudechis porphyriacus*), and erythrocyte cholinesterase at a suitable rate The non-enzymatic blanks were much lower than those of the other substrates Enzymatic hydrolysis of this compound and of acetylcholine was inhibited to the same degree by physostigmine Simultaneous hydrolysis with different concentrations of this non-choline ester and acetylcholine always resulted in competitive inhibition, both with hemolyzed erythrocytes and with snake venom Purified cholinesterase from human erythrocytes (which, contrary to the unpurified hemolyzed erythrocytes, does not split methyl butyrate) also catalyzed the hydrolysis of β -chloroethyl acetate, and gave rise

to competition with acetylcholine The β -Chloroethyl acetate was split more quickly by e-cholinesterases (snake venoms, erythrocytes) than by s-cholinesterases ('pseudo-' cholinesterase) from human serum or guinea-pig parotid glands

Effect of ultrasonic vibrations on the release and inactivation of paramycin L P ZILL (by invitation) and W J VAN WAGTENDONK *Dept of Zoology, Indiana Univ, Bloomington, Ind* Experiments were carried out to determine the feasibility of using high frequency ultrasonic vibrations as a method of disintegrating large numbers of *Paramecium aurelia* as a preliminary step in the isolation of paramycin ('killer' substance of *Paramecium aurelia* 51, variety 4) The instrument used had a maximum input of 1000 watts at a frequency of 450,000 cycles/sec Tests which were run on whole animals demonstrated that complete disintegration was not obtained with maximum input for five minutes Paramycin was completely inactivated during this treatment In order to investigate ultrasonic effects on paramycin itself, experiments were performed, using a brei prepared by repeatedly forcing a suspension of *Paramecium* through a narrow-gauge injection needle It was found that ultrasonic treatment at low intensity increased the activity of the original brei The maximum increase in activity was found to be obtained with a constant current of 80 milliamperes (ca 32 watts) and at a bath temperature not exceeding 6°C All further experiments were performed under identical conditions of vibration (constant current of 80 milliamperes) If vibration was carried out under an atmosphere of air or oxygen, increases in activity up to 185% were observed No increase or decrease in the apparent activity of the brei was found if the vibration took place under an atmosphere of nitrogen When oxygen was passed through the sample during vibration, the inactivation of paramycin proceeded according to a first order reaction

Polarographic micro-method for determination of chloride in whole blood, serum and plasma WALTER J ZIMMERMAN and WILLIAM M LAYTON, JR (introduced by H JENSEN) *Medical Department, Field Research Laboratory, Fort Knox, Ky* A polarographic method has been developed for the determination of chloride concentration in from 0.02 to 0.05 ml of whole blood, plasma and serum Proteins are removed by an adaptation of the Somogyi barium hydroxide-zinc sulfate precipitation, the final dilution of the sample for analysis being 1:100 in 0.1N potassium nitrate The diffusion current, determined by a manual polarograph at an applied voltage of 0.0 volts against a saturated mercurous sulfate half cell has been found to be directly proportional to chloride concentration in the range of concentration used It is unnecessary to calibrate the apparatus in absolute

units or to thermostat the analysis cell, provided the diffusion current is determined for two standard chloride solutions. The method has been tested for accuracy by means of duplicate determinations on 0.05 ml. of plasma with a mean error of 0.6% and by addition of 40 millimoles of potassium chloride with a mean error in recovery of 0.8%. Plasma chloride values obtained by the polaro-

graphic method have been found to correspond with values obtained with identical solutions by a standard volumetric technic. The polarographic method has the advantage of utilizing small volumes of blood, thus allowing frequent serial chloride determinations on small laboratory animals without causing undesirable changes attending excessive blood loss.

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(For possible corrections in any of the following abstracts see the June issue)

In vitro oxidation of morphine by tissue homogenates L G ABOOD and ERNEST KUN (introduced by JULIUS M COON) *Dept of Pharmacology, Univ of Chicago, Chicago, Ill* Isotonic tissue homogenates prepared in Krebs-Henseleit solution oxidize morphine *in vitro* Morphine derivatives in which the phenolic hydroxy group is substituted are not oxidized, but depress the endogenous oxygen uptake The oxidation of morphine sulfate was followed manometrically and colorimetrically by determining the reducing value of the flask contents In 1 hour 70 to 80% of the added morphine was oxidized, a fact which corresponds to an equivalent decrease in the reducing value of the reaction mixture (10-100 mg of tissue homogenate plus 10 mg of morphine sulfate), suggesting that the phenolic hydroxy group is oxidized probably to a quinone On this basis 1 mole of morphine utilizes one-half atom of oxygen, which is in agreement with the experimental findings The oxidized morphine is still pharmacologically active, and it is not further oxidized by tissue preparations Muscle, brain, liver, kidney of rabbit, rat, and guinea pig were all able to oxidize morphine Invertebrate tissues also oxidize morphine The extra oxygen consumption of 10 mg of morphine is depressed 59% by 0.01M NaCN, 83% by 0.01M Na-ScO₂, while no inhibition occurred with 0.01M NaN₃ or 0.001M HgCl₂

Effect of morphine sulfate on cerebral blood flow and metabolism in man BENEDICT E ABREU, HENRY W ELLIOTT, VIOLETTE C SUTHERLAND (by invitation), LESTER MARGOLIS (by invitation) and ALEXANDER SIMON (by invitation) *Divisions of Pharmacology and Experimental Therapeutics and of Psychiatry, Univ of California Medical School, San Francisco, and Langley Porter Clinic, State Dept of Mental Hygiene, San Francisco, Calif* Mean arterial blood pressure (MBP), cerebral blood flow (CBF), cerebral O₂ and glucose uptake and CO₂ output were determined in 5 subjects before and 20 minutes after morphine SO₄, 10 mg, 1 m All were voluntary patients who were in the Langley Porter Clinic for psychiatric care Results expressed as percentage change from the control values are indicated in the table

Pt	Sex	M B P	C B F	O ₂	CO ₂	Glucose
M R	f	-7	-21	-32	-19	0
M P	f	+9	-22	-31	-25	-19
R R	m	+3	-3	+4	-2	+124
S M	m	0	-9	-17	-26	+40
J T	m	+3	+58	+60	+47	+124

The trend of effect as indicated in 5 subjects is for morphine to decrease significantly the CBF (in 2 of 5), O₂ uptake (in 3 of 5), CO output (in 3 of 5), but to increase glucose uptake (in 3 of 5)

Simple method for determining the degree of vascular sympatholytic action RAYMOND P AHLQUIST *Dept of Pharmacology, Univ of Georgia School of Medicine, Augusta, Ga* The increasing use of sympatholytic agents as tools in pharmacological and physiological research has demonstrated the need for a simple method for determining more exactly the degree or extent of sympatholysis present in the experimental animal The proposed method is based on the reduction, by sympatholysis, of the intense vasoconstriction produced by the injection of epinephrine, 0.005 mg/kg, into the femoral artery This dose, which practically always produces a marked pressor response when injected intravenously, has little or no pressor effect when injected into the femoral artery of untreated dogs As sympatholysis develops following the administration of Dibenamine, Priscol or other sympatholytics, this intraarterial constricting effect becomes diminished, and the pressure response to the intra arterial injection becomes more similar to the intravenous response This method gives much more information regarding degree of sympatholysis than does a determination of the presence or absence of epinephrine 'reversal' This is well illustrated by the finding that even though epinephrine 'reversal' is present, the intra arterial injection shows that epinephrine is still capable of producing marked vasoconstriction It has also been found that in some of the visceral vascular beds the vasoconstricting action of epinephrine cannot be prevented by any dose of the sympatholytic agents tested thus far

Sympatholytic action of dibenamine RAYMOND P AHLQUIST *Dept of Pharmacology, Univ of Georgia School of Medicine, Augusta, Ga* Nickerson

et al first showed that epinephrine, even in very high dosage, produced only a depressor response when injected intravenously into cats or dogs following an effective dose of Dibenamine. Although this tends to show that the sympatholytic action of Dibenamine is absolute against epinephrine constriction, results obtained in this laboratory, using direct blood flow measurements and intra-arterial injections of epinephrine, show that the action of Dibenamine is not absolute. Epinephrine continued to produce constriction in the vascular bed of the pregnant dog's uterus following doses of Dibenamine up to 40 mg/kg. In these same experiments the epinephrine also produced uterine contractions after Dibenamine. In some dogs it was found that the femoral intra-arterial injection of epinephrine continued to produce vasoconstriction following high doses of Dibenamine although the usual response in this case is a slight vasodilation. Other vascular beds are still under investigation. These results emphasize the fact that the response to epinephrine following sympatholytic agents is determined by the ratio between the receptors which respond unchanged to epinephrine (vasodilation, uterine inhibition etc.) and the receptors whose response is decreased but apparently not completely abolished (vasoconstriction, uterine stimulation, etc.). These results also indicate that the apparent differences between so-called 'adrenolytic' and 'sympatholytic' action are probably not real since intra-arterial injection of epinephrine corresponds more closely to sympathetic nerve stimulation (other than to the adrenal medulla) than does intravenous injection.

Influence of orally administered rutin on adrenal ascorbic acid storage in guinea pigs. ANTHONY M. AMBROSE and FLOYD DEEDS. *Pharmacology Laboratory, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, USDA, Albany, Calif.* In a previous paper we have shown that guinea pigs maintained on a scorbutogenic diet and receiving suboptimal doses of ascorbic acid (0.2 mg/day) in conjunction with rutin (100 mg/day) survived longer and showed less severe scorbutic symptoms than guinea pigs receiving ascorbic acid or rutin alone. In the light of results by Cotureau *et al* (*Nature* 161: 557, 1948) on the 'sparing action' of vitamin P or catechin on adrenal ascorbic acid, it was felt that this approach to the evaluation of the physiological role of rutin might be more objective. Accordingly, 120 young guinea pigs were divided into 4 groups of 30 each and placed on a scorbutogenic diet. In addition the following supplements were administered orally each day. One group served as control, one group received 100 mg rutin in 0.25 ml propylene glycol, one group received 2 mg ascorbic acid, and another group received 2 mg ascorbic acid and 100 mg rutin. After 12 to 20 days, 8 animals from each

group were sacrificed and adrenal ascorbic acid determined by the method of Roe and Kuether. Our results indicate that rutin appears to play no important role as an 'economizing factor' in the storage of adrenal ascorbic acid under the conditions of our experiments. Smaller doses of ascorbic acid, 1 mg or 0.5 mg/day, clearly demonstrate that as the dose of ascorbic acid is lowered there is an accompanying lowering of the amount of adrenal ascorbic acid stored irrespective of rutin.

Aureomycin protective effect in experimental Rocky Mountain spotted fever with particular reference to intermittent treatment. LUDWIG ANIGSTEIN, DOROTHY M. WHITNEY, and JOE BENINSON (introduced by G. A. EMERSON). *Rickettsial Research Laboratory, Dept of Preventive Medicine and Public Health, Univ of Texas Medical Branch, Galveston, Texas.* Time-dose relationships in intermittent aureomycin therapy of spotted fever is the subject of this study. A total of 30 guinea pigs were infected with spotted fever and subsequently injected subcutaneously with repeated single doses of 10, 20, and 30 mg of aureomycin hydrochloride (Lederle) per guinea pig (weighing 450-500 gm), at 48- and 72-hour intervals. Four injections were given to each test animal covering the incubation and most of the febrile period of the controls. A drastic change of the clinical pattern of the infection was manifested by all treated guinea pigs. The fever was either entirely suppressed or reduced to abortive attacks after long incubation, regardless of time-dose schedule. Whereas the untreated controls developed typical spotted fever and died, all test animals recovered with immunity to reinoculation with spotted fever. Evidence was thus gained that intermittent aureomycin therapy with minimum dosage is equally efficient in spotted fever as the continuous massive treatment. The protective effect of aureomycin and the ultimate immunity of the infected guinea pig express the balance kept by the antibiotic between the rickettsial invader and the host resistance. This was substantiated by the specific antibody response in the mechanism of the rickettsiostatic activity of the antibiotic as found by complement fixation tests. This phase of the problem is under study.

Physiological disposition of aminopyrine in man. JULIUS AXELROD (by invitation) and BERNARD B. BRODIE. *Dept of Biochemistry, New York Univ College of Medicine, New York Univ Research Service, Goldwater Memorial Hospital, and Laboratory of Industrial Hygiene, New York City.* Aminopyrine is quickly and essentially completely absorbed from the gastro-intestinal tract. Only about 2% of the drug is excreted unchanged indicating its almost complete metabolism in the body. Plasma levels decline slowly, about 15 to 20% per hr. The route of metabolism of amino

pyrine was shown to be as follows. About 55% of the drug demethylates to form 4-aminopyrine, which in turn is acetylated and excreted in this form. The other route or routes of metabolism were not ascertained. Preliminary results suggest that 4-aminoantipyrine is an effective antipyretic, antirheumatic and analgesic.

Differentiation of sympathetic from parasympathetic drugs by measurements of phaseboundary potentials. T. C. BARNES and R. BEUTNER, *Hahnemann Medical College and Hospital of Philadelphia, Philadelphia, Pa.* The oil-cell method (Barnes and Beutner, *Science* 104: 569, 1946) provides a basis for the distinction between adrenergic and cholinergic nerves. Previous reports (*Federation Proc.* 4: 112, 1945; *Internat. Physiol. Congress* 1947, p. 60) showed that adrenergic drugs dissolve in triglyceride oils on which they form negative phaseboundary potentials. In contrast, the cholinergic drugs, choline, acetylcholine, mechoyl, pilocarpine, eserine, prostigmine, etc. are inactive on adrenergic oils. Tests show that atropine, curare and etamon also have the solubility of cholinergic drugs. The alipathic sympathomimetics give potentials with the adrenergic oils. (In this abstract all concentrations are 0.05% and potentials negative.) Aranthol gave 19 mv. on tributyrin, octin produced 32 mv. on triacetin and octethyl gave 15 mv. on triacetin. Adrenergic blockade may be competitive negativity since priscol produced 45 mv. on tributyrin, yohimbine 77 mv. on tributyrin and dibenamine 6 mv. on triacetin. Most ergot compounds are difficult to test in the oil cell but ergonovine gave 53 mv. on the adrenergic oil tributyrin. It is interesting that nicotine has the solubility characteristics of adrenergic drugs giving 87 mv. on tributyrin suggesting that the pressor effect is direct adrenergic action. The problem of nicotinic and muscarinic effects is now being investigated by the oil cell method.

Relation of 4-methyl and 4-ethyl substituted imidazoles to 1) histaminic action, 2) antagonism by antihistaminics. W. BARRETT (by invitation), J. FEICKERT (by invitation), B. CRAVER, *Division of Microbiology, Research Dept., Ciba Pharmaceutical Products, Inc., Summit, N. J.* In respect to the relation between chemical structure and histaminic activity as judged by the stimulating action of these compounds upon the smooth muscle of the ileum of the guinea pig, the following observations seem justifiable. The activity appeared progressively to diminish as one or both of the hydrogens of the amino group were replaced by alkyl groups, and the length of these alkyl groups was extended, the replacement of the amino group with other groups diminished or eliminated the activity, the amino methyl derivative of imidazole was devoid of this action unless at least one hydrogen were substituted by an alkyl group,

but otherwise the effect of increasing the length of the alkyl group was the same as in the ethyl series. As to the relation between the chemical structure of these compounds and the antihistaminic activity of Pyribenzamine, the following conclusions appear justifiable. Minimal doses eliminated the histaminic action of the compound in the ethyl series only in the presence of an amino group, whether it were mono- or di-substituted, but in the methyl series the low doses of Pyribenzamine sufficed only if but one hydrogen had been replaced by an alkyl group and not if both had been replaced. Its antagonism in high doses toward other derivatives would appear attributable to its mild anticholinergic activity.

Effect of certain mitotic poisons on uric acid and allantoin excretion in mice. ALLAN D. BASS and ELIZABETH F. PLACL, *Dept. of Pharmacology, College of Medicine, Syracuse, N. Y.* KOPAC (*Tr. N. Y. Acad. Sci.* 8: 5, 1945) has called attention to the relationship of mitotic poisons to nucleoprotein systems. Krizik *et al.* (*U. S. Atomic Energy Commission Report* MDCC 1647) found no increase in uric acid or allantoin in the urine of dogs given lethal doses of X-ray, a well established mitotic inhibitor. These observations appeared to be somewhat at variance in light of the reduction in amount of lymphoid tissue we have observed in mice following administration of such mitotic poisons as nitrogen mustard and urethane. A study was therefore begun to determine in mice the effect of mitotic poisons on the products of nucleoprotein catabolism. C3H or C3H₁ hybrid mice bearing 6C3H1ED tumors were used since these lymphoid tumors gave a large reservoir of lymphoid tissue high in nucleoproteins. Urine was collected from groups of 10 to 15 animals over a period of three hours. Daily doses of colchicine (0.75 mg/kg) and nitrogen mustard (2 or 4 mg/kg) in saline were administered intraperitoneally. Uric acid-creatinine and allantoin-creatinine ratios were determined since only fractions of the 24-hr. output of urine were collected. The uric acid-creatinine ratio was elevated in the 6 to 48 hour period following drug administration. The allantoin-creatinine ratio was elevated to a lesser extent. Less striking but definite changes were observed in non-tumor bearing animals. Blood uric acid levels were also found to be elevated following administration of the agents used. The findings suggest that no major defect in nucleoprotein catabolism is affected by the mitotic poisons studied.

The subcutaneous use of thimerin, a new mercurial diuretic for treatment of congestive heart failure. ROBERT C. BATTERMAN, DAVID UNTERMAN (by invitation) and ARTHUR C. DEGRAFF, *Dept. of Therapeutics, New York Univ. College of Medicine, New York City.* The diuretic effectiveness in terms of predictability of

response, and degree of diuresis was ascertained for a new mercurial diuretic, Thiomerin, N(β -carboxymethyl mercapto-mercuri- γ -methoxy) propyl camphoramic acid disodium salt. In a group of 45 patients with congestive heart failure, the subcutaneous administration of Thiomerin resulted in a satisfactory diuresis in 86.6% of trials as compared with 81.0 and 83.3% of the trials for Thiomerin and Mercuzanthin respectively, given intravenously. The degree of diuresis for each drug regardless of route of administration was equivalent, indicating that complete absorption occurred from the site of subcutaneous injection. In an additional group of 35 ambulatory and 73 bedridden patients, Thiomerin when administered subcutaneously was satisfactory in removing the edema, preventing the accumulation of edema and for maintenance of patients' state of compensation. In all 589 injections of 1 to 4 cc of Thiomerin were given subcutaneously to 143 hospital and ambulatory patients. The drug was well tolerated by the patients in 90% of the trials. Subjective burning at the site of injection occurred in approximately 10% of the number of injections. Tender induration and nodular formation was noted in 4 and 2.5% respectively. Thiomerin when given subcutaneously is an effective and safe diuretic with a predictability of diuretic response and degree of diuresis equivalent to that noted with other mercury diuretics used intravenously and is superior to these preparations used intramuscularly.

Subacute respiratory toxicity of a new insecticide activator in 'aerosol bomb' mixture. ROBERT O. BAUER and WALTER W. JETTER (introduced by GEORGE L. MAISON) *Dept of Pharmacology, Boston Univ School of Medicine, Boston, Mass.* Animals were exposed in a closed chamber to dispersions of insecticide mixtures in air 1 hour a day for 4 weeks. The mixture contained (activator) N-octyl bicycloheptene dicarboximide 2%, (insecticide) DDT 2% in kerosene) 1%, (vehicle) methylene chloride 5%, alkyl naphthalenes 5%, freons 11 and 12, 85%. Two concentrations at 1 gm and 0.1 gm/60 l air were used. Ten guinea pigs and 10 Wistar rats were subjected to each concentration. An equal number served as controls. Hemoglobin, red and white cell counts, urinary albumin and sugar were estimated before and after the experiment. The test animals displayed irregular food consumption. Group growth curves were depressed. Surviving test animals regained lost weight completely. Only 4 fatalities occurred and these were in rats on high concentrations. In both species fine tremors, hyperirritability and conjunctival hyperemia were observed, becoming progressively worse and disappearing after exposure. Female guinea pigs receiving high concentrations developed reversible alopecia. Mild anemia in test guinea pigs

and leucopenia in test rats were encountered irregularly. Incomplete pathologic examinations show 1) moderate to severe interstitial pneumonitis in all test animals, 2) focal necrosis of liver and spleen in high concentration rats, 3) extensive splenic hemosiderosis and hypoplastic bone marrow, chiefly in high concentration guinea pig group. Pneumonitis and conjunctival hyperemia in test animals point to direct chemical irritation, more likely due to the vehicle than the insecticide or activator. Other clinical and pathologic changes may result from the insecticide mixture, nutritional disturbance or a combination.

Survival of plasmodium cathemerium sporozoites in hen blood with addition of canary blood cells. HARRY BECKMAN *Marquette University School of Medicine, Milwaukee, Wis.* Mosquitoes carrying the sporozoites of *P. cathemerium* 3H2 were ground in Locke's solution and added simultaneously to freshly prepared hen plasma to which there had been added to respective portions a) freshly washed canary blood cells, b) freshly washed hen blood cells, c) a mixture of freshly washed canary and hen blood cells. The three mixtures were incubated with gentle agitation at 41.5° C for 1 hour and then portions representing 1 mosquito each were withdrawn and injected intramuscularly into canaries, the peripheral blood of these birds being subsequently examined from the 5th to 16th days, inclusive. Birds failing to become positive were challenged by mosquito bite. Trials employing 4 birds in each group were run at sufficient intervals that at least one complete 3-week sporozoite-to-sporozoite cycle lay between. Of the 16 canaries injected with hen plasma plus canary cells, all developed patent blood infections. Of the 16 injected with hen plasma plus hen cells, none developed patent infections and all became infected when later challenged by mosquito bite. Of the 16 birds injected with hen plasma plus both hen and canary cells, 13 developed patent blood infections. It would appear from this preliminary study that it is the formed elements of canary blood that permit survival of sporozoites in hen plasma, in which alone they do not survive, as earlier shown in this laboratory.

Some metabolic changes observed in seizures induced by certain agenetreated protein in dogs. JULIUS BELFORD (by invitation) and D. D. BONNycastle *Laboratory of Pharmacology, Yale Univ., New Haven, Conn.* The resemblance to human epilepsy of the motor seizure and EEG dysrhythmia occurring in canine 'epilepsy' (caused by certain 'agene' (NCl₃)-treated proteins such as wheat gluten and zein etc.), warranted further study of this seizure phenomena. Five to 8 dogs were employed in each experiment and were classified as being in the normal, pre-fit, fit, (and when feasible) inter-paroxysmal stage. Blood samples

were taken from these animals and examined for various entities. These results were subjected to statistical examination. No significant changes were found at these several stages in serum calcium, magnesium or choline esterase. The intravenous injection of 100 mg of physostigmine, reputed to lower serum choline esterase by 95% did not precipitate convulsions, on the other hand, sub-convulsive doses of metrazol could induce a seizure. No significant differences were found in arterial glucose, but a significant pre fit fall occurred in venous glucose followed by a rise during a seizure. No significant A-V glucose differences were observed although there was a tendency towards negative A-V differences in the abnormal phases. The oxygen content of arterial and venous blood showed no change. However, while the arterial carbon dioxide did not change, the venous carbon dioxide fell significantly at the time of the fit.

Observations on administration of BAL in selenium poisoning. J B BRIGORSKY (by invitation) and DONALD SLAUGHTER, *Dept of Physiology and Pharmacology, Univ of South Dakota School of Medicine, Vermillion, S D*. Braun, Lushy and Calvery, (*J Pharmacol & Exper Therap* 87: 119, 1946) reported that BAL was not only ineffective in the treatment of selenium poisoning but actually increased the toxicity of this element in experimental animals. Because of the prevalence of selenium in the soil of this region, further work seemed pertinent. Twenty young rats were used in each of three experiments, sodium selenite (SS) was administered intraperitoneally, BAL intramuscularly. Animals were injected daily for a period of 14 to 24 days. Varying doses of SS caused no toxic symptoms until a level of 10.0 mg/kg was reached. When a non-toxic dose of BAL (15.0 mg/kg) was administered $\frac{1}{2}$ hour following the injection of a nontoxic dose of SS, (5.0 mg/kg) all animals died. This would seem to indicate that BAL and SS have a synergistic toxicity. Durlacher *et al*, (*J Pharmacol & Exper Therap* 87: 28, 1946) state that BAL is toxic in a 15 mg/kg dose but our work does not bear out this statement. As a matter of fact, histological examination of the liver following BAL alone reveals no pathological changes. Since the liver is considered to be the probable site for detoxification of SS, it is of unusual interest to observe that BAL prevented the necrosis and fat deposits produced by SS alone. Further experimental work to elucidate this seeming paradox is being conducted.

Alteration of the activity of the human gastrointestinal tract with dihydroergocornine methanesulfonate (D H O 180). W D BENNETT (by invitation), L E MORRIS (by invitation) and O SIDNEY ORTH, *Depts of Anesthesiology and Pharmacology, Univ of Wisconsin Medical School, Madison, Wis*. Administration of the dihydroergot

derivatives, particularly dihydroergocornine methanesulfonate (D H O 180—Sandoz), may elicit initially unfavorable side effects such as anorexia, nausea and vomiting. When this drug was given to block possible cardiac irregularities before cyclopropane anesthesia, it was noted subsequently in patients in which laparotomies were performed that the intestines were quite contracted, in fact that they had a ribbon-like appearance. It seemed possible that such evident stimulation of the gastrointestinal tract could be used to advantage clinically if the proper amount of one of these relatively non-toxic dihydroergot compounds was used. Dihydroergocornine has been administered in doses of up to 4 mg total intravenously given in about 4 minutes, 1-2 mg as a single dose intramuscularly, or 3 mg q i d orally. In cases of intestinal distention, intestinal obstruction, and congenital megacolon, this drug has been found to be distinctly advantageous.

Paralysing action of 2-methyl, 2-n-amy-4-hydroxymethyl-1,3-dioxolane (glyketal). F M BERGER (introduced by ARNOLD D WELCH) *Univ of Rochester School of Medicine, Rochester, N Y*. Numerous 2 substituted-4-hydroxymethyl-1,3 dioxolanes possess central depressant properties similar to those of the glycerol ethers or benzimidazoles. 2-methyl, 2-n-amy-4-hydroxymethyl-1,3-dioxolane appeared most active in this respect. This compound in doses of 100-150 mg/kg injected intraperitoneally to small laboratory animals caused paralysis of the voluntary muscles. During paralysis respiration was not depressed and the animals remained conscious. There was a marked decrease of muscular tone. The muscles remained excitable to direct and indirect stimulation and there was no curare-like action. The corneal and pupillary reflexes and the knee jerk remained unaffected. The animals did not react to painful stimuli of moderate intensity. This was probably due to the motor paralysis. The compound had no analgesic action in nonparalytic doses. Paralysis was followed by complete recovery of all functions. After lethal doses death was due to respiratory paralysis. On intravenous injection mild and transient peripheral vasodilatation was caused. The action of acetylcholine was not interfered with. Glyketal also had a local anesthetic and quindine-like action. Its anticonvulsant action against strychnine was of a similar order as that of myanesin. Myanesin and glyketal differed in their metrazol antagonism. Myanesin had little effect on the occurrence of convulsions but protected the animals from death. Glyketal behaved as a true, although inefficient, anticonvulsant by protecting the animals from both convulsions and death.

Ratio of amino acid nitrogen to creatinine in the urine as a sensitive diagnostic test for ura-

num poisoning in the rabbit H BERKE AND A ROTHSTEIN (introduced by H C HODGE) *Dept of Radiation Biology, Univ of Rochester, Rochester, N Y* It has been shown in this laboratory that the ratio of amino acid nitrogen to creatinine in the urine was a sensitive indicator of uranium poisoning in the rabbit During daily exposure to inhalation of uranium dusts, the ratio remained elevated in contrast to the more or less rapid decline of other blood and urine constituents which also became abnormally high soon after exposure to uranium More detailed experiments, therefore, were set up to determine the animal response in terms of this ratio to intravenous administration of uranyl nitrate The minimal dose of uranyl nitrate which gave a statistically significant increase in ratio was 0.02 mg U/kg The ratio was a more sensitive test than either urinary protein or blood NPN Blood amino acid nitrogen remained normal in all animals, indicating that the amino aciduria was renal in origin Multiple injections of UO_2NO_3 at 2- to 3-week intervals at levels of 0.04, 0.1 and 0.2 mg U/kg showed 3 patterns of response as measured by the ratio In general the magnitude of the response was proportional to the dose given At lower levels of administration, the maximal increase in the ratio occurred soon after each injection of uranium followed by lower but still high values The peak values following each injection either remained the same, declined or increased in value depending on the dose given A few animals, observed for 3 months after their last multiple injection, still showed abnormally high values of the ratio, even though histological examination revealed no renal damage directly attributable to uranium

Effect of a number of aralkylamines on tyramine oxidation by amine oxidase FREDERICK BERNHEIM and EDWIN J FELLOWS *Duke Univ School of Medicine, Durham, N C and Temple Univ School of Medicine, Philadelphia, Pa* The present experiments were carried out in order to determine if a correlation existed between the central excitatory and amine oxidase inhibitory activity of a number of benzylamine, beta-aryl-ethylamine and aryl-2-aminopropane modifications In many instances an excellent correlation was observed, however, there were notable exceptions Injection of several 3-amino-1-phenylbutane and 4-amino-1-phenylpentane derivatives and subsequent histopathological examination of the brain and cord of these animals disclosed a destructive effect on certain cells of the nervous system Since enzymatic data on this type compound were not available, it appeared desirable to include them in the present studies All of these agents exhibited a significant amine oxidase inhibitory effect

Chemotherapeutic effect of 8-amino quinolines

in schistosoma mansoni infections in mice RAYMOND N BIETER, ELIZABETH M CRANSTON, WAYNE CHADBURN (by invitation), ASHTON C CUCKLER (by invitation), DOMINIC DEGIUSTI (by invitation), W W BECKLUND (by invitation), and HAROLD N WRIGHT *Dept of Pharmacology, Univ of Minnesota Medical School, Minneapolis, Minn* Two 8-amino quinolines, 6-methoxy-8-B diisobutylaminoethylamino quinoline dihydrochloride (SN2842) and 6-methoxyl-8-diisobutylaminoisopropylamino quinoline dihydrochloride (SN-3501), have been found to possess a relatively high degree of chemotherapeutic activity in schistosomiasis in mice The drugs were administered in the diet in varying concentrations to mice injected intraperitoneally with 100 to 125 cercariae of schistosoma mansoni shed from snails of the species *Australorbis glabratus* Therapy was started 4 to 6 weeks after infection and continued for 1, 2, or 4 weeks Mice were autopsied 1 or 2 weeks after therapy was discontinued The number of worms in the portal and mesenteric veins and liver were counted When mice were treated with SN3501 in a concentration of 0.1% for 2 weeks 85% of 32 mice contained no worms at autopsy, compared with 10% negativity in the controls, and the average number of worms per mouse was 0.25, compared with 8.4 per mouse in the controls With SN2842 in the same concentration and for the same duration of therapy 97% of 39 mice were completely negative for worms, compared with 12% of control mice, and the average number of worms per mouse was 0.1, compared with 9.5 per mouse in the controls Eleven other 8-amino quinolines tested were found to be relatively inactive Neither SN3501 nor SN2842 showed activity as a prophylactic when administered simultaneously with infection for a period of 2 weeks in a concentration of 0.1%

Depressor action of nitrate esters of alkyl glycolates JOSEPH G BIRD (by invitation), HARRY K IWAMOTO, JOHN B HARMON (by invitation) and JOHN C KRAVITZ, JR *Dept of Pharmacology, Univ of Maryland School of Medicine, Baltimore, Md* A series of nitrate esters of glycolic acid esterified with alkyl alcohols varying from methyl to decyl were prepared Certain physicochemical properties associated with pharmacodynamic responses were investigated Depressor responses elicited, in dogs under anesthesia, by these esters were observed It appears that the depressor response in the series studied increased with decreasing water solubility of the higher esters

Quality and potency determination of analgesics D D BONNICASTLE and JOHN A BIRD, JR (by invitation) *Laboratory of Pharmacology, Yale Univ, New Haven, Conn* Experiments are reported in which a constant dose of morphine

applied to the rat tail and the log-ratio of reaction time before and during the influence of a drug was used as a metameter of response to pain. The mean response of groups of such animals was calculated from truncated distributions. The relation of reaction time to degree of stimulus also was studied. The curves illustrating response/log dose relationship for the individual drugs take various forms, and three main types can be recognized. In one group, (e.g. acetylsalicylic acid) the continuous rise in response levels off asymptotically at about 30% increase in reaction time. A second type (e.g. codeine) presents the same picture at lower doses, but with high doses a secondary rise occurs tending towards an asymptote at about 100%. A third form of reaction curve is a direct rise from 0 to an asymptote of 100%. Intermediate forms between these types may be observed. It is suggested that the two asymptotic levels represent respectively the maximum analgetic, (thalamic?) effect and the maximum hypesthetic (cortical?) effect. These two properties of the drug are measured by the two doses at the respective points of inflection in the first and secondary rises. In the description of the pain-relieving activity of an individual chemical compound these two constants may serve to indicate a) simple analgetic potency, and b) narcotic activity, respectively.

Binding of streptomycin to plasma proteins
G. E. BOXER, V. C. JELINEK (by invitation), and A. O. EDISON (by invitation) *Research Laboratories of Merck & Co., Inc., and the Merck Institute for Therapeutic Research, Rahway, N. J.* Elsewhere it has been reported that the clearance of streptomycin is about 70% of the simultaneously measured glomerular filtration rate. The possibility that protein binding made part of the streptomycin present in the plasma unavailable for filtration through the glomerulus was investigated. Two techniques were employed to measure protein binding: 1) Measurement of the distribution of streptomycin on both sides of a cellophane membrane after dialysis of dog plasma against a Ringer solution containing varying amounts of streptomycin. 2) Determination of streptomycin in the ultrafiltrate of plasma containing varying amounts of the drug. The dialysis experiments indicated $33 \pm 3\%$ and the ultrafiltration experiment $35 \pm 4\%$ binding of streptomycin to the plasma proteins of the dog. Within the range tested, 5 to 100 γ /ml, the percentage of streptomycin bound was independent of the concentration of the drug but dependent on the protein concentration. Both albumin and globulins are responsible for the binding. The observed protein binding accounts quantitatively for the observed clearance of streptomycin at about 30% below the simultaneously measured glomerular filtration rate.

Indirect in vitro action of antibiotics in com-

parison with activity of accepted amebicides
JOHN L. BRADIN, JR., and EDER L. HANSEN (introduced by HAMILTON H. ANDERSON) *Division of Pharmacology and Experimental Therapeutics, Univ. of California Medical School, San Francisco, Calif.* A comparative study has been carried out which reveals differences in amebicidal levels between tests performed in cotton-stoppered versus vaseline-sealed cultures (see abstract by Hansen, *Federation Proc.*, this issue). As a base line for the antibiotic studies, the method has been applied also to retest representative amebicides, such as carbarsone, carbarsone oxide, emetine, vioform, diodoquin, and two thioarsenites (C.C. 914 and C.C. 1037). All antibiotics tested to date have shown some degree of bacteriostatic activity, and death of the ameba in cotton-stoppered tubes is shown to be due to inhibition of growth of the associated bacteria rather than to direct action of the antibiotic on *E. histolytica*. It has been shown previously (Bradin, in press) that death of the ameba, following exposure to an antibiotic which produced bacteriostasis, could be correlated with a shift in oxidation-reduction potentials of the cultures from negative to positive levels. Chang (1946) had reported that such positive levels constitute an environment lethal to this protozoan. It is, therefore, suggested that the indirect amebicidal activity, in the case of the antibiotics observed in cotton stoppered cultures, is due, at least in part, to this phenomenon. Data are presented showing oxidation-reduction levels in cotton-stoppered and in vaseline-sealed cultures.

Inhibition of cholinesterase activity of human blood plasma II. Studies with hexaethyltetrapolyphosphate containing C¹⁴
RALPH W. BRAUER and RITA L. PESSORRI (by invitation) *Dept. of Pharmacology and Experimental Therapeutics, School of Medicine, Louisiana State Univ., New Orleans, La.* The mechanism of the interaction between plasma (cholin) esterase and neutral phosphate ester type inhibitors (e.g. hexaethyltetrapolyphosphate (HETP), Tetraethylpyrophosphate (TEPP)), has been studied in a previous paper (Ralph W. Brauer, *J. Pharmacol. & Exper. Therap.* 92: 162, 1948). Work to be reported represents an extension of that earlier investigation along the following lines: 1) HETP containing C¹⁴ in the alkyl groups (alpha carbons) has been prepared. This material is being used to investigate whether the enzyme inhibition involves the fixation of any part of the inhibitor molecule upon the inhibited enzyme. The previous study had shown that the P-containing moiety is not firmly attached to the enzyme, and that enzyme inhibition is paralleled by disappearance of inhibitor. 2) The addition of F⁻ in amounts which do not affect the enzyme to aqueous solutions of HETP or TEPP markedly delays the loss of anticholinesterase activity, ob-

served under these conditions in the absence of fluoride. This reaction is described in detail, and discussed in relation to the enzyme-inhibitor reaction. 3) *In vivo* studies of the rate of recovery of liver and plasma cholinesterase activities of male rats after Di-isopropyl fluorophosphate (TEPP) and p-nitrophenyl thiophosphate administration are reported.

A single injection method for the assay of digitalis in pigeons. H. A. BRAUN and L. M. LUSKY (by invitation) *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.* A comparative study of digitalis assay has been made using a single-injection pigeon method, the USP XIII intravenous infusion cat method, and the intravenous infusion pigeon method (*J. Pharmacol. & Exper. Therap.* 93: 81, 1948). In the single injection method, digitalis dilutions were injected into the alar veins of pigeons at 3 dosage levels. Six pigeons were used on each dose. Death occurred in from 3 minutes to 1 hour. The slope of the dosage mortality curve is very steep (on the order of 40 probits/log dose) and as a result the standard error of the assay is small. This method results in a considerable saving in time over the infusion methods. Assays by the 3 methods have been completed on a number of digitalis preparations of various types with good agreement between methods.

Evaluation of several types of drugs and combinations against anaphylaxis in guinea pigs. BARBARA B. BROWN (by invitation) and HAROLD W. WERNER *Pharmacology Dept., Research Laboratories, Wm. S. Merrell Co., Cincinnati, Ohio.* Eight compounds possessing parasympathetic depressant, sympathomimetic, or direct smooth muscle depressant action were evaluated for antianaphylactic activity in guinea pigs actively sensitized to horse serum. Activity was determined following intraperitoneal injections of each compound and of combinations of each with a maximal noneffective dose of the antihistamine, Decapryn Succinate. The direct smooth muscle depressants, papaverine and Butaphyllamine (theophylline isobutanolamine), considered as a group were the most effective antianaphylactic agents both alone and in combination with Decapryn. Two of the parasympathetic depressants, atropine and Diocyl (diethylaminoethyl 1-cyclohexylcyclohexanecarboxylate), failed to protect against anaphylactic death, but doses of 6 to 14 mg/kg injected with the noneffective dose of Decapryn reduced mortality by about 60%. A new parasympathetic depressant which also has direct smooth muscle depressant action, 2,2-diphenyl-4-dimethylaminomethyl-1,3-dioxolane methobromide, was ineffective alone but reduced mortality to a minimum when combined with Decapryn. Two sympathomimetic amines, Nethamine (N-ethylephedrine) and N-methyl-

N-cinnamyl-2-phenylpropylamine, had no action alone but caused a significant reduction in mortality when employed in 6 to 17 mg/kg doses with the antihistamine. A test of one triple combination composed of noneffective doses of Butaphyllamine, Nethamine, and Decapryn demonstrated marked potentiation of action. These results indicate that certain combinations of drug actions may prove valuable in allergic bronchial asthma.

Adrenergic-like action of acetylcholine on the isolated rabbit heart. H. D. BRUNER and KWANG SOO LEE (by invitation) *Dept. of Pharmacology, Univ. of North Carolina, Chapel Hill, N. C.* The apparent adrenergic action of acetylcholine on atropinized isolated mammalian hearts has been further analyzed by adding tetraethylammonium Br, nicotine, d-tubocurarine, F-933, dihydroergocornine, ergotamine, dibenamine and priscol to the isolated rabbit heart perfused by the Langendorff technique. The findings throughout confirm the possibility suggested by Hoffman *et al.* (*Am. J. Physiol.* 144: 189, 1945) that there are ganglion cells on these hearts whose activities are to be classed as adrenergic. The dose of acetylcholine necessary to produce the adrenergic effect is some 10 to 100 times that to produce an intense typical cholinergic effect. No evidence has been found that acetylcholine has a direct myotropic action on ventricular muscle. Acetyl- β -methyl choline does not elicit the response.

Oral Activity of Analogues of 3-ortho-toloxyl-1,2-propanediol. JOHN C. BURKE, RICHARD K. THOMS, G. LEE HASSERT, JR. and RITA J. WILKINS (introduced by CHARLES R. LINEGAR) *Pharmacological Development Division, E. R. Squibb & Sons, New Brunswick, N. J.* In June 1948 Berger and Schwarz (*J. A. M. A.* 137: 772) reported that 3-ortho-toloxyl-1,2-propanediol (Tolserol) is effective by oral administration for the relief of various spastic conditions. One of the chief limitations to such use is its short duration of action. In an attempt to overcome this difficulty, a large series of compounds, synthesized and screened in Squibb Institute for Medical Research (*Tr. New York Acad. Sci.* 2: 11, 2-5, 1948) for possible intravenous use, were reviewed. Seven of these compounds were selected on the basis of their intraperitoneal potency in mice and their solubility and screened for their depressant action orally in mice. Compounds with lower solubility than Tolserol showed for the most part a slower onset and longer duration of action. Solutions of Tolserol have as much as three times the activity as high concentration suspensions but a shorter duration of action. Two compounds of the series 2-ortho-toloxyl-1,2-propanediol and 3-thymyloxy-1,2-propanediol, showed the most advantageous combination of oral potency and duration of action in mice. The 3-thymyloxy compound proved irritant at 100 mg/kg.

level studies in dogs showed that the absorption of the latter compound was delayed but more prolonged and constant than that of Tolserol

Trichloroethanol as a metabolic product of trichloroethylene THOMAS C BUTLER *Dept of Pharmacology and Experimental Therapeutics, Johns Hopkins Univ, Baltimore, Md* When urine from dogs that have inhaled trichloroethylene vapor is acidified and heated, trichloroethanol is formed, presumably by hydrolysis of the glucuronide, urochloralic acid Trichloroethanol is produced in larger amounts from trichloroethylene by the dog than is trichloroacetic acid, a metabolic product previously identified (Barrett and Johnston, *J Biol Chem* 127 765, 1939, Powell, *Brit J Indus Med* 2 142, 1945) The immediate precursor both of trichloroethanol and of trichloroacetic acid may be chloral hydrate

Influence of alpha-naphthylthiourea (ANTU) on iodine metabolism RICHARD U BIERRUM (by invitation), KENNETH W COCHRAN (by invitation), and KENNETH P DuBOIS *Univ of Chicago Toxicity Laboratory and the Dept of Pharmacology, Univ of Chicago, Chicago, Ill* Previous work in this laboratory has shown that the administration of iodide will protect rats against several lethal doses of ANTU while thyroidectomized rats are not protected by iodide We have, therefore, studied the effect of acute ANTU poisoning on iodine metabolism Thyroids of normal rats fed a synthetic diet had an average iodine concentration of 129 mg % Five hr after a lethal dose (10 mg/kg) of ANTU there was a 50% decrease in thyroid iodine Twenty-four hr after a sublethal dose (4 mg/kg) of ANTU thyroid iodine was one-third the normal level When normal rats were fed a diet containing 5.3 mg I/gm of diet for 2, 5, and 10 days the average iodine values increased to 201, 270, and 297 mg % respectively for the 3 feeding periods Various tissues from thyroidectomized rats contained as much iodine as those from normal rats after similar iodide feeding periods Guinea pigs which have about 1/3 the thyroid iodine concentration of rats exhibited an increase in thyro iodine and a decrease in thyroid weight with an overall loss in total iodine after 200 mg/kg of ANTU Feeding iodide failed to protect guinea pigs against ANTU and failed to increase the thyroid iodine concentration ANTU inhibited the uptake of I^{131} by rat and guinea pig thyroids Doses of 1 and 10 mg/kg of ANTU to rats caused respectively 50% and 90% inhibition and 150 mg/kg to guinea pigs caused 95% inhibition of thyroid uptake of I^{131}

Relation of chemical structure to action of hyaluronidase inhibitor B CALESNICK and K R. BUETNER (introduced by R. BUETNER) *Dept of Pharmacology, Hahnemann Medical College and Keeley Inst, Dwight, Ill* A search was undertaken

for chemically related substances which inhibit hyaluronidase For testing hyaluronidase activity, we used the turbidimetric method by Kass and Seastone which is based on the precipitation of acidified horse serum by potassium hyaluronate Hyaluronate and hyaluronidase were incubated with or without the substance investigated The decomposition of hyaluronate was then tested by adding horse serum Phenol proved to be active 1 mg inactivating 7.5 gammas of hyaluronidase, 1 mg of cresol, 24 gammas, ortho cresol, 37.5 gammas per mg, chlorine substitution products more With longer side chains attached to phenol, the activity rises higher 1 mg of diamyl phenol inactivates 600 gammas (0.6 mg) and hexylresorcinol, 400 gammas (0.4 mg) while the unsubstituted resorcinol is close to phenol in activity In contrast salicylic acid proved to be only feebly inhibiting, 1 mg inhibiting only 5.0 gamma of hyaluronidase These drugs were added to the testing mixture dissolved in alcohol which has no anti-enzymatic effect in the amounts used The therapeutic use of massive doses of sodium salicylate in rheumatic fever may be explained by its low inhibiting power Sodium gentisate, a metabolic product of salicylate, inhibits even less Many anti-histamine drugs are known to restrict the spreading of a subcutaneously injected dye after hyaluronidase, but we found that these drugs do not inhibit hyaluronidase *in vitro* Also, epinephrine which restricts spreading, does not inhibit hyaluronidase but enhances it

Method for assaying potential coronary vasodilators A CAMERON (by invitation), E SORENSSEN (by invitation) and B CRAVER *Division of Macrobiology, Research Dept, Ciba Pharmaceutical Products, Inc, Summit, N J* In the Langendorff preparation of the perfused mammalian heart, primary effects upon the coronary circulation may be difficult to distinguish from effects secondary to changes in rate or the amplitude of the contractions In studying a series of mono and dialkyl amino derivatives of histamine, it was found that a better appraisal of the effects of these derivatives upon the actual coronary circulation could be achieved by perfusing the heart with a recycled perfusion fluid to which one microgram/ml of 2-(Beta-naphthyl)imidazoline hydrochloride (Privine) had been added The addition of 0.035-0.1 IU/ml of Pituitrin served almost as well but was somewhat less consistent in its action This usually allowed the progressive development of a decreased coronary flow which only after many minutes adversely affected the rate and amplitude of the myocardial contractions The addition of histamine or various derivatives of histamine to the perfusion fluid containing the Privine promptly led to a marked increase in the coronary flow, soon thereafter to an increase in rate, and finally to an

increase in the amplitude of the myocardial contractions. This order of changes bespeaks therefore a direct effect of the drugs upon the coronary circulation. This argument is strengthened by the effects of Pituitrin itself upon the isolated heart, since low doses can exert a considerable positive inotropic action, especially upon the hearts from younger animals with a presumed greater reserve of coronary flow. This balancing of two opposed effects has also been observed with Prinine.

Arsenic content of normal hair in the Chicago area. W. J. R. CAMP and V. A. GAST (by invitation) *Dept of Toxicology, Univ of Illinois, Chicago, Ill.* Because arsenic effects symptoms mimicing those of many pathological conditions physicians frequently consider arsenic intoxication in differential diagnosis. Arsenic determination was requested in 18.5% of our cases for the year 1948. Hair is commonly submitted for analysis since it is simpler to collect than a 24 hr urine specimen. In order to establish the amount of arsenic found in the hair of healthy normal individuals in the Chicago area, a series of 13 samples were analyzed by the Gutzeit method with an average of 0.51 μg of arsenic calculated as arsenic trioxide and a range of 0.25 to 0.88 $\mu\text{g}/\text{gm}$ of hair. While these figures are somewhat lower than those of Künkele and Fordyce, Rosen and Myers, they agree reasonably well with those reported by Glaster. Since there is no way to differentiate 'interior' and 'exterior' arsenic the amount normally found will probably vary from area to area depending on the dietary habits of the community, the amount of smoke and soot in the atmosphere, etc. We did not find any clear cut difference with age (6-52 yr), nor between smokers and non smokers, nor between those who did and did not eat sprayed fruit.

Maintenance of *T. equiperdum* in vitro at 38 C. WILLIAM CANTRELL (introduced by E. M. K. GEILING) *Dept of Pharmacology, Univ of Chicago, Chicago, Ill.* A modification of the technique of Geiman, Anfinson, McKee, Ormsbee and Ball for the cultivation of malaria parasites was used to maintain *Trypanosoma equiperdum* in vitro at 38 C. An increase in numbers of about 50% was observed during the first 4 hours when 40 mg/l ascorbic acid and 160 mg/l glutathione were added to the perfusion medium which contained casein hydrolysate, glucose, pyridoxine, nicotinic acid, nicotinamide, riboflavin, thiamin, calcium pantothenate, choline, and the inorganic salts of the medium of Geiman and coworkers. The trypanosomes were maintained in 14 ml defibrinated rat blood as both citrate and heparin had an adverse effect. The blood was placed in a glass tube 5 cm in diameter and 10 cm long with one end covered with cellophane and suspended in 250 ml of the perfusion medium. Small glass covered magnets in the blood phase

and in the perfusion medium were used to promote diffusion through the membrane and to keep the trypanosomes in suspension. The medium was sterilized by filtration.

Comparative studies of dihydroergot derivatives. R. T. CAIRNS (by invitation), H. M. SLECKLE (by invitation) and O. SIDNEY ORTH *Depts of Pharmacology and Neurosurgery, Univ of Wisconsin Medical School, Madison, Wis.* Alterations of some of the pharmacological properties of the ergot alkaloids due to hydrogenation have been reported for dihydro derivatives of ergotamine and ergocornine. The present study is concerned with 2 additional hydrogenated derivatives, viz, dihydroergocristine (D.C.S. 90)- and dihydroergokryptine (D.H.K. 135). Sandoz Comparisons have been made of the 4 derivatives for both acute and chronic toxic quantities, pathological changes in the heart, lung, liver, spleen, and kidney, and pharmacodynamic actions on the cardiovascular system and gastrointestinal tract. Protection was found against cardiac irregularities which occurred spontaneously during the administration of various anesthetic agents or were initiated by epinephrine injections. Augmentation of movement of the gastrointestinal tract was observed.

Cyclic and noncyclic hydrocarbons and cardiac automaticity. C. JILLIFF CARR and JOHN C. KRANTZ, JR. *Dept of Pharmacology, Univ of Maryland School of Medicine, Baltimore, Md.* The inhalation of 12 different hydrocarbons by the dog was found to produce a sensitization of the myocardium to epinephrine. The compounds studied were ethane, propane, propylene, cyclopropane, n-butane, isobutane, cis-trans-butene-2, cyclobutene, cyclobutane, cyclopentane, isopentane, and 2,2 dimethyl-butane. Ethylene inhalation did not sensitize the myocardium under similar experimental conditions. In an attempt to explore further this unique character of ethylene, additional compounds have been tried, including methylene cyclobutene, methyleyclobutane, cyclopentene, spirocyclopropane, ethylene oxide, propylene oxide and acetylene. Mixtures of cyclopropane and ethylene were found to produce a sensitization of the dog's myocardium in a manner similar to that observed with pure cyclopropane.

Curariform action of decamethylene-1,10-bis-trimethylammonium bromide. JULIO C. CASTILLO (by invitation) and EDWIN J. DE BEER *Wellcome Research Laboratories, Tuckahoe, N. Y.* Confirming the findings of Barlow and Ing, and Paton and Zaimis (*Nature* 161: 718, 1948) on the curariform action of polymethylene bisquaternary ammonium salts of the general formula $(\text{CH}_2)_n\text{N}^+(\text{CH}_3)_3\text{N}^+(\text{CH}_3)_3\text{Br}^-$, the decamethylene (C_{10}) member of the series has been found to have a curare-like activity exceeding that of d-tubocurarine chloride (d.t.c.), the potency varying with the species and

the method of test. It is less toxic and it causes less embarrassment of the respiration than d t c. In cats and dogs the intravenous injection of a curarizing dose of C_{10} causes no significant change in blood pressure unless a big dose sufficient to depress the respiration is given, in which case a typical asphyxial rise in blood pressure is observed. The curare-like action of C_{10} is not antagonized by prostigmine in doses adequate to antagonize the effect of d t c, but in certain cases appears to be antagonized by the C_6 member of the series. An interesting finding is the fact that if a dose of C_{10} is preceded by a dose of d-tubocurarine chloride, the curarizing action of C_{10} is markedly inhibited.

N-(p-arsenoso-benzyl)-glycineamide HCl as an amebicide, *in vitro* and *in vivo* in macaques. Y. T. CHANG and R. K. REED (introduced by HAMILTON H. ANDERSON) *Division of Pharmacology and Experimental Therapeutics, Univ of California Medical School, San Francisco, Calif.* The substituted phenyl arsine oxide (NU-1067 supplied by Dr. E. L. Sevringhaus) contains 26% arsenic. It occurs as a stable white powder and is reportedly less toxic for mice, rats, and rabbits than o-phenarsine HCl. It has shown activity against trypanosomes, treponema, and borrelia. Since it is stable in solution, in contrast to many arsenoso compounds, a comparison of its amebicidal activity was made with mapharsen. *E. histolytica*, grown in egg slope medium, after 48 hours' exposure at 37°C (with a single bacterial associate, organism 't'), was killed by mapharsen at 1:40,000. NU-1067 was active within the range of emetine HCl (1:20,000). Mapharsen was active in monkeys only during the period of therapy, when given in toxic amounts (Anderson and Hansen, 1947). NU-1067 was administered at various dose levels to 11 macaques. Two received 25 and 50 mg/kg, intravenously, total for 5 days without clearance of *E. histolytica*. Two were given orally 30 and 100 mg/kg total for 10 days with clearance of amebas for 6 weeks at the higher dose level. Two monkeys, similarly treated with 300 mg/kg, were cleared of amebas during 3 months' follow-up in one. One developed edema of the genitalia and increased urea-N (20 mg %) as well as depressed 'T' waves on the electrocardiogram. At 450 mg/kg, similarly administered, 2 were cleared over 3 months. At 650 mg/kg, similarly given, one was cleared. The other received identical treatment, had increased urea-N (32.5 mg %) and depressed 'T' waves also. At 1.0 gm/kg orally for 10 days, the urea-N after therapy was 20 mg %. Thus, NU-1067, like mapharsen, was amebicidal *in vitro*, but in macaques amounts that cleared natural infections were within the toxic range.

Action of diphenhydramine (benadryl) in reversing the epinephrine reversal of blood pressure. GRAHAM CHILN and DAVID RUSSELL (by in-

itation) *Research Laboratories, Parke, Davis and Co., Detroit, Mich.* Diphenhydramine, 5 to 10 mg/kg, was found to reverse the 'epinephrine reversal' of blood pressure of a dog, previously receiving an adrenergic blocking agent. A number of the para-substituted derivatives of diphenhydramine and cocaine in larger doses can produce the same effect. Since these compounds also potentiate the pressor effect of epinephrine, this and the action in reversing the 'epinephrine reversal' of blood pressure may be similar in nature. The blocking of the vasodepressor receptors is suggested as a possible mode of action of these compounds on the vasopressor response to epinephrine.

Comparison of the cardiac action of thevebioside and nerifolin with thevetin. K. K. CHEN, ROBERT C. ANDERSON (by invitation), and FRANCIS G. HENDERSON (by invitation) *The Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, Ind.* Since the isolation of thevetin in our laboratory from the nuts of *Thevetia nerifolia*, a newer glycoside, nerifolin, has been independently obtained by Frèrejacque (*Compt. rend.* 221: 645, 1945) and Helfenberger and Reichstein (*Helvet. Chim. Acta* 31: 1470, 1948). By careful hydrolysis of thevetin with strophanthobase, the latter succeeded in splitting 2 molecules of glucose, one by one, and separating in crystalline form 2 simpler glycosides, thevebioside and nerifolin. The last was identical with that occurring in nature. The digitalis-like action of either thevebioside or nerifolin was easily demonstrated by the systolic standstill of the frog's ventricle, and the characteristic changes of the cat's electrocardiogram. By comparison with thevetin, nerifolin was found to be about $4\frac{1}{2}$ times as active in cats, and 3 times as active in frogs, as shown in the table. Thevebioside showed a higher potency in frogs, but was not much different from thevetin in cats. The median emetic doses were determined in both cats and pigeons.

	Thevetin	Thevebioside	Nerifolin
Cat Mean LD $\mu\text{g/kg}$	889.0 ± 31.0	1004.0 ± 103.8	196.1 ± 10.3
Frog SD $_{50}$ $\mu\text{g/g}$	6.35 ± 0.20	3.78 ± 0.57	2.03 ± 0.19
Cat EmD $_{50}$ $\mu\text{g/kg}$	300.0	161.0	64.7
Pigeon EmD $_{50}$ $\mu\text{g/kg}$	211.8 ± 13.8	141.0 ± 19.0	110.6 ± 11.6

Prevention of fluoroacetate poisoning by acetate donors. MAYNARD B. CHENOWETH, EDWARD B. SCOTT (by invitation) and S. LOUISE SEKI (by invitation) *Dept of Pharmacology, Univ of Michigan Medical School, Ann Arbor, Mich.* The depression of contractility of isolated, perfused rabbit hearts or intestine induced by sodium fluoroacetate (FA) can be prevented by prior addition of sodium acetate. However, little or no protection of the intact rabbit can be obtained with even very large doses of sodium acetate. Administration of acetate

as monoacetin in doses of 2-4 gm/kg one-half hour before a certainly lethal dose of FA (0.5 mg/kg) prevented any symptoms of FA poisoning in 27 of 36 rabbits, while of 26 rabbits receiving the same dose of FA, 26 died. Alkalosis induced by sodium bicarbonate, fumarate or hydroxide greatly shortens the time to death of FA poisoned rabbits, probably explaining failures with sodium acetate. Five dogs receiving 0.1 mg/kg FA died in an average of 330 minutes while of 14 dogs receiving a total of 5 gm/kg monoacetin before and after the same FA dose, 5 survived without showing symptoms of FA poisoning. Average time to death of remainder of this group was 600 minutes. Monoacetin administration during the latent period characteristic of FA, or acute stages of poisoning, is without much effect. Monoacetin appears to be specific against FA and its congeners. That it acts by delivering a useful acetate moiety is indicated by the fact that it is equally or more effective than equimolar amounts of sodium acetate in increasing the acetylation of PABA by the rabbit. Some other acetate donors appear to be effective FA antagonists and will be reported upon.

Effect of local anesthetics on the motility of isolated rabbit ileum. GEORGE P. CHINN (introduced by ROBERT A. WOODBURY) *Dept of Physiology and Pharmacology, Albany Medical College, Albany, N. Y.* The spasmolytic action of a number of local anesthetics was studied using segments of isolated rabbit ileum. Most of the tests were made with Nupercaine, Pontocaine, Butacaine, Larocaine and Procaine. With the exception of Procaine these drugs decrease the normal motility of the ileum and relieve the spasm induced by Acetyl Choline and Barium Chloride. Their effectiveness on the intestine parallels their action as local anesthetics. 0.02% Procaine increases the amplitude of contractions, 0.04% increases amplitude and tone, 0.06% decreases the amplitude with increased tone, 0.1% decreases both amplitude and tone, acting like the other local anesthetics. The stimulating action of Procaine is not inhibited by Atropine. The action of these drugs appears to be neurotropic and musculotropic and is completely reversible on changing the bath. The question as to whether part of their effect is due to their action as local anesthetics is difficult to answer. Many synthetic antispasmodics also possess local anesthetic properties. The rapidity with which they often act in relieving the pain of peptic ulcer suggests that their success may be due in part to this local anesthetic action. Preliminary clinical trials with the local anesthetics indicate that they are effective in relieving pylorospasm, cardiospasm and the pain of peptic ulcer.

Action of synthetic antispasmodics and local anesthetics on human gastric motility. GEORGE P. CHINN (by invitation) and ROBERT A. WOOD-

BURY *Dept of Physiology and Pharmacology, Albany Medical College, Albany, N. Y. and Dept of Pharmacology, Univ of Tennessee Medical College, Knoxville, Tenn.* The synthetic antispasmodic, diethylamino ethyl phenyl thienyl acetate, Asymatrine, was found to be very useful in the relief of cardiospasm, pylorospasm, the pain of peptic ulcer and other gastro enteric conditions. Some patients, however, volunteered the information that Asymatrine often produced a painless increased gastric motility. The action of Asymatrine and other antispasmodics on the motility of the human stomach was studied with intragastric balloons. Using Urecholine as the spasmogenic drug, Atropine, Homatropine, Papatrine, Triisentin and Syntropin were spasmolytic in that order. Asymatrine, however, sometimes increased and sometimes decreased the motility. A comparison of the spasmolytic action of these drugs using isolated rabbit ileum with Barium Chloride and Acetyl Choline as spasmogenic agents, showed that Asymatrine was better than the others against Barium but not as good as Atropine or Homatropine against Acetyl Choline. A study of the local anesthetic action of these compounds showed that Asymatrine produced the best anesthesia of the rabbit cornea. On the basis of these apparently paradoxical results and a study of the spasmolytic action of a series of local anesthetics, Procaine, Butacaine, Larocaine, Pontocaine and Nupercaine were tried clinically for the relief of the pain of peptic ulcer, pylorospasm and cardiospasm with gratifying results. It is suggested that local anesthetics be given a clinical trial in other conditions for which antispasmodics have been used.

Toxicology and pharmacology of lupulon. YIN-CH'ANG CHIN (by invitation) and HAMILTON H. ANDERSON *Division of Pharmacology and Experimental Therapeutics, Univ of California Medical School, San Francisco, Calif.* Lupulon, an antibiotic derived from hops (*Humulus lupulus*, L.) has been reported to be active against experimental mouse infections of *Mycobacterium tuberculosis* (Chin, Y. C., et al., *Proc Soc Exp Med*, in press). The results so far obtained in toxicologic and pharmacologic studies follow. Intragastric LD₅₀'s in mice, rats and guinea pigs were 1500, 1800 and 130 mg/kg, respectively. The intramuscular LD₅₀ was 600 mg/kg for mice and 330 mg/kg for rats. Convulsions were observed in animals which received lethal or sub-lethal intragastric or intramuscular doses. Daily intragastric administration of lupulon for 12-14 days resulted in a slight suppression of growth of young rats which received 450 and 300 mg/kg, but not in those given 150 mg. A slight decrease in weight of rabbits occurred after 300 mg/kg daily, and an increase of weight occurred in guinea pigs after 30 mg/kg/day over 14 days. Hemoglobin, RBC and WBC determinations in these ani-

mals were within the normal range. Intramuscular doses of 60 mg/kg/day for 30 days were tolerated without gross effects in mice. Lupulon, 4% added to diet, caused deaths in 50% of mice within 18 days, while 75 and 90% survived 40 days on food which contained 2 and 1%, respectively. All mice lost weight, which was less with lower doses. Neither hemolysis of rabbit erythrocytes nor loss of motility of leukocytes occurred *in vitro* within the effective antibiotic range. Respiratory stimulation was observed following intravenous injection of lupulon in both anesthetized and unanesthetized animals.

Ganglionic blocking action of diethylaminoethanol BYRON B. CLARK and MAURICE M. HELPERN (by invitation) *Dept of Pharmacology, Tufts College Medical School, Boston, Mass.* Recent quantitative studies show that diethylaminoethanol (DEAE) is rapidly formed after the intravenous injection of procaine, and that DEAE has therapeutic actions in man similar to procaine intravenously, with less toxicity (Brodie and co-workers, unpublished). Evidence for a ganglionic blocking action was obtained from the following observations on pentobarbitalized dogs and cats. DEAE-HCl injected intravenously in 30-60 seconds produces a prompt vasodepression of about 50% lasting 5-45 minutes with doses of 28 to 212 mg/kg. In the cat the inhibition of the contraction of the nictitating membrane to preganglionic stimulation paralleled the vasodepression, while postganglionic stimulation remained fully effective. The pressor response to splanchnic nerve stimulation was inhibited during DEAE vasodepression in adrenalectomized dogs, but typical pressor responses were obtained with the adrenals intact, and with injected epinephrine. Atropinization did not alter the vasodepressor response to DEAE. The heart rate is decreased about 10-25% during the vasodepression, and the vasodepressor and chronotropic effects on the heart of vagal stimulation are inhibited. In addition, the chronotropic effect of epinephrine on the heart appears to be inhibited (DEAE has been shown to protect against epinephrine-cyclopropane ventricular tachycardia in dogs—Brodie). DEAE has a positive inotropic effect on the isolated rabbit heart. On isolated intestine and uterus DEAE was found to have no significant cholinergic, anticholinergic, antihistaminic activity, or spasmolytic action against barium chloride. It does not antagonize the inhibitory action of epinephrine on intestine, but may have an adrenolytic action in high concentrations on rabbit uterus. The present evidence indicates that a major action of DEAE is to block transmission at autonomic ganglia.

Effect of fluoroacetate on oxidative processes in frog muscle DONALD A. CLARKE (by invitation), JAMES F. TOOLE (by invitation) and WALTER F.

RIKER *Dept of Pharmacology, Cornell Univ Medical College, New York City* The exposure of intact frog sartorii to varying concentrations of methyl fluoroacetate (1080) results in the disappearance of the recovery heat associated with contraction, the initial heat as well as the mechanical response remains unaltered. This indicates that fluoroacetate interferes with oxidative recovery processes in contracting muscle. Concentrations of sodium or methyl fluoroacetate as high as 10^{-3} M are without effect on the oxygen consumption of resting muscle. In contrast, however, a fluoroacetate concentration of 10^{-3} M produces a profound depression of the excess oxygen consumption which is associated with the action of caffeine. This suggests that fluoroacetate, like azide and hydroxylamine, is capable of differentiating the resting from the activity oxidative processes. In contrast to azide it does not inhibit the cytochrome oxidase system. To define further the locus of action, various intermediates of the carbohydrate cycle were added to the caffeine stimulated muscles poisoned with fluoroacetate. The substances tested were lactate, succinate, pyruvate, glucose, fumarate, acetate, ketoglutarate, tartrate, and ATP. Of these, only pyruvate significantly antagonized the effects of fluoroacetate. A concentration of 10^{-2} M sodium pyruvate restored completely the activity oxygen consumption of the fluoroacetate poisoned muscles.

Loss of As from tissues by volatilization RANDOLPH CLEMENTS, J. EUGENE HILL and HERSHAL G. TREE (by invitation), P. L. EWING and G. A. EMERSON *Dept of Pharmacology, Univer of Texas Medical Branch, Galveston, Texas* As-balance studies were made with mice and rats treated repeatedly with oral doses of K arsenite, Fe arsenate and Fe arsenate plus kaolin, or with water and sludge samples from a Texas stream which had been heavily polluted with Na arsenite and then treated with FeSO_4 and lime. Recovery of As from animals analyzed immediately after oral or intraperitoneal treatment with any of these dosage forms was essentially quantitative by either the A O A C official Gutzzeit or bromate method. On repeated treatment, the balance between the amount given and the amount recovered in the excreta plus tissue As left a deficit of 30-60%, depending upon dosage. Less than 1% of the 'lost' As was recovered from the air by bubbling through concentrated HNO_3 , with a group of rats maintained in a bell jar for two weeks and given daily injections of 5 mg/kg of As_2O_3 as K arsenite. After absorption of inorganic As, the As must be in part converted into volatile substances which either are not absorbed by efficient bubbling through HNO_3 or are retained in the tissues and are volatilized on treatment with H_2SO_4 and HNO_3 .

Toxicity of N-p-chlorophenyldiazothourea

(promurit) to mammals KENNETH W COCHRAN (by invitation) and KENNETH P DuBois *Univ of Chicago Toxicity Laboratory and the Dept of Pharmacology, Univ of Chicago, Chicago, Ill* N-p-chlorophenyldiazothiurea (Promurit) has been employed as a rodenticide in Germany. The chemical similarity of Promurit to alphanaphthylthiourea (ANTU) made it of interest to compare the toxicity and pharmacological effects of the two compounds. For injection propylene glycol solutions of Promurit were employed. The approximate LD_{50} values in mg/kg after intraperitoneal injection were: female albino rats, 0.2; mice, 1.35; guinea pigs, 1.9; and rabbits, 1.75. The approximate LD_{50} for Promurit given orally to female rats was 0.28 mg/kg. The compound is, therefore, more toxic than ANTU. The outstanding pathological change in acutely poisoned animals was pleural effusion. Repeated doses of Promurit resulted in the acquisition of some tolerance. It was acceptable to rats and produced 100% mortality when offered overnight in the diet at concentrations as low as 0.05%. Some increase in blood glucose and a depletion of liver glycogen followed acute poisoning by Promurit. The rodenticide caused a transitory inhibition of tyrosinase and produced a 50% inhibition of peroxidase at a final concentration of 1×10^{-4} M. Feeding iodide to rats before the injection of Promurit resulted in a two-fold increase in resistance toward the rodenticide. Thiosorbitol was ineffective therapeutically against Promurit poisoning while thiourea given prior to Promurit protected against several times the lethal dose. The extremely high toxicity of Promurit and the lack of an antidote appear to limit its possible usefulness as a rodenticide.

Effect of epinephrine on toxicity of procaine
VERSA V COLE and H R HULPIEU *Dept of Biochemistry and Pharmacology, Indiana University, Indianapolis, Ind*. All drugs were given intravenously on a per kg basis. Dogs anesthetized 45 minutes with diethyl ether were infused continuously with 2 mg/min procaine hydrochloride until death. There were 4 groups of 10-12 dogs each as follows: I-controls, II-3 micrograms epinephrine every 5 minutes, III-3 μ g/min for the first 10 minutes of procaine, IV-2.5 μ g/min continuously with the procaine. Two other groups of 5 each anesthetized with thiopental sodium were given 3.5 mg/min procaine hydrochloride until death. Group V were controls, VI received continuous epinephrine as IV. Survival time varied greatly, but averages in minutes were: I-21, II-19, III-17, IV-38, V-56, VI-44. The differences in time between respiratory failure and heart failure in minutes were: I-1.55, II-1.58, III-1.25, IV-0.65, V-2.0, VI-1.1. The blood levels of procaine in mg % at heart failure were: I-3.7, II-3.7, III-2.6, IV-4.4, V-10.7, and VI-7.7. The levels of para-amino benzoic acid in

blood in mg % were: I-0.74, II-0.70, III-0.42, IV-1.54, V-4.9, and VI-4.5. Continuously administered epinephrine in the dose given has a favorable effect on resistance to procaine under ether anesthesia. This effect is not observed under anesthesia with thiopental.

Tetraethyl ammonium and the response of the isolated rabbit heart to nicotine
J M COON, P R SALERNO (by invitation), and L E ELLINWOOD (by invitation) *Dept of Pharmacology, Univ of Chicago, Chicago, Ill*. The isolated rabbit heart gives a biphasic response to a single treatment with 1 mg of perfused nicotine. A complete standstill of about 1 minute duration ceases abruptly to be followed by a marked acceleration and an increase in amplitude lasting about five minutes. Smaller doses of nicotine produce only acceleration. Larger doses elicit a longer period of standstill followed by a more intense stimulation. It is thought that the depression is due to a stimulation of parasympathetic ganglia and that the acceleration may be due to axon reflexes induced in the postganglionic sympathetic innervation. If this were true tetraethyl ammonium (TEA) might be expected to abolish both phases of the cardiac response to nicotine. Perfusion of the isolated heart with 6×10^{-5} M TEA for 10 minutes abolished the inhibitor but not the accelerator response to nicotine. However, 20 minutes perfusion with this concentration of TEA also abolished the accelerator response. Discontinuation of the TEA perfusion was followed by a rapid recovery of the accelerator response, while the inhibitory effect returned more slowly upon washing out with Ringer-Locke solution. If the mechanism of the nicotine action is as described above these findings indicate that the threshold for parasympathetic ganglionic stimulation is higher than that for postganglionic sympathetic fiber stimulation. This would explain the pure cardiac acceleration elicited by small doses of nicotine. Furthermore, the TEA paralysis of the sympathetic fibers appears to be more readily reversible than that of the parasympathetic ganglia.

Comparative activities of five antispasmodics
B CRAVER, W BARRETT (by invitation), A CAMERON (by invitation), A EARL (by invitation), F ROTH (by invitation) *Division of Macrobiology, Research Dept, Ciba Pharmaceutical Products, Inc, Summit, N J*. The activities of 3 known antispasmodics: Trasentine, Trasentine-6H and atropine were compared with those of two new drugs, N-dimethyl thymoxy acetamidine hydrochloride (Su-198), and Thymoxyethyl-diethylamidine hydrochloride (Su-216) in respect to the functions noted. They were examined in respect to the dose in μ g/kg required to inhibit salivation in the cat induced: 1) by histamine, 2) by pilocarpine, and 3) by faradization of the chorda tympani nerve. In order of decreasing activity they were: atropine,

Trasentine-6H, Su-198, Su-216 and Trasentine For each of the 6 drugs, the doses required were essentially the same irrespective of the sialogogic stimulant. The same doses of each drug that inhibited salivation eliminated the spasm of the nictitating membrane induced by subcutaneously injected pilocarpine. To eliminate the hypotension induced by 0.5 y/kg of Mecholyl, approximately a 10-fold increase in the dose of each drug (compared to anti-sialogogic doses) was required. This relationship was strictly quantitative and larger doses of Mecholyl produced hypotension. The order of spasmolytic activity of the 5 drugs remained the same in respect to this function as well as in respect to their ability to eliminate the brief bradycardia and hypotension induced by faradization of the peripheral ends of the severed vagi, but for this latter function only about 5 times the anti-sialogogic doses were needed. The respective LD₅₀'s (I V rats) for the 5 drugs in mg/kg are Su-216—65, Su-198—45, Trasentine-6H—c 37, Trasentine—c 27, and atropine—c 100-125. The problem of attempting to predict the therapeutic usefulness of antispasmodics will be discussed.

Efficacy and toxicity of simaroubidin in experimental amoebiasis ASHTON C. CUCKLER and CARL C. SMITH (introduced by HANS MOLITOR) *Merck Institute for Therapeutic Research, Rahway, N. J.* A standardized procedure has been developed for the evaluation of potential amoebicides in young rats infected with *Endamoeba histolytica*. One of the compounds evaluated in this way, and which is more active therapeutically than many of the currently used amoebicides, is simaroubidin, a crystalline material isolated from the plant *Simarouba amara*. Single oral doses of 25 mg/kg do not have significant amoebicidal activity, whereas single doses of 100 mg/kg % CD₅₀. Single doses of 200 and 400 mg/kg % CD₁₀₀. Six daily doses of 2 mg/kg and 10 mg/kg are ineffective, but doses of 50 mg/kg have significant (>CD₅₀), although not complete, effectiveness. A drug diet of 0.01% given *ad libitum* for 6 days has only slight amoebicidal activity, drug diets of 0.04% and 0.16% % CD₅₀. The acute and chronic toxicities of simaroubidin have been determined for several species. The LD₅₀ for rats and mice of single oral doses was 800 ± 100 mg/kg. In a dog single oral doses of 100 mg/kg produced loss of appetite, but an oral dose of 400 mg/kg was lethal in 2-3 days. Repeated daily oral doses of 500 mg/kg were lethal for 9/10 rats within 10 days, and 250 mg/kg killed 3/12 rats within 21 days. Doses of 50 mg/kg for 21 days produced no mortality and only slight effect on growth of young rats. When the drug was placed in the diet at 0.16% and fed to rats for 21 days the growth was significantly depressed and 1/12 rats died, but no gross pathologic changes were observed at autopsy.

Comparative muscle-relaxing activity of seven

glycerol ethers on isolated nerve-muscle preparations HAROLD DAVIS (by invitation), CHARLES H. HINE and FRANK J. MURPHY (by invitation) *Division of Pharmacology and Experimental Therapeutics and Dept. of Anesthesiology, Univ. of California Medical School, San Francisco, Calif.* Two methods of producing muscular contractions have been adapted for *in vitro* comparison of muscle-relaxing agents. Contractions of frog recti abdomini, suspended in frog Ringer solution (Torda and Wolff, 1944), were recorded. The muscle was stimulated either by physostigmine (2×10^{-4}) plus acetylcholine (10^{-7}) or by tetanic current (100-200 V). In each case the endpoint was arbitrarily designated as 50% reduction of normal contraction following addition of the compound. The duration of exposure to the solution was 30 minutes or less. This technique yielded consistent results. Seven modifications of glycerol ethers, procaine, and Intocostrin were tested. The greatest dilution of each compound which produced an endpoint are listed in the accompanying table. Since the results obtained *in vitro* paralleled those secured in mouse assay procedures, the *in vitro* techniques may serve as a guide to possible muscle-relaxing activity *in vivo*.

Compound	AcCh Eserinized Muscle Stimulation		Electrical Nerve-muscle Stimulation	
	Dilution V/V or W/V	Molarity mM/liter	Dilution V/V or W/V	Molarity mM/liter
Intocostrin (20 u/ml)	10^{-3}	—	10^{-3}	—
Procaine HCl	5×10^{-4}	1.8	5×10^{-4}	1.8
Monocyclohexyl	10^{-4}	0.61	5×10^{-4}	3.0
α, γ isopropyl ethyl	Contraction before endpoint was reached			
α, γ isopropyl tolyl	10^{-3}	0.065	5×10^{-4}	0.33
Phenyl monoacetate	10^{-3}	5.5	5×10^{-4}	2.75
Phenyl diacetate	5×10^{-4}	2.3	5×10^{-4}	2.3
Dibutyl monoacetate	10^{-4}	0.4		
o Toxyl (Myonasin)	10^{-3}	5.5	5×10^{-4}	2.75

Antiepileptic action of Marijuana-active substances JEAN P. DAVIS and H. H. RAMSEY (introduced by LOUIS S. GOODMAN) *Dept. of Pharmacology, Univ. of Utah College of Medicine, Salt Lake City, Utah, and Utah State Training School, American Fork, Utah.* The demonstration of anti-convulsant activity of the tetrahydrocannabinol (THC) congeners by laboratory tests (Loewe and Goodman, *Federation Proc.* 6:352, 1947) prompted clinical trial in 5 institutionalized epileptic children. All of them had severe symptomatic grand mal epilepsy with mental retardation, three had cerebral palsy in addition. Electroencephalographic tracings were grossly abnormal in the entire group, three had focal seizure activity. Their attacks had been inadequately controlled on 0.13 gm of phenobarbital daily, combined with 0.3 gm of Dilantin per day in 2 of the patients, and in a 3rd, with 0.2 gm of Mesantoin daily.

Two isomeric 3 (1,2 dimethyl heptyl) homologs

of THC were tested, Numbers 122 and 125A, with ataxia potencies 50 and 8 times, respectively, that of natural marihuana principles. Number 122 was given to 2 patients for 3 weeks and to 3 patients for 7 weeks. Three responded at least as well as to previous therapy, the 4th became almost completely, and the 5th, entirely seizure free. The optimum dosage was 1.2 to 1.8 mg daily. One patient, transferred to 125A after 3 weeks, had prompt exacerbation of seizures during the ensuing 4 weeks, despite dosages up to 4 mg daily. The 2nd patient transferred to 125A was adequately controlled on this dosage, except for a brief period of paranoid behavior 3½ weeks later, similar episodes had occurred prior to cannabinol therapy. Other psychic disturbances or toxic reactions were not manifested during the periods of treatment. Blood counts were normal. The cannabinols herein reported deserve further trial in non-institutionalized epileptics.

Anti-anemic agents and cholinesterase activity
JOHN EMERSON DAVIS *Pharmacological Laboratory, Univ of Arkansas, Little Rock, Ark.* Considerable evidence is accumulating which indicates that an increase of cholinesterase activity may play an important part in the therapeutic remission of pernicious anemia induced by the administration of liver extract or folic acid. As a further contribution to this evidence, the author has tested the effects of liver extract and folic acid upon the cholinesterase activity of powdered liver (Viobin 40° liver). One mg of pteroylglutamic acid or 0.1 unit of crude liver injection was added to a suspension of 10 mg of powdered liver in 2 cc of tap water. The mixture was incubated at 37° C for 3 hr with occasional agitation or shaking, and then its cholinesterase activity was measured by an electrometric titration method, previously described (*Proc Soc Exper Biol & Med* 63: 287, 1946). When acetylcholine bromide was used as substrate, both liver extract and folic acid caused an average 7-fold increase in the initial hydrolysis rate produced by the powdered liver. When Meecholy chloride was used as the substrate, folic acid increased the enzyme activity of the powdered liver by only about 4-fold while the liver extract increased the activity by 20-fold. Under the conditions of our experiment, it seems that liver extract causes an activation, or perhaps formation, of an enzyme which has some of the properties of a specific cholinesterase, while folic acid increases an enzyme activity which resembles that of a non-specific cholinesterase.

A fatal case of Bartonella Canis treated with penicillin
JOHN EMERSON DAVIS *Pharmacological Laboratory, Univ of Arkansas, Little Rock, Ark.* Since reports of Bartonella Canis infection in splenectomized dogs are quite rare, it seems pertinent to report a severe spontaneous case which showed many of the signs and symptoms of human Oroya Fever. About 90 days after splenectomy had been

performed, one of our dogs appeared to be quite emaciated and was found to have a severe anemia. He had a low grade fever (102.3°), leukocytosis (23000), weakness and pain, a red cell count of 770,000, 2.5 gm % of hemoglobin, a hematocrit of 10.5%, an icteric index of 112, and reticulocytosis (up to 40%). About 75% of the erythrocytes were parasitized by organisms which appeared to be identical with Bartonella Canis. Five cc of blood from this animal was transferred into a second splenectomized dog which, 80 days later, was very anemic (1.9 million RBC) and had parasitized cells, but slowly and spontaneously recovered. Penicillin was given to the original bartonella dog over a 3-day period (10,000 u every 6 hr, I.M.). On the 3rd day of treatment, the blood was cleared of parasites, the icteric index was greatly reduced, and temperature and leukocyte count were normal. By the 4th day, the erythrocyte count had more than doubled, but on the 7th day parasites began to reappear in the blood, and the anemia subsequently became more severe. The dog died in coma, 26 days after his condition had been discovered.

Thiopental hypnosis in rabbits on experimental diets
B. DEBOER *Dept of Pharmacology, St Louis Univ, School of Medicine, St Louis, Mo.* The addition of NaHCO₃ to the diet of rabbits fed whole oats *ad libitum* was accompanied by a shortening of the sleeping time following 2 intravenous injections of 25 mg/kg thiopental an hr apart. Previous information (*Proc Soc Exper Biol & Med* 68, 1948) indicated that the sleeping time of rabbits on a diet of oats for one month was 60% longer than that of rabbits on a diet of Purina rabbit chow. Maintaining these animals upon the experimental diet as long as 20 weeks did not significantly change the effects of thiopental beyond that occurring in 4 to 8 weeks. The addition of NaHCO₃ to the whole grain diet (1% in the daily water supply, which was sufficient to render the urine alkaline) reduced the sleeping time after one week to within 25% of the previous control time. Intravenous injection of 300 mg/kg NaHCO₃ (3 cc of a 10% solution/kg of animal, resulting in approximately 1 gm/animal) preceding the administration of thiopental was without effect the duration of sleep. The effect of diet upon pentobarbital sleeping time was found to be similar. Animals fed rabbit chow averaged 36 and 66 minutes sleeping time after the first and second injections of 25 mg/kg pentobarbital one hr apart. Animals fed whole oats for one month showed only partial recovery during the first hour after the injection and slept an average of 185 minutes after the second injection given at the end of the first hr.

Pentobarbital hypnosis in guinea pigs on ascorbic acid-deficient diet
B. DEBOER and K. O. DEBOER (by invitation) *Dept of Pharmacology, St Louis Univ School of Medicine, St Louis, Mo.* For

58 male and 21 female guinea pigs on a normal diet the sleeping time was 210 minutes when given 25 mg/kg pentobarbital via the peritoneal cavity. The average weight of these animals was 480 grams. After 14 days upon a vitamin C deficient diet the duration of pentobarbital hypnosis was 50% longer than that of controls and after 21 days on the diet 65% longer. The weight of these animals decreased 19% after 14 days and 22% after 21 days on the deficient diet. Returning the animals to a normal diet for 21 days restored the weight to nearly normal and the duration of sleep to within 10% of the previous normal. Ascorbic acid 20 mg/kg given intramuscularly immediately preceding the pentobarbital injection had no apparent effect on the duration of sleep of animals on the deficient diet. Ascorbic acid given 20 or 100 mg/kg 1 hour prior to the injection of the barbiturate in animals 14 days upon the deficient diet had but little effect but in those 21 days upon the diet the sleeping time was 58% above the normal after 20 mg/kg ascorbic acid and 42% above normal after 100 mg/kg ascorbic acid instead of the 65% increase previously noted. In 9 animals on the deficient diet for 14 days a single injection of 100 mg/kg of ascorbic acid was accompanied by a decrease in the duration of sleep (when tested 7 days later) to about 10% above the pre-experimental control sleeping time.

Certain physico-chemical and toxic properties of phenol-camphor preparations. WM B DEICHMANN and T MILLER (by invitation) *Division of Pharmacology, Dept of Physiology and Pharmacology, Albany Medical College, Albany, N Y*. The solubility at 23°C of phenol in mineral oil was found to be 0.1%, in aromatic mineral oil (mineral oil containing traces of eucalyptus and safrol) 0.33%. A 10° increase of temperature raised the solubility about 6 times, to 0.65 and to 1.87%, respectively. Addition of 40 ml of water to 25 ml of a saturated solution of phenol in mineral oil (in a 100 ml separatory funnel having a diameter of 3.88 cm), or to phenol in aromatic mineral oil brought about a redistribution of phenol. At equilibrium, the water phases contained 60 and 50% of phenol respectively. Similar equilibration of a solution of 4.75% phenol and 10.86% camphor in aromatic mineral oil against water led to a phenol concentration in the aqueous phase of 1.026%. Doubling the phenol in the camphor-mineral oil mixture also doubled its concentration in the aqueous phase. The lethal dosages (LD₅₀) of phenol solutions administered as single doses by stomach tube to albino rats were as follows in terms of phenol content: 0.5 gm/kg when given as a preparation of 3.56% phenol in aromatic mineral oil (heated to 34°C), 0.72 gm/kg as a solution of 4.75% phenol in water, and 1.06 gm/kg as a 4.75% solution of phenol and 10.86% camphor in aromatic mineral oil. Immersion of the tails of albino rats in solutions containing

various quantities of phenol continuously up to 8 hours, established the following concentrations that killed approximately 50% of one strain of albino rats: 2.0% phenol in aromatic mineral oil, 4.4% phenol in water, and 7.9% phenol plus 10.86% camphor in aromatic mineral oil. With another strain of albino rats the corresponding LD₅₀ values were 2.7% phenol in aromatic mineral oil, 6.6% phenol in water, and 11.5% phenol plus 10.86% camphor in aromatic mineral oil.

Effects of epinephrine, ephedrine and 1-(m-hydroxyphenyl)-N²-methylethylene diamine (NU-1683) on the coronary blood flow. ADAM B DENISON, JR (by invitation), BEN J LAWRENCE, JR (by invitation) and HAROLD D GREEN *Dept of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N C*. Wayne *et al* have reported at this meeting that 1 µgm of epinephrine, 1 mg of ephedrine and 1 mg of NU-1683 produce approximately at 25-50% reduction in flow in the hind leg when injected into the femoral artery. This paper presents the effects of intracoronary artery injections of similar amounts of these drugs upon the coronary blood flow. In dogs anesthetized with pentobarbital, the heart was exposed by removal of the anterior halves of the left 4th and 5th ribs. Heparin plus, in some experiments, chlorazol fast pink, served as anticoagulant. Flow of blood from a carotid into the anterior ramus descendens was measured with a Gregg-Green orifice meter and differential manometer. Epinephrine in the above dosage caused a 50% increase in flow at the end of diastole. The above doses of NU-1683 and ephedrine had no effect. With rise in arterial pressure, which frequently occurred as the drugs reached the systemic circulation, flow increased with all three drugs.

Biosynthesis of radioactive bufagin containing C¹⁴. JOHN DOULL (by invitation), KENNETH P DUBOIS, and E M K GEILING *Dept of Pharmacology, Univ of Chicago, Chicago, Ill*. An investigation of the optimum conditions for the biosynthesis of radioactive bufagin with C¹⁴ was undertaken to obtain labeled bufagin for studying the distribution and fate of this cardiac glycoside in mammals. Since C¹⁴ as carbonate was rapidly excreted in the expired air it was more desirable to feed an organic form of carbon, algae (supplied by Dr W F Libby) grown in the presence of C¹⁴ and containing 406 uc C¹⁴/gm were, therefore, employed. Tropical toads (*Bufo agui*) were used for the biosynthesis. They were kept in an enclosed system through which CO₂-free air was passed allowing the expired CO₂ to be collected in alkali and measured. Two weeks after feeding C¹⁴ the secretion was collected from the parotid glands and the animals were sacrificed for quantitative measurement of the C¹⁴ in the tissues. One toad receiving 50 uc C¹⁴ as radioactive algae contained 8.9% of the administered

dose in the tissues, 11.8% was exhaled as CO_2 and 79.3% was excreted in the urine and feces. Two weeks after 100 μC of C^{14} 29.4% of the original dose was present in the tissues, 17.9% was exhaled as CO_2 and 52.7% was excreted in the urine and feces, mainly in the feces. Of the C^{14} in the tissues the liver contained 16.9%, skeletal muscle and bones 60.0%, small intestine 10.4% and the remainder was rather evenly distributed throughout the other tissues. Radioactive bufagin and epinephrine were obtained from the parotid secretion.

Comparative percutaneous toxicity of 3-mercapto-1,2-propanediol (thioglycerol) and ammonium thioglycolate JOHN H. DRAIZE, ELSIE ALVAREZ (by invitation) and MARIE WOODARD (by invitation) *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.* Ammonium thioglycolate is used extensively in the 'cold-hair wave process'. To a lesser extent thioglycerol is similarly employed. The thioglycolate 'hair waving' procedure is sometimes varied by the application of heat. Ninety-day percutaneous toxicity experiments on the rabbit were undertaken with 7% ammonium thioglycolate (with solution at room temperature and also heated to 47°C), and with a 6% thioglycerol waving solution at room temperature. All waving solutions were adjusted to a $\text{pH } 9.2 \pm 0.1$. Blood examinations, urinary sulfur excretion, and systemic effects in subjects were recorded. Histological examination of tissues were made. Sensitization studies were conducted on the guinea pig. When 4.0 ml/kg/day (a total of 65 doses) of the waving solutions were injected mortalities of 74, 61 and 0% were obtained with ammonium thioglycolate (47°C), ammonium thioglycolate (room temperature) and the thioglycerol respectively. The percentage increase of urinary sulfur reached peak figures of 147, 105 and 83 for ammonium thioglycolate (47°C), ammonium thioglycolate (room temperature) and thioglycerol respectively. Symptoms of poisoning in animals had a rapid onset with all agents and upon discontinuance of treatment recovery was prompt. Extensive skin damage was always associated with serious poisoning. While thioglycerol showed less gross evidence of toxicity it produced a high incidence of sensitization and evidence of pathologic changes in the thyroid gland.

Experimental skin sensitization to sulfonamides and penicillin ROBERT H. DREISBACH *Dept of Pharmacology and Therapeutics, Stanford Univ. School of Medicine, San Francisco, Calif.* Since both sulfonamides and penicillin have been suspected clinically of increasing skin sensitization to each other attempts were made to test this experimentally. Sensitization of the Arthus type was attempted in 12 rabbits by giving 6 weekly subcutaneous injections of 1 cc 0.8% (saturated) sulfanilamide, or 0.06% (saturated)

sulfathiazole, in 0.9% saline solution in separate groups of 6 rabbits each, controlled by similar injections of 0.9% saline. No noticeable swellings or reactions occurred. In order to test possible increased susceptibility to penicillin sensitization in the sulfonamide injected areas of these rabbits, a total of 4 injections of 10,000 units each of crystalline penicillin G in 1 cc of saline solution were made at weekly intervals. Three of 11 rabbits showed a positive Arthus phenomenon, chiefly minor swellings at the injection sites. In our experience, as many as 10 of 12 normal rabbits may show sensitivity reactions to crystalline penicillin G alone. Following the penicillin injections, the same 12 rabbits were given 2 injections at weekly intervals of sulfanilamide or sulfathiazole into the penicillin-sulfonamide areas, in the same manner as before, but no reactions occurred. In another attempt to produce sensitization, cutaneous applications were made of 1% solutions of sulfanilamide or fathiazole in carbitol (known for epidermal penetration) at weekly intervals on separate groups of 6 rabbits each. No sensitization occurred after 8 applications, although the sulfonamides were absorbed (present in urine). According to these methods, therefore, sulfonamide treated skin is not demonstrably sensitized nor does such treatment increase penicillin sensitization.

Inhibitory action of bufagin on carbohydrate oxidation KENNETH P. DUBOIS, JOHN DOULL (by invitation), and E. M. K. GEILING *Dept of Pharmacology, Univ of Chicago, Chicago, Ill.* In connection with studies on the mechanism of action of cardiac glycosides the effect of bufagin on enzymatic reactions was investigated. Bufagin was isolated from the parotid secretion of the tropical toad, *Bufo aqua*, by the method of Jensen and Chen (*J Biol Chem* 87:755, 1930). Since this glycoside has a very low solubility in aqueous media preliminary experiments were carried out by addition of the crystalline compound to achieve saturation of the test system. For further experiments 50% ethanol solutions of bufagin were prepared and further diluted with water for use, appropriate ethanol controls were included. Addition of crystalline bufagin to isotonic brain homogenates resulted in marked inhibition of the oxidation of glucose. The amount of inhibition was consistently greater than 65% for rat, guinea pig and mouse brain homogenates. Further tests showed that a final concentration of $2 \times 10^{-4} \text{ M}$ bufagin resulted in 50% inhibition of the respiration of isotonic brain homogenates. The respiration of brain slices was inhibited to about the same extent as homogenates by similar concentrations of the glycoside. The rate of oxidation of glucose by heart slices from guinea pigs, rats and mice was decreased about 75% by saturating the test system with bufagin. The oxidation of glucose by liver and kidney slices was only

inhibited to the extent of about 15% by saturation of the media with the drug. Bufagin had no effect on anaerobic glycolysis nor on the cytochrome oxidase and succinic dehydrogenase systems.

Bromate distribution in blood. A. L. DUNN (by invitation) and A. R. MCINTYRE, *Dept. of Physiology and Pharmacology, Univ. of Nebraska, College of Medicine, Omaha, Nebr.* The distribution and fate of ingested bromates has not been studied quantitatively previously. In these studies the distribution of bromate in the red cells and serum was determined following the administration of approximately $\frac{1}{2}$ of the delayed M. L. D. per os in the dog. After 75 mg of KBiO_3/kg gram by stomach tube in 100 cc of water followed by another 100 cc of water, blood was collected from a jugular vein at intervals of $\frac{1}{2}$ hour, 1 hour, 2 hours, 4 hours and 24 hours. No anesthetic and no anticoagulant were used. The blood was defibrinated, centrifuged, the serum drawn off, the red cells washed in isotonic saline and hemolyzed, and protein-free filtrates were then prepared. Bromate was determined by the decolorization of Evans blue. The decolorization of this dye has a marked specificity for bromate over the other halates, and was previously described by us (*J. Lab. & Clin. Med.*, in press). This method was modified in such a manner that the bromate was determined spectrophotometrically. The findings indicate that bromate in the above dosage is absorbed rapidly from the gastrointestinal tract and the peak concentration is found in the blood filtrate within one hour following ingestion. The concentration remains at a high level for 3 hours and falls to a negligible amount at 24 hours. No bromate was found in the saline-washed cells.

Renal clearance of streptomycin. A. O. EDISON (by invitation), V. C. JHINER (by invitation) and G. E. BOXER, *Merck Institute for Therapeutic Research and The Research Laboratories of Merck & Co., Inc., Rahway, N. J.* The renal clearance of streptomycin in dogs was obtained by fluorometric determination of the drug in the plasma and urine. The clearance was measured in 5 experiments over 6 equal one-half-hour periods after a single intramuscular injection of the drug. The normal glomerular filtration was determined simultaneously by measuring the renal clearance of thiosulfate ions since the streptomycin interferes with the determination of inulin, mannitol and creatinine. The clearance of streptomycin was on the average 70% below the simultaneously determined glomerular filtration rate. Wide variations in urine flow and the concentration of the drug in the plasma did not influence the clearance, apparently excluding tubular reabsorption. The clearance measured from the rate of disappearance from the plasma and from the calculated volume of distribution of streptomycin was found to agree with the clearance obtained in

the conventional manner from the concentration in plasma and urine and the rate of urine flow. The implications of this finding with respect to the volume of distribution and the manner of excretion are discussed. The clearance calculated from the plasma concentration of the drug was found to be identical for man and dog per unit of surface area.

Biliary excretion and intestinal reabsorption of C^{14} -labeled methadone hydrobromide. L. L. EISENBRANDT, I. A. ABDOU and T. K. ADLER (introduced by H. W. ELLIOTT), *Division of Pharmacology and Experimental Therapeutics, Univ. of California Medical School, San Francisco, Calif.* Using the standard techniques of tracer investigations, C^{14} incorporated into methadone was administered subcutaneously into rats for the purpose of determining methadone excretion via the digestive tract. Elliott, et al. (*J. Pharmacol. & Exper. Therap.* in press) in their distribution studies showed relatively large amounts of radioactive methadone in the liver, intestine and in feces. In the present study the bile duct was cannulated and bile collected for the duration of analgetic effects which usually lasted about 2 hours following 15 mg of methadone. After injection into the left hind leg, the bile became radioactive in approximately 10 minutes. The peak of radioactivity was reached in 60-90 minutes and decreased as analgetic effects diminished. Although much of the radioactive methadone was excreted in the feces, reabsorption in the small intestine appeared likely. Radioactive bile collected from one animal (donor), and 1 ml was then injected into the duodenum of a second rat which was also cannulated for bile collection. Samples of bile from the recipient became radioactive in about 10 minutes, and this animal showed definite signs of analgesia. Apparently, radioactive methadone was excreted through the intestinal route and, in addition, portions were reabsorbed and distributed throughout the body in sufficient amounts to produce analgesia.

Respiration of human brain slices and the effects of morphine and methadone on oxygen uptake. H. W. ELLIOTT (by invitation), V. C. SUTHERLAND and E. B. BOLDREY, *Divisions of Pharmacology and Experimental Therapeutics and of Neurological Surgery, Univ. of California Medical School, San Francisco, Calif.* Samples of cerebral cortex were obtained from patients undergoing surgical removal of various types of brain tumors. The tissue was immediately iced, transferred to a cold box and prepared for respiration studies. Pieces of cortex were isolated with scissors, sliced freehand with a razor and template, weighed and placed in Warburg flasks. The data obtained from 7 human brains studied to date follow. The QO_2 of human cerebral cortex slices in glucose-Ringer- PO_4 , 30 minutes after equilibration at 37°C , was approximately 1.0 on a wet weight

basis. One hundred fifty minutes later the values for oxygen uptake had fallen 20–30%. For purposes of comparison 30-minute QO_2 's for dog and rat cortex were 1.7 and 2.3, respectively. These species differences are to be expected because of the inverse relationships between metabolic rate and body size. Human cortex slices exhibited a striking difference in behavior from rat cortex slices when O_2 uptake was determined in Ringer- PO_4 containing no glucose. Rat cortex QO_2 's fall very rapidly in the absence of substrate but human cortex respiration is not affected. QO_2 's were just as high and as well maintained over a 3-hour period in unfortified Ringer- PO_4 as when glucose was added. The nature of the substrate apparently stored in human cortical tissue is as yet undetermined. To some vessels morphine and methadone were added after a 90-minute control period. The drug levels studied were 1) 0.002M morphine HCl, 2) 0.0005M methadone HCl and 3) 0.002M methadone HCl. The O_2 uptake of both rat and human cerebral cortex slices in glucose-Ringer- PO_4 was not affected by 1), stimulated by 2) and strongly inhibited by 3). The respiration of human brain slices in Ringer- PO_4 without added glucose was not affected by 1) and inhibited by 2). A comparison of the findings on rat and human brain indicates that the effects of 0.0005M methadone are on glucose utilization and may be a manifestation of enzyme inhibition rather than stimulation.

Metabolism of isopropyl alcohol by the perfused liver. FRED W. ELLIS, *Department of Pharmacology, Univ. of North Carolina, Chapel Hill, N. C.* Rabbits, fasted for 48 hours, were anesthetized with 40 mg/kg of pentobarbital sodium, intravenously, and prepared for liver perfusion by a modification of the technique of Lundsgaard, Nielsen and Orskov (*Skandinav Arch f Physiol* 73: 296, 1936). Fresh defibrinated, oxygenated rabbit blood diluted to 70 per cent with 0.9 per cent saline was perfused through the *in situ* liver, maintained at body temperature, at the rate of 72 cc/min for a duration of 2 hours. At certain time intervals, the concentrations of sugar, acetone and lactic acid (and alcohol, when present) in the perfusion fluid were determined and liver samples were obtained for glycogen analyses. When isopropyl alcohol was added to the perfusion fluid, there occurred a distinct increase in acetone (a known oxidation product of isopropyl alcohol), as well as a less marked rise in sugar concentration. There was a corresponding decrease in the concentration of isopropyl alcohol. Changes in lactic acid and glycogen values did not seem to be significantly different from the control data. Upon the substitution of ethyl alcohol in the perfusion fluid there was no increase in acetone or sugar, and the rate of disappearance of the alcohol appeared to be somewhat slower than in the case of isopropyl alcohol. Lactic acid concentration remained prac-

tically constant as compared with the progressive decrease in the control and isopropyl alcohol experiments. There was no significant change in liver glycogen.

Photoelectric estimation of 1-carnosine and 1-histidine. G. A. EMERSON and PAUL L. EWING, *Dept. of Pharmacology, Univ. of Texas Medical Branch, Galveston, Texas.* The reaction of Pauly (*Ztschr f physiol Chem* 42: 508–18, 1904) may be adapted to photoelectric colorimetry of carnosine and histidine with certain modifications of the visual method of Koessler and Hanke (*J Biol Chem* 39: 497–519, 1919) proposed by Kuen, Eggleton and others. A Coleman model 11 spectrophotometer was used, at λ 470 m μ . Molar color values of carnosine and carbobenzoxy-carnosine are higher than that of histidine if estimated immediately after diazotization, but approach that of histidine in 30 and 60 minutes, respectively, after which fading is negligible at a room temperature of 32°C, although the apparent spectral transmittance curves still differ somewhat for the secondary minima. Excellent agreement with the Lambert-Beer law obtains with 20–100 α of carnosine or histidine, and the method is suitable for estimations with neutralized Cl_3CCOOH extracts of tissues.

A comparative study of anticonvulsant activities of antiepileptic drugs by electro-shock methods in cats, rats and mice. CHARLES R. ENSOR (by invitation) and GRAHAM CHEN, *Research Laboratories, Parke, Davis and Company, Detroit, Mich.* The anticonvulsant activities of nine antiepileptic drugs were determined with 2 electro-shock procedures, 1) by the increase of electro-convulsive threshold in the cat (Putnam and Merritt) and 2) by the abolition of extensor tonic seizures in rats and mice (Toman, Swinyard and Goodman). The values obtained with the 2 methods agree well in the cat and the mouse in the following order of potency: Dilantin, Mesantoin, Nirvanol, phenobarbital, Mebaral, Phenurone, Carbromal, Epidon and Tridione. In the rat, on the other hand, the protective doses of Mesantoin and phenobarbital were found to be smaller than those of others. The laboratory data are compared with the clinical results for these drugs in the treatment of grand mal epilepsy. They appear to show a fair agreement.

Pharmacological studies of phenacetylurea (Phenurone), an anticonvulsant drug. GUY M. EVERETT (introduced by R. K. RICHARDS), *Dept. of Pharmacology, Abbott Research Laboratories, North Chicago, Ill.* Phenacetylurea (Phenurone) has outstanding anticonvulsant properties against Metrazol and supramaximal shock seizures in mice, rabbits and cats with non-sedative doses (300 mg/kg). The drug also raised convulsive threshold to minimal electroshock seizures in rabbits. The toxicity is low, the LD_{50} by IP or oral administration being 2

to 3 gm/kg Slight ataxia appears at 400 mg/kg The EEG in unanesthetized cats and rabbits remained unchanged with doses up to 1 gm/kg Higher doses produce high voltage slow wave activity The duration of anticonvulsant action in mice is 3 to 5 hours The duration remained unchanged in double nephrectomized mice, but was significantly prolonged (20 hours) by CCl_4 liver damage and hepatectomy In man Phenurone causes signs of stimulation more often than sedation The drug has been found effective in some resistant grand and petit mal cases and is particularly useful in psychomotor epilepsy

Comparative effects of adenosine, isoguanosine and related compounds on smooth muscle PAUL L EWING, FRITZ SCHLENK and G A EMERSON *Dept of Pharmacology, Univ of Texas Medical Branch, Galveston, Texas, and Dept of Bacteriology, Iowa State College, Ames, Iowa* Isoguanosine (2-oxyadenosine) was found to be about 3 times as active as adenosine in depressing the anesthetized rabbit's blood pressure after intravenous injection, in inhibiting rhythmic contractions of isolated rabbit or hamster intestine, and in stimulating the hamster uterus On the isolated guinea pig uterus, isoguanosine was 60-100 times as effective a stimulant as adenosine The related pyrimidines, cytosine and isocytosine, while much less active than adenosine, show the expected biochemorphic relationships Although *in vitro* studies have demonstrated the production of glutamine and inosinic acid from ATP, glutamate and ammonia with tissue enzymes, we were unable to find any gross evidence of the reverse process, i e, the production of active amounts of adenylic acid from a combination of inosinic acid and either glutamine or asparagine as amino donors

Action of some enzyme inhibitors on the activity of the isolated intestine A FARAH, R ANGEL (by invitation) and T C WEST (by invitation) *Dept of Pharmacology, School of Medicine, Univ of Washington, Seattle, Wash* Isolated intestinal strips of the rabbit were placed in glucose-free Tyrode solution After 30-60 minutes the intestinal strip showed a marked reduction in the height of spontaneous contractions and usually a loss in tone In confirmation of results of previous workers, the addition of glucose, mannose, acetate, pyruvate and a number of fatty acids produced a recovery of the spontaneous contractions The enzyme inhibitors malonic acid, p-chloromercuric benzoate, sodium fluoroacetate, dinitrophenol, propionic acid, sodium cyanide and sodium azide were studied The effect of some of these inhibitors was dependent on the type of substrate employed The results will be presented and discussed in the light of our knowledge of the intermediary metabolism of carbohydrates and fatty acids

Method for determining hydroquinone excretion

in the urine DAVID W FASSETT *Laboratory of Industrial Medicine, Eastman Kodak Company, Rochester, N Y* Because of the current interest in hydroquinone as an antioxidant for use in foods, and in the possible use of sodium gentisate in rheumatic fever, a study has been made of the excretion of hydroquinone in the urine of man and animals The lack of specificity and sensitivity of available methods has been overcome by a modification of the procedure described by Sterner, Oglesby and Anderson for quinone vapors (*J Indust Hyg & Toxicol* 29 60, 1947) The intense color formed by the simple addition of hydroquinone to an alkaline phloroglucinol solution has a characteristic absorption spectrum with two maxima at 420 and at 515 millimicrons Catechol gives an equally intense but entirely different color, and resorcinol and phenol give no color under the same circumstances Conjugated hydroquinone is estimated after hydrolysis of the urine with normal sulfuric acid Amount of less than one microgram can be detected Hydroquinone in both free and conjugated form (mostly the latter) is normally present in the urine of men, rats, guinea pigs, and cats About 20-50 mg/24 hours is estimated to be a normal value for man, although this will vary with the type of diet After ingestion of hydroquinone by mouth or by injection, large amounts are excreted in the conjugated form starting in an hour or two, indicating that the body eliminates hydroquinone rapidly Compounds such as nor-dihydroguaiaretic acid also give a characteristic color with alkaline phloroglucinol but are less rapidly excreted

Coenzyme I—Quinine relationships in bacterial growth and metabolism R M FEATHERSTONE and J R PORTER (by invitation) *Depts of Pharmacology and Bacteriology, College of Medicine, State Univ of Iowa, Iowa City, Iowa* It has been reported by others that coenzyme I will reverse the inhibitory effects which quinine has on the metabolism of *Escherichia coli* In the work to be reported, turbidimetric measurements of growth, methylene blue reduction studies, experiments using standard manometric techniques, and microbiological assays for coenzyme I have been utilized in an investigation of quinine-coenzyme I relationships as they pertain to the growth and metabolism of several bacterial species The results indicate that the apparent coenzyme reversal of quinine inhibition of methylene blue reduction by bacterial cells may be due to an oxidation of the coenzyme molecule This oxidation is not inhibited by the concentrations of quinine used and it proceeds independently of the retarded glucose oxidation It has also been shown that numerous other growth factors fail to reverse the quinine inhibition of growth or respiration of the several bacterial species studied

Local anesthetic activity of a series of aminoalkoxy isoquinolines EDWIN J FELLOWS and EDWARD MACKO (by invitation) *Dept of Pharmacology, Temple Univ School of Medicine, Philadelphia, Penna* The following compounds were tested for local anesthetic activity after topical application to rabbits' eyes 1-(beta-Dimethylaminoethoxy)-isoquinoline Monohydrochloride (I), 1-(beta-dimethylaminoethoxy)-3-propylisoquinoline Monohydrochloride (II), 1-(beta-Dimethylaminoethoxy)-3-butylisoquinoline Monohydrochloride (III), 1-(beta-Diethylaminoethoxy)-3-methylisoquinoline Monohydrochloride (IV), 1-(beta-Di-n-butylaminoethoxy)-isoquinoline Monohydrochloride (V), 1-(beta-N-piperidylethoxy)-3-methylisoquinoline Monohydrochloride (VI), 1-(beta-N-piperidylethoxy)-3-ethylisoquinoline Monohydrochloride (VII), 1-(3-Diethylaminopropoxy)-3-ethylisoquinoline Monohydrochloride (VIII), 1-(beta-Benzylaminoethoxy)-3-ethylisoquinoline Monohydrochloride (IX) All of the present derivatives exhibited anesthetic activity Derivatives nos II, IV, VI, VII, VIII and IX produced anesthesia for periods of 26 to 94 minutes in 0.01% concentration However, no III was outstanding in that an average of 75 minutes anesthesia was noted after application of 0.001% solution In comparative studies anesthesia was noted for a period of 18 minutes after 1.0% cocaine and for an average duration of 69 minutes after 0.01% dibucaine hydrochloride

Influences of progesterone and testosterone on reactivity of rabbit myometrium to oxytocin and vasopressin PAUL P C FENG (by invitation) and R A WOODBURY *Division of Pharmacology, Univ of Tennessee, Memphis, Tenn* Investigations were conducted on isolated uterine strips from rabbits, varying in age from 3 months to 3 years, with or without previous pregnancies Twenty-one rabbits were sensitized by injecting 0.01-0.02 mg of stilbestrol daily for a period of 7 days (Control studies showed that such treatment produced full sensitization of the myometrium to oxytocin and vasopressin) After sensitization 1 uterine horn was removed to test its reactivity to oxytocin and vasopressin These results were then compared with the reactivity of the remaining horn removed after 5 days additional treatment with various combinations of stilbestrol and either progesterone or testosterone In 8 rabbits, a stilbestrol/progesterone ratio of 1:20 to 1:100 decreased the reactivity of sensitized myometrium to oxytocin and vasopressin to similar degrees varying from five-fold to one hundred and fifty-fold In 13 rabbits, a stilbestrol/testosterone ratio of 1:100 to 1:400 decreased the reactivity to oxytocin and vasopressin from 2-fold to 330-fold However, testosterone decreased to a greater extent the reactivity to vasopressin in 9 of the 13 rabbits and to the

same extent in 3 of the remaining 4 In stilbestrol-sensitized and non-sensitized rabbits, the oxytocic activity of vasopressin was roughly 20 times that as specified in the current N N R These results are comparable with those reported by Bachinski and Allmark on normal rabbits (*J Am Pharmacol Assoc* 34:73, 1947)

Action of 4-amino, N 10-methyl, pteroylglutamic acid in mice, rats, and dogs F C FERGUSON, JR (by invitation), J B THIERSCH (by invitation), and F S PHILIPS *Dept of Pharmacology, Cornell Univ Medical College, and Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York City* The intraperitoneal LD_{50} of 4-amino, N 10-methyl, pteroylglutamic acid in mice was 94 ± 8.8 mg/kg in a single dose and 1.94 ± 0.30 mg/kg/day given for 5 successive days, and in rats, 12.6 mg/kg acutely (with much variation) and 1.11 ± 0.34 mg/kg/day for 5 successive days Orally in rats the acute LD_{50} was 180 ± 45 mg/kg Signs of intoxication included weight loss, anorexia, diarrhea, dehydration, and weakness, complicated by secondary infections, with death after 3 days Rats sacrificed within 72 hours after large acute doses and after chronic doses given for 60 days, showed lesions in the bone marrow and intestines, the lymphoid tissues remaining relatively unaffected The intestinal mucosa showed hyperemia, edema, desquamation and leucocytic infiltration The marrow became fluid and hypocellular in the myeloid and erythroid elements The blood showed progressive normochromic normocytic anemia, reticulocytopenia, and leucopenia Dogs receiving various doses were followed with serial blood counts and sternal marrow aspirations The course of intoxication and the lesions seen were similar to those in rats In addition, megaloblasts appeared in the marrow, the lymphoid tissues appeared reduced and ulcerative colitis was prominent

Comparison of the chronic toxicity of alpha, beta and gamma isomers of benzene hexachloride O G FITZHUGH, A A NELSON and OMA L HOLLAND (by invitation) *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D C* Following our observation that technical grade benzene hexachloride was more toxic than could be accounted for by the gamma content, a chronic experiment on the individual isomers was started Albino rats, 21 days old, 20 to a group, were fed dosages of 10, 100 and 800 ppm of the alpha, beta and gamma isomers in a stock diet for their lifetime Additional dosage levels of 5, 50 and 1600 ppm gamma and 50 ppm alpha were used Results showed that the beta isomer was more than twice as toxic as the gamma and that the alpha isomer was of the same order of toxicity as the gamma All rats receiving the 800 ppm beta isomer died within the first 10 weeks of

the experiment, whereas some of those on 1600 ppm gamma survived for more than a year. Retardation in growth occurred at the 800 ppm dosage level for each isomer. Lower dosage levels did not affect growth. Rats on the high dosages of the gamma isomer had severe convulsions. At 100 ppm all isomers produced slight but distinct histological changes in the liver, and, with the alpha and gamma, in the kidney also. The beta affected the liver in a greater degree at a given level. The gamma and alpha isomers produced no damage to the liver or kidney at the 10 ppm and the beta at this level showed a questionable injury to the liver.

Mercury content of human tissues from routine autopsy material. ROBERT B. FORNEY (introduced by H. R. HULPIEU) and R. N. HARGER, *Dept. of Biochemistry and Pharmacology, Indiana Univ. School of Medicine, Indianapolis, Ind.* Using the method of Laug and Nelson (*J. Assoc. Off. Agr. Chem.* 25:390, 1942) mercury analyses were run on kidneys and livers from 117 consecutive autopsies. These comprised group A, 92 persons with no known mercury medication, and group B, 25 persons with a history of the administration of mercury diuretics. In group A the frequency distribution of mercury concentration expressed as mg % of the fresh organ was, kidneys: 9, none (< 0.01 mg %), 20, 0.01-0.10, 28, 0.11-0.50, 22, 0.51-1.00, and 11, 1.01-12.7; and liver: 18, none, 37, 0.01-0.10, 26, 0.11-0.50, 5, 0.51-1.00, and 3, 1.01-1.72. Concentrations of mercury above 0.1 mg % were found in 67.5% of the kidneys and 34.5% of the livers. In group B the distribution of mercury levels was, kidney: 1, 0.94, 12, 1.1-5.0, 6, 5.1-10.0, 3, 12.0-15.0, 1, 21.7, and 1, 27.5; and liver: 15, 0.11-0.50, 4, 0.51-1.00, and 3, 1.1-2.5. In all of the cases in group A and in most of the cases in group B there was no microscopic evidence of the pathological changes associated with acute mercury poisoning, although a few of group B showed mild changes. In 24 of the cases separate analyses were run on the cortex and medulla of the kidney. In 22 of them the cortex/medulla ratios varied from 1.1 to 6.8. The highest level of mercury was found in the kidney with the liver next, followed by the spleen.

Respiration apparatus for rabbits and its use in the study of piperidine analgesics. R. H. K. FOSTER and B. DEBOER, *Dept. of Pharmacology, St. Louis University School of Medicine, St. Louis, Mo.* The apparatus, which eliminates the use of respiratory valves, consists of a closed two-compartment rabbit holder, a 40 gallon air reservoir with a blower for introducing fresh air, 2 damped float recorders and an air circulating fan. A perforated rubber diaphragm fitting snugly around the rabbit's neck separates the two compartments of the rabbit holder. The larger compartment, holding the rabbit's body, is connected to one float re-

corder for registering rate and depth of respiration, the smaller, holding the rabbit's head, is connected in turn to the soda lime container, the air reservoir, the small air circulating fan and thence back to the head compartment. An outlet from the air reservoir is connected through a fine capillary to the second float recorder for recording oxygen consumption. The damping effect of the capillary prevents oscillations due to breathing and results in practically a smooth line. Morphine sulfate and dl- α -1,3-dimethyl-4,4-phenylpropionoxypiperidine hydrochloride (Nu-1196) were injected in doses of 2 and 5 mg/kg. Both drugs produced about equally a marked decrease in rate and minute volume but tidal volume and oxygen consumption showed no significant changes from the control period. The onset was slightly faster with Nu-1196 and recovery definitely faster. Other members of the series are under investigation.

Effects of posterior pituitary hormones on carbohydrate metabolism. ALEXANDER MACL. FRASER, *Dept. of Pharmacology, McGill University, Montreal, Canada.* The hyperglycemic action of pituitary extract is well known, it has been attributed to the oxytocic hormone in the case of the dog. Hyperglycemia following administration of glucose or adrenaline decreases blood inorganic phosphate, due to increased insulin secretion, but workers have shown that pituitary extract increases blood inorganic phosphate, and that pitressin antagonizes the fall in inorganic phosphate caused by insulin. In the present work, the effects of highly purified preparations of the oxytocic and pressor hormones on the sugar and inorganic phosphate of the blood were studied in dogs. The results show a drop in phosphate following oxytocic hormone, and a rise following the pressor hormone. Blood sugar rose after injection of each hormone. The hyperglycemic action of the pressor preparation cannot be accounted for, quantitatively or qualitatively, by its contamination with oxytocic hormone, therefore a hyperglycemic action is attributed to the pressor hormone. It appears likely that the decrease in plasma phosphate after the oxytocic hormone is due to increased insulin secretion, which is caused, in turn, by the hyperglycemia. Consideration of the doses of the pressor hormone required to produce the above changes, leads to the suggestion that the increases in phosphate and sugar following administration of this hormone are due to tissue anoxia, which results from the vasoconstriction, anoxia is known to produce such changes. Hyperglycemic doses of the oxytocic hormone are within a more physiological range.

Rate of disappearance of isomers of benzene hexachloride from fat depots in rats. JOHN P. FRAWLEY (by invitation) and O. GARTH FITZHUGH, *Division of Pharmacology, Food and Drug*

Administration, Federal Security Agency, Washington, D C Previous experiments in this laboratory have shown that the chronic storage of the beta isomer of benzene hexachloride was considerably greater than that of the other isomers and that the principal storage took place in fat tissue. Data are presented here to show the various rates of disappearance of the isomers from the fat tissue. Rats were fed the isomers of benzene hexachloride at 100 ppm and 500 ppm until the maximum storage level was reached. Fat tissues from both sexes on the isomers were analyzed at the maximum level of storage, and repeatedly thereafter until the level fell below the sensitivity of the method of analysis. The method was based on the conversion of the benzene hexachloride to 1,2,4-trichlorobenzene and the estimation of this compound by means of the ultraviolet spectrophotometer. The results obtained from the female rats are summarized in the following table.

Disappearance of Isomers of BHC in Female Rats

Concentration Original Diet	Weeks Control Diet	Conc of Isomers (Mg/g)			
		alpha	beta	gamma	delta
ppm 100	0	0 177	1 014	0 102	0 128
	1	0 142	0 860	0	0
	2	0	0 837		
500	0	1 180	No	0 281	0 376
	1	0 281	survivals	0 039	0 188
	2	0 158		0	0

The disappearance of the isomers from fat tissue of male rats is accomplished sooner than in female rats.

Effects of indophenols on glycolysis JAMES A. FREEK (introduced by G. L. GEMMILL) *Dept of Pharmacology, Univ of Virginia Medical School, Charlottesville, Va* A study was made of the effect of some indophenols on glycolysis in frog muscle extracts. The rate of glycolysis was determined by the liberation of carbon dioxide from a bicarbonate buffered solution in a Warburg vessel. The following compounds completely inhibited glycolysis at 0.001 to 0.008 M: 2,6-dibromo-3'-carboxyindophenol, 2,6-dichloro-3'-chloroindophenol, 2,6-dibromoindophenol and 2,6-dichloro-3'-methylindophenol. The following compounds partially inhibited at 0.008 to 0.03 M: 2,6-dichloroindophenol, 2,6-dibromo-3'-methoxyindophenol, 2,6-dibromo-2'-methyl-5'-isopropylindophenol, 3'-methyl-6'-isopropylindophenol, 2'-methyl-5'-isopropylindophenol, 3'-methylindophenol, 2'-methylindophenol, 2,6-dibromo-3'-methylindophenol, 2,6-dibromo-3'-methylindophenol, indophenol, and 3'-naphtholsulfonate. 2,6-Dichloroindophenol, 2,6-dibromo-3'-carboxyindophenol and 3-chloroindophenol at 0.0001 M increased the amount of carbon dioxide production.

This activating effect was dependent on the normal activity and reducing capacity of the extract. The inhibition produced by 2,6-dibromo-3'-carboxyindophenol and 3'-naphtholsulfonate was not reversed by glutathione or cysteine. Inhibition by 2,6-dibromo-3'-carboxyindophenol was reversed in the presence of added ATP. There was no quantitative relationship between inhibition and the oxidation-reduction potentials of the indophenols.

Responses to epinephrine and norepinephrine after dibenamine WALTER A. FREYBURGER (by invitation), LUIS R. CAPO (by invitation), and GORDON K. MOE *Dept of Pharmacology, Univ of Michigan, Ann Arbor, Mich* In the intact dog dibenamine 'reverses' the pressor action of epinephrine in doses which depress but do not abolish or reverse the pressor action of norepinephrine. 'Reversal' of epinephrine is thought to represent unmasking of its vasodilator activity. Since a depressor action of epinephrine is rarely apparent in the pithed animal, one might expect that the pressor effects of the two amines would be depressed to an equal degree by dibenamine in this preparation. This was found to be the case. In a series of intact anesthetized dogs dibenamine, in a dose which reduced the maximum response to epinephrine to a pressure rise of 5 mm Hg followed by a fall, reduced the pressor action of norepinephrine by only 50%. In the pithed cat, pressor responses to the two amines were reduced to exactly the same degree by dibenamine. If the vasodilator action of epinephrine depends upon an intact autonomic nerve supply, interruption of such pathways by tetraethylammonium (TEA) should abolish vasodilator responses. TEA was found to abolish the pressure fall normally observed after small doses of epinephrine. The depressor response to epinephrine which occurs following dibenamine, however, was only occasionally prevented by TEA. Although the difference may be only quantitative, there is a possibility that the vasodilator effect of epinephrine following a reversing agent differs from the normal vasodilator effect demonstrable with small doses.

Paper chromatography of flavonoid pigments II. Separation and quantitative estimation of rutin and quercetin. THOMAS B. GAGE and SIMON H. WENDER (introduced by ARTHUR A. HELLBaum) *Chemistry Dept, Univ of Oklahoma, Norman, Okla* The qualitative separation and identification of micro quantities of 11 flavonoid pigments by paper partition chromatography has been previously reported by the authors (*Science* in press). A micro method of quantitatively estimating the amounts of certain flavonoid pigments initially present in a mixture has now been developed. Mixtures of rutin and quercetin have been separated and quantitatively determined.

by the following procedure Whatman No 1 filter paper strips, 2.5×50 cm were spotted by means of a micro pipette with 5-10 μ l of a solution containing 10-40 μ g of each pigment. The strips were chromatographed in a one-dimensional apparatus with such solvent combinations as ethyl acetate-water or n-butanol-acetic acid-water (40-10-50 vol %) Blank strips were treated similarly in order to provide solvent blanks for the preparation of absorption spectra curves. The strips were air-dried, and the pigment zones located by their fluorescence in ultraviolet light. The isolated pigment zones and the corresponding areas of the blank strip were cut out and the paper extracted in closed containers with solvents such as 5 cc of ethanol containing 0.02 cc of concentrated HCl. The Beckman model DU quartz spectrophotometer, fitted with micro absorption cells, was used for obtaining spectral data. Rutin and quercetin were identified by the shape of their characteristic ultraviolet absorption spectra curves and the amount recovered was calculated from their extinction values at 362.5 and 375 $m\mu$ respectively. Further studies are in progress with combinations of 2 and 3 flavonoid pigments often found associated together in natural products.

Studies on furan compounds toxicity and pharmacological action of furfuryl alcohols
JOHN E. GAJEWSKI and WILLIAM R. ALSDORF (introduced by C. H. HINE) *Naval Medical Research Institute, Bethesda, Md.* Upon oral administration of a 2.0% aqueous solution of furfuryl alcohol, the LD-50 for white rats is 275 mgm per kilo. Administered intravenously as a 10% aqueous solution to rabbits, the LD-50 is 650 mgm per kilo. The marked difference between oral and intravenous toxicity can probably be explained on the basis of destruction of furfuryl alcohol by the gastric juice, since this substance is very unstable in an acid medium. This difference does not exist with the more stable hydrogenated analog, tetrahydro-furfuryl alcohol, (oral LD-50 for white rats, 4500 mgm per kilo, intravenous LD-50 for rabbits, 725 mgm per kilo). Either alcohol chronically administered to rats in the drinking water over a period of 20 days results in marked anorexia and weight loss. Intravenous injection of either alcohol into rabbits produces a flaccid paralysis, which becomes permanent with large doses. Recovery from smaller doses is complete in a period of about thirty minutes. On smooth muscle furfuryl alcohol exerts a slight antispasmodic effect. In dogs, upon intravenous injection, the depression of respiration is not accompanied by any changes in blood pressure until doses near the lethal are given. In all species death results from respiratory paralysis.

Metabolism of radioactive nicotine A. GANZ (introduced by F. E. KELSEY) *Dept of Pharmacology, University of Chicago, Chicago, Ill.* The

biosynthesis of C^{14} labeled radioactive nicotine has been reported by Geiling, Kelsey, McIntosh and Ganz in *Science* 108: 558, 1948. Using radioactive nicotine with a specific activity of 57,000/min/mg, rats and mice were injected intravenously and the distribution of the drug among the various organs and body fluids was studied. Distribution and fixation studies on the isolated heart of the guinea pig were also carried out with simultaneous analyses being made of the perfusion fluid. The relationships between the tissue data, the perfusate analyses and the kymograph records were investigated.

Effects of naphthoquinones and related compounds on glycolysis CHALMERS L. GEMMILL *Dept of Pharmacology, University of Virginia Medical School, Charlottesville, Va.* The study of the action of some of the naphthoquinones and related compounds on muscle glycolysis reported last year (*Federation Proc.* 7: 220, 1948) has been extended. A detailed investigation was made of the effects of various concentrations of sodium 1,2-naphthoquinone-4-sulfonate on glycolysis of glycogen to lactic acid in muscle extracts of frogs. With small amounts (1×10^{-4} Molar) of this compound a slight stimulation of glycolysis was observed, while larger amounts caused a progressive decrease in glycolysis, with practically complete inhibition occurring at concentrations of 6×10^{-4} Molar. Several of the compounds having Vitamin K activity gave partial inhibition of glycolysis in amounts of 0.002 Molar or less. These compounds were 2-methyl-1,4-naphthoquinone (Eastman), sodium-2-methyl-1,4-naphthohydroquinone diphosphate (Parke Davis Co.) and 2-methyl-4-amino-1-naphthol hydrochloride. 2-methyl-3-hydroxy-1,4-naphthoquinone (Phthiocol) gave partial inhibition of glycolysis in a concentration of 0.001 Molar. A polarographic examination (carried out by Mr. J. G. Hilton) of the sodium 1,2-naphthoquinone-4-sulfonate in the muscle enzyme solution revealed that from 80 to 90% of this compound is destroyed or bound in its reaction with the glycolytic mechanisms.

Chemotherapeutic efficacy of nitrofurazone (furacin) in experimental trypanosomiasis of the mouse N. J. GIARMAN (by invitation), B. A. RUBIN and E. L. MCCAWLEY *Laboratories of Pharmacology and Bacteriology, Yale Univ. School of Medicine, New Haven, Conn.* Single oral or subcutaneous doses of 500 mg of nitrofurazone per kg of body weight were capable of curing mice which had been given a massive, lethal infection of *Trypanosoma equiperdum* (one million organisms injected intra-abdominally). There were no therapeutic failures at this dose level in 120 mice, some of which were observed for 180 days after infection. The average survival time of infected but un-

treated animals was 84 hours. The chemotherapeutic index for nitrofurazone, calculated on the basis of $LD_{50}/ED_{95} \times 100$, is 107 when the drug is administered orally, and 117 when it is given subcutaneously. These indices compare favorably with those of such trypanocidal antimonials as fuadin and stibenyl, which were calculated in the same manner from the data of Chen *et al.* (*J Infect Dis* 76: 144, 1945) on intramuscular therapy of trypanosomiasis, as 87 and 143 respectively. When the infecting inoculum was increased to 10 million organisms, the dose of 500 mg/kg was still capable of curing 100% of the experimental animals. When treatment was withheld for 60 hours, at which time the first inoculated control animal had succumbed to the infection, the dose of 500 mg/kg subcutaneously cured 50% of the animals. Nitrofurazone has a low order of toxicity. Doses of the drug in the magnitude of 30 to 50 mg/kg given intravenously to nembutalized dogs and cats failed to produce any profound toxic changes in the blood pressure, EKG, EEG, or respiration. The LD_{50} for nitrofurazone administered orally to the mouse is 737 mg/kg, leaving a convenient margin of safety from the effective dose of 500 mg/kg.

Pharmacological studies on actidione. ANDRES GOTH and FABIAN J. ROBINSON (by invitation). *Dept of Physiology and Pharmacology, Southwestern Medical College, Dallas, Texas.* The antibiotic actidione has been shown to be markedly inhibitory for certain pathogenic fungi (Whiffen, A. J., *J Bact*, 56: 283). It also showed a marked species variation in its toxicity for mammals. Despite its toxicity it seemed of interest to study blood levels of the antibiotic in several species of animals. A solution of crystalline actidione was injected intraperitoneally into rats, dogs, and guinea pigs. The citrated plasma of these animals was tested by a serial dilution method in tryptose phosphate broth. The test organism was *Saccharomyces pastorianus*, ATCC 2366, and the inoculum was adjusted to give a final concentration of 1:250 of a 24 hour culture. In the rat the injection of 1 to 10 mg/kg of actidione was followed in 30 minutes by plasma levels of 5-20 dilution units per cc. The inhibitory activity decreased in 90 minutes but was still demonstrable. In the dog and the guinea pig the results were essentially the same as in the rat. These data indicate that actidione is rapidly absorbed from the intraperitoneal site in the rat, dog, and guinea pig and is demonstrable in plasma for at least 90 minutes. Since inhibitory blood levels can be obtained following the injection of sublethal doses of actidione, the possibility exists that the compound may exert a chemotherapeutic effect against susceptible pathogenic fungi in experimental animals and man.

Modified ultraviolet spectrophotometric method for quantitative determination of barbiturates

THELMA C. GOULD, ELIZABETH L. MAIO, and KARL P. BOWMAN (introduced by CHARLES H. HINE). *Divisions of Pharmacology and Experimental Therapeutics and of Psychiatry, Univ of California Medical School and Langley Porter Clinic, State Dept of Mental Hygiene, San Francisco, Calif.* Diethyl ether rather than chloroform was used to remove the barbiturate from the sample of blood. A semi-micro, continuous extractor permits direct extraction of from 0.5-5 ml sera. This procedure is less time-consuming than previous methods and has simplifications regarding apparatus, procedure, and glassware. By extraction at the normal pH range of serum, all sulfonamides thus far tested, which may contribute to ultraviolet absorption, are eliminated. The major problem arising in spectrophotometric determination of barbiturates is the ultraviolet absorption by other extractable materials present in blood sera. In experimental work, the optical density of sera can be determined prior to administration of barbiturate, and correction made. In forensic toxicology, however, where no blank specimen is available, this is not possible. To account for this possible error, the optical density of normal human sera was determined in 100 samples. This variation was from 0.040 to more than 0.200 at a wave length of 245 mμ, with the majority of sera falling within the range of optical density of 0.100-0.150, with a mean of 0.130. When this value is used as a blank reading in barbiturate-containing serum, the error involved would probably not exceed 0.5 mg%. The pH should be carefully adjusted between 9.5 and 10.0 when reading optical density, to detect the typical lactam-barbiturate curve. This is not obtained otherwise, and its maximum may be obscured by the serum blank.

Synergistic chemotherapeutic effect of penicillin and aurothioglucose (Solganal). ARNOLD GRANDAGE, LEILA DIAMOND, MARVIN ROSENKRANTZ, JEANNE H. CHASE, RICHARD TISLOW and ERWIN SCHWENK (introduced by H. B. Haag). *Biological Research Labs, Schering Corporation, Bloomfield, N. J.* The chemotherapeutic effects of aurothioglucose (Solganal) and penicillin were studied in albino mice infected with *Borrelia novyi* (culture obtained through the courtesy of Dr. R. J. Schmitzer, Hoffmann-La Roche, Nutley, New Jersey). A shortened assay procedure similar to that described by Buck, Farr and Schmitzer (*Science* 104: 307, 1946) was used. Mice inoculated intraperitoneally with standardized dilutions of blood in Tyrode solution from mice carrying infection of *Borrelia novyi* were treated in separate experiments with graded doses of penicillin or aurothioglucose and checked for clearance of the blood from *Borrelia novyi* at the end of 24 hours. A satisfactory dose response relationship was obtained with both drugs. Also a satisfactory dose

response relationship between graded inocula and clearance was found using 1 dose of aurothioglucose or penicillin. On the basis of these preliminary experiments 4 short-term experiments, each with groups of 6 animals per dose level, were run to determine the effect of combined treatment with aurothioglucose and penicillin. Aurothioglucose was injected intraperitoneally 4 hours before and crystalline sodium penicillin G immediately after infection. The results of each of the 4 experiments revealed a synergism of the 2 drugs. According to the combined data 8 U/gm of penicillin cleared 37% and 0.05 mg/gm of aurothioglucose 9% of the mice, whereas a combined treatment with the 2 drugs at these dose levels resulted in a 75% clearance. Similarly 8 U/gm penicillin and 0.09 mg/gm aurothioglucose cleared 37% and 13% respectively and the 2 drugs in combination 93% of the animals. These data indicate that combined therapy in this dosage range produced more than an additive effect.

Toxicity of ozone under simulated industrial conditions. M. G. GRAY, *Labys of Arthur D Little, Inc., Cambridge, Mass.* The use of ozone as an 'air-purifier' in garages has directed attention to the limited data regarding its actual toxicity in concentrations of the order of 1 ppm. An experimental study is reported in which rats, mice and rabbits were exposed to a) ozone, b) to exhaust fumes from an internal combustion engine and c) to an atmosphere combining a) and b). This was done in a gas chamber designed to simulate conditions in a service garage. The duration of exposure was equivalent for the species used to more than 1 year of average working time for garage employees. Neither ozone (1 ppm), motor exhaust fumes (containing 50 ppm carbon monoxide) or a combination of these produced significant changes in the physical condition, nutritional status or gross findings at autopsy. No evidence was obtained to support claims that ozone decreases the toxicity of carbon monoxide. The study was carried out in a gas chamber designed to permit the blending of several gases with normal atmospheres while maintaining constant physical conditions. By use of interchangeable panels, the size of the chamber can be altered to suit the requirements of several kinds of experimental work.

Xanthine studies 1) effects of aminophyllin, 1,3 diethyl 8 bromoxanthine and 1,3 dimethyl 8 chloroxanthine on cardiac output. D. M. GREEN, W. C. BRIDGES, A. D. JOHNSON, J. H. LEHMANN, F. GRAY, and L. FIELD (all by invitation), *School of Medicine, Univ of Washington, Seattle, Wash.* 1,3-diethyl-8-bromoxanthine and 1,3-dimethyl-8-chloroxanthine have been compared with aminophyllin as to effect on cardiac output. The subjects consisted of 45 patients with myocardial insufficiency. Output was measured by the Fick

method. Total of output determinations numbered 236, of which 118 followed intravenous administration of a test drug. Aminophyllin was the most active compound and produced an average increase in output of 12% in doses of 250 mg. 1,3-diethyl-8-bromoxanthine was qualitatively similar to aminophyllin but the effect on output was approximately $\frac{1}{3}$ as intense. 1,3-dimethyl-8-chloroxanthine was followed by a small net decrease. The actual effect on output was probably greater than indicated by comparison of post-injection with pre-injection values, for a fall in output of approximately 10% was observed when repeated determinations were made in close succession on controls. None of the 3 compounds uniformly affected cardiac output in a single direction. The percentage change following injection varied from plus 185 to minus 45%. On the average, patients with high pre-injection outputs manifested a fall in output following administration of the test drug, while patients with low pre-injection outputs showed a rise. Many individual variations were observed, however. Output changes were due largely to altered stroke volume. Increased output was associated with a greater fall in intra-atrial pressure, a decreased arteriovenous oxygen difference and a rise in systemic blood pressure.

Xanthine studies 2) effects of aminophyllin, 1,3 diethyl 8 bromoxanthine and 1,3 dimethyl 8 chloroxanthine on water and sodium excretion. D. M. GREEN, W. C. BRIDGES, A. D. JOHNSON, J. H. LEHMANN, F. GRAY, and L. FIELD (all by invitation), *School of Medicine, Univ of Washington, Seattle, Wash.* The renal actions of 1,3-diethyl-8-bromoxanthine and 1,3-dimethyl-8-chloroxanthine were first observed by Newman and associates. In the present study these compounds have been compared with aminophyllin as to locus and intensity of effect. The subjects consisted of 25 hospital patients. Basal levels of renal function were measured during 2 20-minute clearance periods prior to intravenous injection of the drug. Drug effects were estimated by comparing control values with those obtained during 2 20-minute clearance periods following administration. All 3 compounds increased sodium and water excretion. Clearance data indicated these changes to be due almost entirely to a fall in percentage of tubular reabsorption. Aminophyllin was the most active of the compounds and doubled sodium output in 250 mg doses. The rise in water excretion was less than half as great. The glomerular filtration rate averaged an increase of approximately 15%. 1,3-diethyl-8-bromoxanthine was $\frac{1}{3}$ - $\frac{1}{4}$ as active as aminophyllin in augmenting sodium output. The effect on water excretion was proportionately less. Filtration rate changes were small and variable in direction. The renal effects of 1,3-dimethyl-8-chloroxanthine were inconstant and so weak as to

indicate the compound to be nearly inert. Analysis of aminophyllin action in relation to pre-injection levels of renal function showed the intensity of diuretic effect to be proportional to basal glomerular filtration rate. This relationship suggests that the effectiveness of the xanthines as diuretics depends on the renal functional reserve of the patient.

In vitro oxidation of oxophenarsine hydrochloride RAY E. GREEN (introduced by A. L. Tatum) *Dept. of Pharmacology, Univ. of Wisconsin Medical School, Madison, Wis.* The breakdown of oxophenarsine hydrochloride (mapharsen) on oxidation in air and 100% oxygen has been studied on various samples of the compound. The changes in LD-50 and minimal curative dose (M.C.D.) during the oxidation were determined for the albino rat and for *T. equiperdum* infection in the same species. An increase in the LD-50 and the M.C.D. has been noted on oxidation for both the aqueous solutions and the powdered compounds. When an aqueous solution is oxidized in pure oxygen for 24 hours or longer, a two- to three-fold increase in LD-50 (from 16 to 45 mg/kg) results and a dose $2\frac{1}{2}$ times the M.C.D. of the unoxidized compound is ineffective. The oxygen uptake in pure oxygen at 37°C has been followed manometrically and a linear relationship between oxygen consumption and time exists up to 25 hours of oxidation. Approximately 90% or more of the total decrease in toxicity occurs during this period. The effects of variations in pH on the rate of oxidation have been observed qualitatively over the range of pH 2.5 to 7. Increasing the pH value of the solution increases the rate of oxidation. At pH 2.5 and 25°C after 95 hours aeration, no demonstrable change in toxicity or therapeutic effectiveness was noted. On the other hand, at pH 7, for example, oxidation occurred very rapidly. In all instances examined, mapharsen upon oxidation became less toxic and correspondingly less effective as a trypanocide.

Effect of cholinesterase inhibitors on the permeability of dog erythrocytes in vitro MARGARET E. GREIG and WILLIAM C. HOLLAND (by invitation) *Dept. of Pharmacology, Vanderbilt Univ. Medical School, Nashville, Tenn.* In this laboratory it has been found that the intravenous administration of methadon to dogs frequently was followed by an increase in erythrocyte fragility and occasionally by a hemoglobinuria. Methadon also produced an increased hemolysis of dog and human erythrocytes *in vitro*. In metabolic experiments *in vitro* we have found that methadon inhibited the glycolysis of glucose, and the oxidation of lactate, pyruvate and succinate by brain (Arch. Biochem. 17, 129, 19, 441, 1948). Methadon also inhibited cholinesterase activity. Wilbrandt (Pflüger's Arch. f. d. ges. Physiol. 243, 519, 1940) reported that sodium fluoride and sodium iodoacetate produced changes in permeability of erythrocytes

which he attributed to their being glycolytic inhibitors. These compounds are, however, also inhibitors of cholinesterase activity. We have found that in addition to methadon certain more specific cholinesterase inhibitors which are reputed to have no effect on glycolysis, e.g., physostigmine, under certain conditions also produced changes in fragility of dog erythrocytes. The effect of these drugs was influenced markedly by the ionic composition of the medium.

New potent analgesic agent E. G. GROSS, M. A. BROTMAN (by invitation), S. F. NAGYFY (by invitation), W. W. SAWTELLE (by invitation) and L. L. ZAGER (by invitation) *Depts. of Pharmacology, Anesthesiology, Obstetrics and Gynecology, Urology and Surgery, State Univ. of Iowa, Iowa City, Iowa.* A new analgesic agent, 3-hydroxy-N-methyl morphinan hydrobromide (Nu-2206, Hoffmann-LaRoche, Inc.) is being studied experimentally and clinically in the above named departments. In the department of pharmacology, the analgesic potency of Nu-2206 has been studied using the Wolff-Hardy technique with normal human subjects, and the compound appears to have approximately four times the analgesic potency of morphine sulfate. With the highest dose used, 3 mg given subcutaneously, slight dizziness, nausea, vague gastro-intestinal symptoms and sedation were observed to a lesser degree than with a comparable analgesic dose of morphine sulfate. No euphoria was experienced by any of the subjects receiving the new agent. Blood pressure changes were slight and probably can be accounted for by the quieting of the subject. Respiration was only slightly depressed. Preliminary clinical reports indicate that the compound has about 3 times the potency of morphine sulfate when used in patients with incurable carcinoma and as a post-operative analgesic agent. The drug has also been used with moderate success in doses as high as 12 mg intravenously as the sole preanesthetic agent for nitrous oxide anesthesia, and there is some indication that the analgesia persists for a greater duration than is usual with other analgesic compounds.

Effect of diethylaminoethyl ester of 1-phenylcyclopentane-1-carboxylic acid hydrochloride, 'parpanit', on decerebrate rigidity, spinal reflexes and skeletal muscle CHARLES M. GRUBER, CHARLES P. KRAATZ (by invitation), CHARLES M. GRUBER, JR., and JOAN E. COPELAND (by invitation) *Dept. of Pharmacology, Jefferson Medical College, Philadelphia, Penna.* Seventeen decerebrated cats were used to study the effect of Diethylaminoethyl ester of phenylcyclopentane-carboxylic acid hydrochloride (parpanit) upon muscular rigidity. Four decapitated cats were used to determine the effect of parpanit upon reflexes, and 4 decerebrated cats and 11 urethanized dogs

to learn the effects of this drug upon motor nerve endings and on skeletal muscle, normal and denervated. Parpanit, trasentin and syntropan injected intravenously in adequate doses relieve the muscular rigidity of decerebrated cats. For this purpose these drugs were found superior to either atropine or scopolamine. The reflexes caused by mechanical stimulation of the back and paws in the spinal cat can be depressed by adequate doses of parpanit, trasentin and syntropan given intravenously. The height of the contractions of the anterior tibial muscle is increased with intravenous injections of parpanit. The intra-arterial injections of parpanit, trasentin and syntropan increase the height of the contractions of the anterior tibial muscle when their doses are small and rapidly injected and when their doses are large and slowly injected. Large doses rapidly injected cause a decrease in the activity of the muscle preceded and followed by increases above the height of the control. Parpanit, trasentin, syntropan, atropine and intocostin cause dilatation of the blood vessels in skeletal muscle when injected intra-arterially. The increased and decreased muscular activity following injections of parpanit and trasentin are direct effects of these drugs on the muscle cells, independent of the increase in blood flow. The depression of decerebrate rigidity by parpanit is due to central action and not a curare-like action on the neuromuscular junction.

Ganglionic blocking action of 'Dibutoline'

CARL C. GRUHZIT (by invitation) and GORDON K. MOE, *Dept of Pharmacology, Univ of Michigan, Ann Arbor, Mich.* Dibutoline, a quaternary ammonium compound with atropine-like properties, produces cardiovascular actions resembling those of tetraethylammonium. The ganglionic blocking action of Dibutoline was demonstrated in studies on arterial pressure, femoral arterial blood flow, and transmission through the stellate and superior cervical ganglia in dogs and cats. Intravenous injection of the drug causes a fall of pressure with an increased femoral blood flow, while intra-arterial injection causes little or no direct action on the vessels. Intravenous infusion blocks the respiratory and pressor actions of nicotine and high doses of acetylcholine and potentiates the pressor action of epinephrine. The effects of pre-ganglionic stimulation of the cardiac sympathetic nerves on heart rate and of the cervical sympathetic trunk on the nictitating membrane are blocked by Dibutoline, while the effects of post-ganglionic stimulation are uninhibited. The doses necessary to produce ganglionic blockade greatly exceed those said to produce atropine-like effects and would be encountered only with the highest doses used clinically.

Relative local emetic activity of glycosides of the digitalis series in cats. ROMULO GUEVARA (by

invitation), WALTER MODELL, THEODORE GREINER (by invitation), MELVIN MOORE (by invitation), ABRAHAM BLUMER (by invitation), HAROLD EVANS (by invitation) and HARRY GOLD, *Dept of Pharmacology, Cornell Univ Medical College, New York City.* All glycosides of the digitalis series exert local irritant action in the gastrointestinal tract. Whether this action is equally well developed in different glycosides has never been satisfactorily examined. Four glycosides, ouabain, scillaren A, scilliroside, and lanatoside C were compared. These were selected because they were available in pure form, their molecular weights and structures are known, and they are poorly absorbed from the gastrointestinal tract. Each of a series of doses of each compound was administered by stomach tube to a group of cats on an empty stomach (in all over 250 cats), and the animals were placed under continuous observation for a period until the first sign of vomiting or for at least 12 hours. The percentage of animals in which there was an emetic response for each of the series of doses was plotted against the log of the dose. The results show that the property of local emetic activity is developed to widely different degrees in the molecular structure of the various digitalis glycosides, and the ones tested here show the following order of increasing approximate potencies: ouabain-1, scillaren A-4, lanatoside C-10, scilliroside-10. That the emetic action was local was assumed from the fact that intravenous assay following emesis disclosed a systemic action of only from about 10 to 20% of the fatal dose. Local emetic action delayed in some cases for as long as 10 hours was an unexpected observation. There was no relation between the cardiac potency and the local emetic action.

Study of coca addiction. CARLOS GUTIERREZ-NORIEGA, *Dept of Pharmacology, Universidad Nacional Mayor de San Marcos, Lima, Peru.* The use of coca leaves is limited chiefly to the Andes regions of South America, from Colombia to Argentina. In regions where coconism exists, 25 to 30% of the total population are coca chewers. The number of coca chewers of South America may be estimated to be 8 millions. The amount of coca leaves annually consumed is, at least, 14,000 tons. The individual daily dose varies between 10 and 100 grams, with 30 grams as a mean value. The extraction of alkaloids during a period of coca chewing varies from 66% to 96%, with 86% as a mean value, in respect to the total amount of alkaloids contained in coca leaves. The total amount of alkaloids consumed varies from 80 to 400 mg, with 180 mg as a mean value. There is no increase in tolerance to cocaine among the coca chewers. The differences between the amounts of alkaloids ingested by the addicts of short-standing and of long-standing use are very small. The daily dose

of coca leaves does not increase during the span of life of the addicts. The duration of the coca chewing habit is more responsible than the size of the daily dose as a cause of the chronic symptoms found among coca chewers.

Toxicity to rats of diallyl acetic acid and other compounds containing the allyl radical ERNEST C HAGAN (by invitation), GEOFFREY WOODARD (by invitation), and ARTHUR A NELSON *Div of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D C* Rectal suppositories containing the bismuth salt of diallyl acetic acid have been held responsible for a number of deaths in children. Implications are that the acid is the responsible chemical. The oral LD50 of diallyl acetic acid (DAA) for rats is in the neighborhood of 630 mg/kg, diallyl phthalate 770, diallyl adipate 750, and allyl alcohol 60. Intravenously DAA has the same LD50 as by oral administration. Symptoms of poisoning by these compounds are depression, arching of the back, ruffled coat, and generalized weakness with anorexia. Deaths, particularly with DAA, are delayed, some occurring several weeks after acute dosage. At autopsy gross examination of the organs shows the livers and kidneys to be pale. In addition the livers present a nutmeg appearance. Microscopically both organs show principally fatty degeneration which is generally more severe in the liver than the kidney. Data obtained from feeding DAA at 25, 50, and 100 ppm for 6 months to Osborne Mendel rats demonstrate growth suppression in female rats at all levels, and in male rats at 50 and 100 ppm. Females show as much inhibition at 25 as at 50 ppm, while males at 25 ppm show growth comparable to that of their control animals. The capacity for detoxification of pentobarbital and phenobarbital was studied in rats which had received 100 ppm DAA for from 1-2 months. With each barbiturate hypnosis was more prolonged in treated animals than in their litter controls.

Correlation between toxicity by inhalation and physical properties of beryllium oxide R H HALL, S LASKIN, G SPRAGUE and B H BROWN (introduced by H C Hodge) *Dept of Radiation Biology, Univ of Rochester, Rochester, N Y* The toxicity of inhaled BeO appears to depend upon the physical properties of the material, which, in turn, are related to the method of preparation. The processes currently employed in the production of BeO involve calcining a beryllium salt at high temperatures. Dogs, guinea pigs, rabbits and rats have been exposed 6 hours daily for 12-15 days to an atmosphere containing approximately 85 mg/m³ of BeO aerosol. Even at this relatively high concentration, one grade of BeO appeared to be virtually nontoxic. This material was fired at 1350°C and was composed of unit particles having an arithmetic mean diameter of about 0.7μ, with

aggregates of low porosity (5.2%). A second grade of BeO gave definite evidence of toxicity. This material was fired at 1100°C and comprised unit particles of about 0.5μ diameter, with aggregates of markedly greater porosity (35%). Two of 20 rats died during the exposure period, while 3 of 5 rats, held for observation following exposure, showed a marked increase in their leukocyte count due to an absolute increase of neutrophils and lymphocytes. One of 4 dogs showed a transient low blood oxygen tension following exposure, and the lungs of this animal which was killed terminally exhibited extensive lesions. Dogs and rats exposed to an aerosol of BeO produced by calcining Be(NO₃)₂ at 400°C exhibited weight loss during exposure, 1 of the 2 dogs showed a significant reduction in blood oxygen tension, and 8 of 40 rats died. This material was made up of unit particles of about 0.4 microns mean diameter and aggregates of very high porosity (134%). The small sizes were confirmed by x-ray diffraction analysis which also revealed trace quantities of a foreign substance identified as silica. On heating of the material at 1000°C, grain growth occurred followed by the disappearance of the silica lines.

Effect of mercurial diuretics on the succinic dehydrogenase system of the kidney C A HANDLEY *Dept of Pharmacology, Baylor Univ College of Medicine, Houston, Texas* The succinic dehydrogenase activity of rat kidney cortex homogenate was inhibited 20-50% by the intravenous administration of non-lethal doses of mercurial diuretics. The dosage range used was 0.1 to 0.2 cc/kg (4-8 mg/kg of combined mercury), the range generally used for diuresis in laboratory animals. One hour after the injection, the kidneys, liver and heart were removed and 5% homogenates of each organ prepared for assay. In none of the experiments was the succinic dehydrogenase activity of heart or liver reduced below that of the controls. When the mercurial diuretic was added directly to Warburg flasks containing homogenates of kidney cortex, liver and heart from untreated animals, so that the concentration of mercury was 5×10^{-6} to 10^{-6} M, a comparable inhibition of succinic dehydrogenase activity occurred in all the homogenates. It is concluded that during mercurial diuresis, sufficient mercury is accumulated in the kidney cortex to partially inhibit the succinic dehydrogenase activity.

Amebicidal action of agents inhibitory to associated bacteria in vitro EDER L HANSEN (introduced by Hamilton H Anderson) *Div of Pharmacology and Experimental Therapeutics, Univ of California Medical School, San Francisco, Calif* *E. histolytica* has not yet been cultivated in the absence of bacteria. Agents that affect trophozoites may do so either directly or indirectly by acting on associated bacteria (Brickett & Bliz

nick, 1947) It is now possible to detect indirect (bacteriostatic) action by modification of the conventional testing procedure, so that the intense reduction produced by associated bacteria is preserved Such reducing conditions are necessary for survival and multiplication of trophozoites, since slight disturbance of bacterial growth can result in death of the ameba Tubes containing dilutions of any agent found to be 'amebacid' when tested by conventional procedure were sealed with melted petrolatum (Shaffer & Frye, 1948) Survival of trophozoites in sealed cultures, after 48 hours at 37°C, indicated that apparent 'amebacid' action observed in cotton-stoppered tubes was due to inhibition of bacteria and not necessarily to direct action on the ameba The method has been applied to Locke's egg slants and to the two liquid media of Hansen & Anderson (1948), using as bacterial associates organism 't' or a 2-membered flora With the latter flora selective action of an inhibitory agent further complicated evaluation of amebacid activity Aureomycin HCl was 'apparently amebacid' in liver-proteose-peptone medium at 1/500,000, however in sealed cultures, trophozoites survived in concentrations of 1/50,000 At higher concentrations organism 't' was so greatly inhibited that satisfactory reducing conditions were not established even in sealed cultures Emetine HCl which exhibited no bacteriostatic action, was amebacid at the same concentrations in both cotton-stoppered and petrolatum-sealed cultures

Comparison of effects of injection of pyrogenic solutions by intravenous and intramuscular routes WILLIAM D HARKNESS (by invitation) and BERT J Vos *Div of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D C* Rabbits were injected either intravenously or intramuscularly with a pyrogenic solution prepared from *Pseudomonas aeruginosa*, and their rectal temperatures were observed at hourly intervals for 5 and 5 hours respectively The rise in temperature following intramuscular injection was more gradual than that following intravenous injection and frequently had not reached a maximum even at 5 hours Within certain limits a linear relationship existed between log dose of pyrogen and the maximum rise in temperature observed during the experimental period However, at least 4 times as much pyrogen was needed by intramuscular as by intravenous administration to produce a given rise in temperature

Pathologic changes in guinea pigs produced by diets deficient in the anti-stiffness factor PAUL N HARRIS and ROSALIND M WULSEN (by invitation) *The Lilly Research Labs, Eli Lilly and Co, Indianapolis, Ind, and the Dept of Zoology, Oregon State College, Corvallis, Ore* It was found by Wulsen and Bahrs (*Am J Physiol*

133 500, 1941) that maintenance of guinea pigs upon pasteurized milk or skim milk diets resulted in stiffness of the wrists Diets of skim milk powder dissolved in skim milk or water and supplemented with ferric chloride, copper sulfate and adequate amounts of the known vitamins have a similar effect, and were used in the studies herein reported Hamsters kept upon such diets remained normal, but guinea pigs developed widespread anatomical lesions These consisted in necrosis of skeletal muscle with a variable amount of macrophage infiltration and foreign body giant cell formation and calcification of necrotic fibers, focal necrosis and calcification of the myocardium, deposition of calcium salts in the smooth muscle of the gastrointestinal tract, and in the kidneys, liver, aorta and peripheral arteries, and development in the muscles and adjacent to bones and joints of abscesses that often became calcified

Skin penetration of radioactive anticholinesterases—a direct quantitative method of study E ROSS HART, JOSEPH H FLEISHER (by invitation), and AMEDEO S MARRAZZI *Toxicology Section, Medical Div, Army Chemical Center, Md* Any study of the ability of a compound to penetrate the skin necessitates some means of determining its concentration in the blood Chemical methods that are sufficiently specific and sensitive are often lacking Moreover, they require repeated sampling thus disturbing the animal economy and providing only discontinuous determinations Pharmacological methods are of limited usefulness because of their inherent lack of precision and their indirectness, latent periods, compensatory mechanisms etc, all introducing complications Radioactive tracer methods offer the advantages of being highly sensitive and specific and, as utilized here, requiring no sampling, being essentially direct and continuous Increased sensitivity and specificity can be attained by measuring the radioactivity of venous blood draining from the area of application thus avoiding dilution in the entire blood volume and minimizing the possibilities of chemical alteration between penetration and determination The rabbit ear seems almost ideally suited to our purpose By applying tagged compounds to the distal portions and placing a Geiger-Mueller tube over the proximal portions of the marginal vein, we have accomplished the conditions outlined above Information concerning routes of penetration can be obtained from radioautographs of cross sections of the ear The technique is described in detail and the results of its application to DFP are presented as an example

Streptomycin content of brain and other tissues after parenteral injection J E HAWKINS, JR, G E BOXER, and V C JELINEK (by invitation) *Merck Inst for Therapeutic Research and The Research Labs of Merck & Co, Rahway, N J*

The streptomycin content of blood, cerebrospinal fluid, brain, liver, lung and spleen was determined by the fluorometric method in 3 cats killed 24, 48 and 72 hours respectively after receiving a subcutaneous injection of streptomycin CaCl_2 -complex equivalent to 400 mg of streptomycin base/kg of body weight. The blood concentration at 24 hours was too high for the tissue concentrations to be meaningful. At 48 and 72 hours, when the blood and cerebrospinal fluid concentration had fallen to 1-2 $\mu\text{g/ml}$, the viscera contained 9 to 38 $\mu\text{g/gm}$ (wet weight) but the brain only 2-4 $\mu\text{g/gm}$. This experiment shows that streptomycin tends to persist in the viscera when it has virtually disappeared from the body fluids, but has no special predilection for the brain. In a second experiment similar determinations were made in 4 cats which had received daily doses of streptomycin equivalent to 100 mg of base/kg for 7 to 28 days. All but one showed the characteristic vestibular disturbance. Blood and cerebrospinal fluid taken 24 hours after the last dose of streptomycin contained 1-3 $\mu\text{g/ml}$ and the brain only 1-2 $\mu\text{g/gm}$, while the concentration in lungs, liver, spleen and salivary glands ranged from 7 to 41 $\mu\text{g/gm}$, and in the kidneys from 119 to 234 $\mu\text{g/gm}$. The significance of these observations is discussed in relation to the therapeutic and neurotoxic actions of streptomycin. It is evident that the neurotoxic action does not depend upon a selective accumulation of the drug in the brain.

Pharmacology and toxicology of thiodipropionic acid and its dilauryl and distearyl esters LLOYD W. HAZLETON and REBECCA C. HELLERMAN (by invitation) *Hazleton Labys, Falls Church, Va.* Thiodipropionic acid ($\text{S}[\text{CH}_2\text{CH}_2\text{COOH}]_2$) and its dilauryl and distearyl esters have antioxidant properties of interest in the preservation of edible fats and oils. The acid is slightly soluble in water while the esters are insoluble over a wide range of temperatures. In rats, mice and guinea pigs the acute toxicity of these compounds is of a relatively low order of magnitude. Infused intravenously in anesthetized dogs the lethal dose of the acid is in excess of 10 gm/kg. The acid possesses no characteristic pharmacological properties and death in these animals is apparently due to hemodilution and acidosis. Two-year rat feeding experiments were essentially negative for the acid and distearyl ester in levels up to 3.0% of the diet. The 3.0% dilauryl level showed an increase in mortality toward the end of the period. In another series a mixture of the acid and dilauryl ester was heated in lard at 190°C for 30 minutes. The diets as fed contained 10% lard plus 0.11% and 1.1% of the acid-ester mixture. At the lower level there was no significant effect on growth or mortality during the 2-year period. At the higher level there was significant mortality during the first year. The

immediate cause of this increased mortality was respiratory infection. The possible role of the diet in this phenomenon was not established. Based on a 2-year expectancy, all the groups showed from 83% to 99% of the theoretically perfect score with the exception of the two groups discussed which showed 75% and 37% respectively.

A new adrenergic blocking agent FRANCIS G. HENDERSON (by invitation) and K. K. CHEN *The Lilly Research Labs, Eli Lilly and Co., Indianapolis, Ind.* A study was made with ethyl β -chloroethyl β -*o*-benzyl) phenoxyethylamine HCl. Its adrenergic blocking activity was found to be approximately 7.5 times that of dibenamine, but its toxicity greater than that of the latter, the LD_{50} in mice by intravenous injection being 33.81 ± 1.28 mg/kg as against 88.45 ± 3.02 mg/kg for dibenamine. A dose of 2 mg/kg intravenously reversed the pressor response ordinarily produced by 25 μg of epinephrine HCl in the dog under barbiturate anesthesia. When a dose of 5 mg/kg was given intravenously the reversal action persisted for over 30 hours. A reversal of the pressor action of epinephrine also occurred 25 hours following the oral administration of this compound in the dose of 20 mg/kg mixed in an acacia suspension. A dose of 5 mg/kg intravenously prevented the rise in blood pressure commonly produced by intravenous injections of 1 mg of the following aliphatic amines: 2-aminoheptane sulfate, 2-amino-4-methylhexane sulfate, and 2-methylamino-1-cyclopentylpropane HCl. No epinephrine reversal could be demonstrated on the cat's uterus. When administered to dogs by slow intravenous injection in doses ranging from 2 to 10 mg/kg, the compound per se caused no significant changes in blood pressure, respiration or electrocardiogram.

Effect of myanesin on extrapyramidal facilitatory and inhibitory systems E. HENNEMAN (by invitation), A. KAPLAN (by invitation) and K. UANA *Depts. of Psychiatry and Pharmacology, Univ. of Illinois College of Medicine, Chicago, Ill.* Hyperactivity of stretch reflexes due to overactive or unopposed extrapyramidal facilitation constitutes spasticity. Demonstration of the origins of separate spinal paths for facilitation and inhibition of myotatic reflexes in two distinct regions of the brain stem (Magoun) suggested investigation of the effects of myanesin on these systems. Bipolar stimulating electrodes carried in a stereotaxic apparatus were used to explore the bulbar reticular formation in lightly nembutalized cats. Regions yielding complete inhibition (medial, medullary sites) or satisfactory facilitation (lateral, pontine sites) of the knee jerk with low voltage were located. Myanesin was administered intravenously. Small doses reduced facilitation or inhibition. Larger doses abolished either effect but left the knee jerk, as judged by amplitude, unaffected.

The two antagonistic systems were influenced equally so far as could be judged. The time course of drug action was determined by following the recovery of such systems. Inhibition and facilitation of the knee jerk resulting from cerebral cortical stimulation were abolished with much smaller doses, suggesting that longer or more complex circuits (such as are usually involved in spasticity) are more vulnerable to myanesin. Purely spinal facilitatory or inhibitory reflex arcs were also investigated by stimulating peripheral skin or muscle nerves in decapitate preparations. From this type of evidence and from electrical studies of segmental spinal reflexes it appears that myanesin relieves spasticity by reducing tonic extrapyramidal facilitation of stretch reflexes, whatever its source. Because facilitation dominates inhibition in spasticity it is presumably more affected by the drug.

Cardiovascular action of magnesium sulfate solutions JAMES P. HENSEN (introduced by R. P. Ahlquist) *Dept. of Pharmacology, Univ. of Georgia School of Medicine, Augusta, Ga.* The cardiovascular actions of magnesium sulfate solutions, administered intravenously and intra-arterially, were investigated in the anesthetized dog. Arterial pressure was recorded by means of either an optical manometer or an ordinary mercury manometer while the femoral arterial or venous blood flow was recorded by a rotameter or 'Bubblemeter'. The results indicate that magnesium sulfate solutions lower the arterial pressure by two different mechanisms: 1) peripheral vasodilation and 2) myocardial depression. The peripheral dilation was found to be due to 1) a direct action of the magnesium ion (an effect proportional to the dose administered) and 2) some osmotic action (an effect proportional to the concentration of the injected solution). This osmotic effect was most pronounced with intra-arterial injections and could be duplicated by intra-arterial injections of hypertonic sodium chloride solutions. No evidence was obtained to indicate that the vasodilating action of magnesium sulfate was due to ganglionic blockade in a manner analogous to tetraethylammonium.

Pharmacologic properties of 2-[N-p'-tolyl-N-(m'-hydroxyphenyl) - aminomethyl] - imidazole hydrochloride (C-7337), a sympatholytic ELIZABETH A. HEROLD (by invitation), ANNE CAMERON (by invitation), ALFRED EARL (by invitation), FRANK ROTH (by invitation), JAMES SMITH (by invitation), NICOLINE SMITH (by invitation), ELIZABETH SORESENSEN (by invitation), BRADFORD N. CRAVER *Div. of Microbiology, Research Dept., Ciba Pharmaceutical Products, Inc., Summit, N. J.* This drug was synthesized by Marxer and Miescher and reported by Meier and Yonkman (*Federation Proc.*, this issue). Doses of 0.2 to 1.0 mg/kg have produced 'epinephrine reversal' in dogs, cats, guinea pigs and rats. C-7337,

in sufficient dosage, produced the hypotension characteristic of sympatholytics. Other augmentatory actions of epinephrine negated by C-7337 in the same range of doses were 1) contraction of nictitating membrane and 2) salivation in the cat. About twice the doses required for adrenolysis sufficed for sympatholysis. The spasmolytic effect of epinephrine upon canine Thiry-Vella loops and upon the feline uterus was unaltered by the drug. It produced complete relaxation of the nictitating membrane in respect to such drugs as Priscol and Privine, whereas atropine produced only partial relaxation; this could be but partly explained by its very weak antihistaminic and anticholinergic activities. In Warburg experiments cardiac tissue from the cat was as sensitive to the inhibitory action of C-7337 on respiration as hepatic tissue from the cat or rat (100 γ /ml — minimal inhibitory concentration). The LD₅₀ in rats is 55 mg/kg intravenously. In the dog 125 mg/kg orally produced depression, tachycardia, defecation, salivation, emesis and recovery; 60 mg/kg intravenously produced the same symptoms and final recovery. Warren, Woodbury and Trapold (*this issue Federation Proc.*) will report an intensive study of the effects of its chronic administration to dogs.

A rapid method for the determination of inulin in blood and urine AEME HIGASHI (by invitation) and LAWRENCE PETERS *Dept. of Pharmacology, School of Medicine, Western Reserve Univ., Cleveland, Ohio*. Bacon and Bell (*Biochem. J.* 42: 397, 1948) used a modified Selwanoff reaction, devised by S. W. Cole, to determine fructose in cadmium sulfate filtrates of blood. They mentioned its application to the determination of inulin. We failed to recover added inulin consistently from blood and urine by this method. However, when a trichloroacetic acid (TCA) rather than a CdSO₄ filtrate was used, and temperature was controlled within 0.5°C, accurate recoveries resulted. To 2.0 ml of plasma or urine filtrate (80% TCA), containing 7.5–30.0 γ of inulin, is added 3.0 ml of 0.15% resorcinol in alcohol (95%). With the tubes immersed in ice water, 3.0 ml of conc. HCl, containing 7.5 mg of FeCl₃/l, are added with shaking. The tubes are heated in a water bath for 20 minutes at 79.0–79.5°C. After rapid cooling, the orange-red color is read in a Klett colorimeter (54 filter). Standard solutions are analyzed with unknowns on all occasions. Plasma containing no inulin gives blank readings equivalent to 0.7–1.4 mg %. Urine from subjects undergoing clearance studies is diluted beyond the point where blank values are obtained. Glucose produces additional color requiring corrective calculation only at high concentrations, such as those obtained during Tm determinations. Numerous recovery experiments have yielded a maximum error of 2.5%. Simultaneous inulin and creatinine clearance values for dogs agree well.

In one experiment the average I/C ratio for 9 consecutive clearance periods was 1.036 (range, 0.99–1.08, S.D. 0.032)

Lack of antagonism of methionine to barbiturates J. EUGENE HILL (by invitation), P. L. EWING, G. A. EMERSON and MELVILLE SAHYUN, *Dept. of Pharmacology, Univ. of Texas Medical Branch, Galveston, Texas, and The Sahyun Lab., Santa Barbara, Calif.* The nutritional significance of methionine suggests a possible antagonism by it of agents detoxified in the liver. White mice treated with tolerated doses of 0.1–0.5 gm/kg of DL-methionine intramuscularly or intraperitoneally were given repeated intraperitoneal doses of hexobarbital or thiopental at short intervals until approximately 50% of untreated controls died, or single intraperitoneal doses of the LD₅₀ of pentobarbital or phenobarbital. In each case, a larger proportion of the methionine-treated mice died than of the controls. With pentobarbital, mice fasted 24 hours again showed no protective effect of methionine. Methionine either is present in excess even in fasted mice or else is not involved in the major degradation reactions of barbiturates in the liver.

A polarographic study of certain quinones JAMES G. HILTON (introduced by C. L. GEMMILL), *Dept. of Pharmacology, Univ. of Virginia Medical School, Charlottesville, Va.* The polarograph has been used as a qualitative and quantitative instrument in the study of certain quinones. The quinones examined were hydroquinone, 2-methylhydroquinone, 2,3-dimethylhydroquinone, 2,3,5-trimethylhydroquinone, 1,4-naphthoquinone, 2-methyl-1,4-naphthoquinone, 2-methyl-3-hydroxy-1,4-naphthoquinone (Phthiocol), 3-hydroxy-1,4-naphthoquinone (Lawsone) and sodium 1,2-naphthoquinone-4-sulfonate. These quinones were studied both in bicarbonate and in glycolytic enzymatic mediums. All of the quinones showed definite half-wave potentials in the bicarbonate-carbon dioxide solution and gave diffusion currents which were in direct proportion to the concentrations. A detailed study was made of the half potentials of 2-methyl-3-hydroxy-1,4-naphthoquinone (Phthiocol) at various levels of oxidation of this compound. A close correlation was obtained between these potentials and the oxidation-reduction potentials previously described by Ball (*J. Biol. Chem.* 106:515, 1943). In the glycolytic enzymatic systems, the half potentials were shifted and the diffusion current heights were either reduced or totally missing. It is concluded from this work that the polarograph is an accurate instrument for determining the concentration of certain quinones of as low as 1×10^{-4} M concentration in a bicarbonate medium and is suitable also for determining oxidation-reduction potentials of these compounds in bicarbonate

buffered solutions. From these results in the glycolytic enzymatic systems, it is concluded that the half potential shift was due to a change in the oxidative level of the quinone system and the diffusion current variations were due either to combination or to destruction of part or all of the quinone.

Acute toxicity of hydroquinone HAROLD C. HODGE, ELLIOTT A. MAYNARD (by invitation) and JAMES H. STERNER (by invitation), *Div. of Pharmacology and Toxicology, Dept. of Radiation Biology, Univ. of Rochester School of Medicine and Dentistry, and Eastman Kodak Company, Rochester, N. Y.* The characteristics of acute hydroquinone poisoning have been observed. Dying animals did so promptly, those surviving 20 minutes almost invariably recovered completely. Convulsions were commonly observed, the heart frequently beat after respiration ceased. Estimations of the LD₅₀ have been made for a number of species. Following intraperitoneal administration, mice, rats, guinea pigs and rabbits have similar LD₅₀'s. After oral administration, cats were most susceptible, rats and rabbits less so, and mice most resistant. Cats can, however, ingest approximately the lethal dose daily for nearly a month if the HQ is mixed in the diet. Fasting rats were twice as susceptible as fed rats to oral doses. The LD₅₀ (orally to rats) of HQ in propylene glycol was considerably greater than in water solution. Rats were maintained for a period of one month on a) a common laboratory mixed diet and b) a commercial rat ration, the acute LD₅₀'s (oral administration) were identical regardless of previous diets. The acute toxicity of commercial photographic-grade hydroquinone was the same as that of a carefully purified sample when each was administered intraperitoneally.

Relationship of chemical structure to biliary concentration in experimental cholecystography JAMES O. HOPPE and S. ARCHER (introduced by A. M. Lands), *Biology Div., Sterling-Winthrop Research Inst., Rosenslaer, N. Y.* Replacement of one of the hydrogens from the methyl group of 4-amino-3,5-diiodotoluene by 2-substituted n-alkanoic, cycloalkyl-alkanoic or aryl-alkanoic acids yielded compounds which were excreted into the bile of cats following oral administration in sufficient amounts to make the gallbladder opaque to x-radiation. Optimum results were obtained with the n-alkanoic acid derivatives when the number of carbon atoms attached to the phenyl nucleus approached seven. The cycloalkyl-alkanoic acid derivatives were ineffective because of poor absorption. The most satisfactory compound was found among the aryl-alkanoic acid derivatives in the form of the sodium salt of β -(4-amino-3,5-diiodophenyl)- α -phenyl propionic acid (WIN 593-2). The density of the shadows produced by WIN

508-2 appeared to be approximately 15 times as great as those of iodoaliphonic acid at comparable dose levels. Upon oral administration to both cats and dogs, WIN 508-2 appeared to be relatively non-irritating to the gastro-intestinal mucosa, readily evacuated from the gallbladder following a fatty meal and to produce a minimum of vomiting and diarrhea. The acute oral toxicity of the free acid in mice was less than that of iodoaliphonic acid whereas that of the sodium salt, WIN 508-2, was slightly greater because of more rapid and efficient absorption.

Metabolic studies in beryllium patients JOE W. HOWLAND and CHRISTINE WATERHOUSE (introduced by H. C. Hodge) *Dept of Radiation Biology, Univ of Rochester, Rochester, N. Y.* Systemic manifestations of weakness, easy fatigability, anorexia, and weight loss have played an important role in the symptomatology of many patients with beryllium granulomatosis. Metabolic studies were carried out on 4 patients with beryllium poisoning in an attempt to determine whether or not these patients were able to utilize and store nitrogen on an adequate dietary intake. Three of the 4 patients studied were malnourished, had lost between 30 to 40 pounds in weight during their illnesses. Yet none was able to establish a significant positive nitrogen balance on diets adequate in calories and protein. Fecal nitrogen was high on all 3 patients. Under the anabolic stimulus of testosterone, one patient had a negligible nitrogen storage response, however, another stored moderate amounts of nitrogen under this regime. The implications of these findings particularly in reference to anoxia and to the systemic effects of beryllium poisoning are discussed.

Effect of quinidine in cyclopropane and cyclopropane-epinephrine induced arrhythmias R. A. HUGGINS (by invitation), R. A. MORSE (by invitation), D. W. CHAPMAN (by invitation), C. A. HANDLEY, and L. F. SCHUHMACHER (by invitation) *Depts of Pharmacology, Medicine and Anesthesiology, Baylor Univ College of Medicine, Houston, Texas*. The protective action of intravenous quinidine sulfate and lactate against the onset of epinephrine induced cardiac arrhythmias under cyclopropane anesthesia has been evaluated as to rapidity of onset, duration of action and minimum effective dose. The dosages used were 1, 5, 10, and 20 mg/kg. The intravenous administration of quinidine sulfate or lactate in all dosage levels tested markedly reduced the incidence of this type of cardiac arrhythmia. No difference was found between the two forms of quinidine. The onset of the protective action was rapid, less than 0.5 minute after the completion of the injection, and independent of the dosage. Injections of epinephrine up to 10 minutes after the administration of quinidine caused no arrhythmias, regardless of the size

of the dose. After 10 minutes, the duration of the protecting properties of quinidine tends to vary directly with the size of the dose, lasting for an average of 24 minutes with 1 and 5 mg/kg, and 1 to 3 hours with 20 mg/kg.

Failure to demonstrate biological competition between hypnotic and non-hypnotic barbiturates WALTER W. JETTER, ROBERT P. BRITAIN and MARY K. NUTER (introduced by George L. Maison) *Dept of Legal Medicine, Harvard Medical School, and The Massachusetts Dept of Mental Health, Boston, Mass.* Sodium salts of non-hypnotic barbituric acid derivatives (BAD) as follows: 1) 5-monoethyl, B. A., 2) 5-monophenyl, B. A., 3) 5-mono sec butyl, B. A., 4) 5,5-ethyl, nitropropyl, B. A., 5) 5,5-methyl (1 methyl butyl), B. A., 6) 5,5-methyl (1 methyl butyl) 4-imino, B. A. furnished in small quantities by Abbott Laboratories, North Chicago, were tested for their analeptic properties against depressant barbiturates in the following way. Twenty-seven rabbits were anesthetized by intravenous pentobarbital (40 or 50 mg/kg) and 26 by phenobarbital (150 or 175 mg/kg). Four rabbits died within less than one hour after receiving the depressant barbiturate alone. The surviving group of 49 rabbits contained 24 animals that received pentobarbital and 25 that received phenobarbital. Fifteen of these (9 pentobarbital and 6 phenobarbital) served as controls and received no treatment. Each of the remaining 34 animals then received intravenously one of the six BAD test compounds in dosage of 50, 100, or 150 mg/kg. Each compound was tested in at least 4 rabbits (BAD no. 5 was not tested against phenobarbital because of insufficient supply). It was clear that in no instance did the injection of the test BAD compound significantly decrease the depth or duration of the anesthesia caused by the previously injected depressant barbiturate. It was concluded that the BAD compounds tested were not successful biological competitors against pentobarbital or phenobarbital under the conditions of the experiment.

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production. Inhibition of the same order of magnitude occurred when Hexose diphosphate was the substrate, when Fluoride is present in concentration of 0.02 M, and when arsenate is substituted for phosphate in the system. These results indicate that these compounds interfere with the conversion of Hexose diphosphate to Phosphoglyceric acid. Studies of the mechanism of this inhibition are in progress. The effect of twenty 8-aminoquinoline analogues of Pamaquine and Pentaquine on acid production in these systems was studied. Alteration of chemical structure modified the ability of these compounds to inhibit acid production, but there was little correlation between inhibition of acid production and antimalarial activity.

Effects of ions and enzyme inhibitors on breakdown of pentobarbital by rat liver slices. I. B. KAHN, JR. (introduced by J. M. Coon) *Dept of Pharmacology, Univ of Chicago, Chicago, Ill.* Although it is well established that some barbiturates are detoxified by tissues *in vitro*, the nature of the process is not well understood. It was therefore decided to study the effects of various ions upon the action of rat liver slices on pentobarbital. The ions studied are known to be effective in activating or inhibiting enzymatic reactions of various types. Approximately 200 mg of liver slices were placed in each of two 250-ml Erlenmeyer flasks, A and B, each containing 90 ml of Krebs Ringer bicarbonate buffer with glucose. A was boiled for 5 minutes while B was maintained at 37°. Both were placed, in a water bath at 37°, and 10 ml pentobarbital, 0.6 mg/ml in Krebs-Ringer bicarbonate buffer added. Air was bubbled through for mixing and oxygenation. Ten-ml aliquots were taken from each at 0, 30, 60, and 120 minutes, and analyzed for unchanged barbiturate by a modification of the method of Goldbaum (*J Pharmacol* 94: 68, 1948). Controls in normal Krebs-Ringer bicarbonate buffer with glucose showed 10% destruction of pentobarbital in 60 and 120 minutes in flask B, while flask A remained constant. Doubling the concentration of potassium and omitting calcium had no effect. The effects of high and low concentrations of Na, K, Ca, and Mg, and of heavy metals, iodoacetate, and cyanide are also reported.

Effect of acetate donors on creatinine levels in blood and urine in dogs. A. KANDEL (by invitation) and MAYNARD B. CHENOWETH *Dept of Pharmacology, Univ of Michigan, Ann Arbor, Mich.* Five unanesthetized dogs were injected intramuscularly, and one intravenously, with monoacetin (glycerol monoacetate). Bladder urine was collected by catheter for a period of 10 hours. As a control, urine was collected over periods of the same length but without administration of monoacetin. The intramuscular injection of monoacetin resulted in a 25% increase in the total

amount of creatinine excreted. An 80% increase was observed in the animal in which a 5% solution of monoacetin was given intravenously. Creatinine was not excreted during control periods, but appeared in the urine only after monoacetin was administered. Both creatine and creatinine are excreted in greatest amounts during the last 2 hours of the collection period. Intramuscular injection of propylene glycol in one of the animals revealed no increase in urinary creatinine. A bilaterally nephrectomized dog was given 1 gm/kg monoacetin intramuscularly in 1 gm/kg doses every 2 hours. Blood collected at hourly intervals showed a steady increase in the creatinine level. A marked augmentation in creatinine accumulation was observed during the 9th and 10th hours. Further experiments are being conducted to establish the nature of the increase in production of creatine congeners by acetate donors.

Chronic effects of large doses of acetanilid in dogs and rats. ALEXANDER G. KARCZMAR (introduced by Theodore Koppányi) *Dept of Pharmacology, Georgetown Univ School of Medicine, Washington, D. C.* This is an extension of a study of acute toxicity of acetanilid (700 mg/kg) in dogs (*Federation Proc* 6: 311, 1947). Daily oral administration of 100 mg/kg of acetanilid caused no withdrawal symptoms after 3 weeks (2 dogs). Administration of 150, 100, and 200 mg/kg (2, 2, and 7 weeks, Payne, *J Pharmacol Exper Therap* 53: 401, 1935) was followed by daily methemoglobin cycles (duration 8 hr) with peaks of 25, 15 and 35% of total hemoglobin respectively. Discontinuation of acetanilid in 1 dog after 11 weeks caused no withdrawal symptoms. Increase of acetanilid in 3 other dogs to 400 (3 days) and 600 (4 days) mg/kg gave a methemoglobin peak of 65%. These levels of acetanilid administration resulted ultimately in reversible hypochromic anemia. There was drowsiness and analgesia, but no withdrawal symptoms. Tolerance did not develop since methemoglobin, lethal dose and the time of death corresponded to those of acute experiments (o.c.). Dogs killed during the chronic experiments showed earlier, bone marrow hyperplasia and reversible changes in the liver and, later, non-specific changes in the central nervous system, cortex and visceral organs (Hicks and Karczmar, to be published). Daily intraperitoneal administration to rats of $\frac{2}{3}$ of the lethal dose of acetanilid (up to 4 weeks) gave similar results with a methemoglobin peak at 35%. Withdrawal symptoms developed neither in dogs (contrary to Payne, o.c.) nor in rats independently of the administration schedule employed.

Duration of action of anticholinesterases in dogs. ALEXANDER G. KARCZMAR (by invitation) and THEODORE KOPPANYI *Dept of Pharmacology, Georgetown Univ School of Medicine, Washington, D. C.* Potentiation of pressor responses to

acetylcholine in atropinized dogs (pentobarbital sodium anesthesia) is used in this laboratory as a qualitative and quantitative assay of anticholinesterases (Science 106 492, 1947). This assay lends itself to establishing the duration of action of anticholinesterases (maximally potentiating doses used throughout) since the animal survives under test conditions for any desired time. It was found by means of this method that physostigmine and neostigmine are short-acting anticholinesterases with a duration of potentiation up to 6 hours. A thiophosphate type of anticholinesterase, 0-0-diethyl p-nitrophenol thiophosphate, belongs here also with a 5-hour duration of action (Anat Rec 101 89, 1948). A compound with an alleged specific action on pseudocholinesterase, dimethylcarbamate of (2-hydroxy-5-phenyl-benzyl) trimethylammonium bromide proved to be a short-acting (2 hours) true anticholinesterase. Organic phosphate esters are either long- or intermediate-acting. DFP belongs to the first category with complete extinction of potentiation of pressor effects after one week. Hexaethyl tetraphosphate (HETP) and tetraethyl pyrophosphate (TEPP), possibly active factor of HETP, cease to act after 12 hours. In all cases, anticholinesterase activity decreases rapidly at first and slowly after 50% of the effect disappears. Determination of anticholinesterase activity in terms of nicotinic effects of acetylcholine measures the biological reversibility of esterase inhibitors. It generally confirms the results obtained *in vitro* and seems free of errors due to dissociability of the esterase-anticholinesterase complex during *in vitro* procedures, (Goldstein J Gen Physiol 27 529, 1944).

Relation between chemical constitution and estrogenic action in a group of azomethines. H. H. KEASLING and F. W. SCHUELER (introduced by E. G. Gross) College of Pharmacy and Dept of Pharmacology, College of Medicine, State University of Iowa, Iowa City, Ia. This work is based on the working hypothesis that estrogenic compounds should consist of a relatively inert molecular structure with two active hydrogen-bond forming groups located at an optimum distance of 14.5 Å units (measured on Fisher-Hirschfelder-Taylor Atomic Models). Since the 4,4'-dihydroxy derivatives of stilbene ($-\text{CH}=\text{CH}-$) and azobenzene ($-\text{N}=\text{N}-$) (Schueler, F. W., Science 103 221 (1946)) show an estrogenic response, a study of the intermediate type of linkage as represented by the azomethines ($-\text{CH}=\text{N}-$) was carried out. In order to investigate the importance of variations in hydrogen-bond forming power, substitutions were made in the 4,4' positions of the basic structure, benzylidene aniline. All 16 possible combinations of the 4 substitution groups, hydrogen, hydroxyl, methyl, and methoxyl, were prepared and tested. The dihydroxy derivative best fulfills the requirements or

the hypothesis, the methoxy derivatives represent blocking of the hydrogen-bond forming groups, and the methyl and hydrogen substitutions are groups of low or no hydrogen-bond forming power. The estrogenic tests were carried out using ovariectomized rats, 4,4'-Dihydroxy benzylidene aniline produced a full estrogenic response in 12.5 mgm doses administered subcutaneously, and in 25γ doses intravaginally. None of the other derivatives produced an estrogenic response in 25-mg doses given subcutaneously. An investigation of other aspects of the general pharmacology of azomethines as well as the substitution of other ring linkages for the azomethine linkage in 4,4'-dihydroxy-diphenyl derivatives (e.g., $-\text{CO}-\text{NH}-$, $-\text{CH}_2-\text{O}-$, $-\text{SO}_2-\text{NH}-$, $-\text{CH}_2-\text{NH}-$, $-\text{S}-$, $-\text{S}-$, $-\text{CH}(\text{C}_2\text{H}_5)-\text{N}(\text{C}_2\text{H}_5)-$, $-\text{CO}-\text{CO}-$, $-\text{CH}(\text{OH})-\text{CO}-$, and $-\text{CO}-\text{O}-$) is now in progress.

Liver as the major organ involved in the detoxication of thiopental by the dog. A. R. KELLY (by invitation) and F. E. SHIDEMAN, Dept of Pharmacology, Univ of Michigan, Ann Arbor, Mich. Previous work from this laboratory, using *in vivo* methods, has established the liver as the major organ involved in the detoxication of thiopental in 3 animal species (mouse, rat, and man). *In vitro* experiments have also demonstrated the ability of both rat liver slices and mince to degrade this thiobarbiturate. Evidence presented here implicates the liver as the major site of detoxication of thiopental in the dog. One approach employed was a comparison of rate of disappearance from plasma of thiopental in normal dogs and in animals with reduced portal blood flow (Eck fistula). In both groups, plasma levels declined at the same rate for the first 8 minutes following injection of 20 mg/kg of the drug. At 2.2 mg % the curve for the Eck fistula animals leveled off and fell very gradually until the righting reflex was regained at 6½ hours at a level of 1.29 mg %. The normal curve dropped much more abruptly, the righting reflex returning 56 minutes after injection at approximately the same plasma level (1.2 mg %) as in the Eck fistula group. The second experimental method consisted of a comparison of plasma decay curves in the heart-lung, heart-lung-liver, and heart-lung-kidney preparation. The heart-lung preparation (morphine-chloralose anesthesia) showed a very gradually falling plasma thiopental curve, so that, after 70 minutes, approximately 25% of the drug had disappeared, over the same period of time, in the heart-lung-liver preparations, 2½ times as much was removed. Preliminary experiments indicate that heart-lung-kidney preparations may degrade thiopental to some extent although not nearly as rapidly as heart-lung-liver preparations.

Effects of podophyllotoxin and picropodophyllin on intact intestine and salivary secretion of non-

anesthetized dogs. MARGARET G. KELLY (by invitation), ALDO P. TRUANT (by invitation) and PAUL K. SMITH *Dept of Pharmacology, George Washington Univ School of Medicine, Washington, D C* A comparative study has been made of the pharmacological effects of podophyllotoxin and picropodophyllin (isomeric compounds obtained from podophyllin) on the intact intestine of non-anesthetized dogs, with Thiry-Vella fistulae. At the same time salivary secretion was investigated. The activity of the freed loop of the intestine was recorded by the classic rubber balloon and tambour method. Both compounds were injected intravenously in the form of a fine suspension in saline. Fifteen to 30 minutes after injection of 0.5 and 0.75 mg/kg of podophyllotoxin in dogs weighing 10 to 15 kg an irregular decrease in amplitude of contractions occurred, with a slight decrease in tone which at times increased. The rhythmicity of contractions remained normal. Normal gut activity returned in 3 hours at the above dose levels. At a dose of 5 mg/kg of picropodophyllin no effects were seen on the gut but salivation was increased. However, respiratory rate was intermittently interrupted by periods of dyspnea. When a dose of 7.5 mg/kg was used a regular increase in the amplitude of contractions was demonstrated with an increase in tone. In certain experiments intermittent quiescent periods occurred. In all the experiments with picropodophyllin, at the latter dose level, profuse salivation was seen which was blocked completely by 0.1 mg/kg of atropine. A dose of 0.5 mg/kg of atropine was not completely effective in blocking the gut effects of picropodophyllin. In a total of 20 experiments no catharsis was seen and no animals died.

Use of C^{14} in studies of metabolism of plant products. F. E. KELSEY *Dept of Pharmacology, Univ of Chicago, Chicago, Ill*. Some of the technical problems arising in a program of biosynthesis of radioactive drugs and their use in metabolic studies are discussed. A simple Geiger tube for the determination of the radioactivity of very weak samples is described. Briefly it consists of a bell-shaped tube, the open end of which is fitted with a sliding-tray arrangement for the insertion of the sample within the sensitive area of the counter. The counting gas, which flows continuously through the counter at atmospheric pressure, is helium saturated with ice-cold absolute alcohol. The available space on the sample holder is approximately 20 cm³. The absence of a window and the position of the sample permits the counting of all betaparticles escaping from the sample. High sensitivity is essential for this work because of the dilution of the C^{14} in the process of the biological synthesis of the drugs by the plant and the further dilution by the C^{12} of the experimental animal used for the metabolic studies. Methods of han-

dling the biological materials are given with illustrations from experiments on the metabolism of radioactive digitoxin.

Actions of calcium and tetraethylammonium ions on the rat diaphragm preparation. CHARLES J. KENSLE (introduced by McKen Cattell) *Dept of Pharmacology, Cornell Univ Medical College, New York City*. The rat diaphragm preparation stimulated through its phrenic nerve at rates of 5 or 30 per minute failed rapidly when placed in a calcium-free Ringer's solution but not when in potassium-, magnesium- or phosphate-free Ringer. The threshold concentration of calcium required for minimal activity varied from 1.5 to 6.5×10^{-4} M while that for maximum activity varied from 7×10^{-4} M to 1.2×10^{-3} M. High concentrations (2.5×10^{-2} M) of calcium depress activity. Tetraethylammonium bromide (TEA) has been found to substitute for calcium on a molar equivalent basis in restoring maximum activity in calcium-deficient solutions providing the calcium concentration has been adjusted to that required for minimal activity. TEA did not substitute for calcium at subthreshold levels and did not appreciably lower the calcium threshold. The sensitivity of the diaphragm preparation to the curarizing agents D-tubocurarine chloride (DTC) and dihydrobetaerythroidine HCl (HBE) was greatly increased by lowering the calcium concentration. Neostigmine was not an effective antagonist of DTC at low calcium concentrations. TEA antagonized the action of DTC and HBE and to a lesser extent the failure produced by acetylcholine following treatment with DFP. Triethylphenylammonium chloride showed slight antiscaric activity and calcium replacement value but triethylsulphonium iodide, triethylethanolammonium chloride, and dimethyldiethanolammonium chloride showed neither.

Reversible blocking action of DFP at the neuromuscular junction of the rat diaphragm preparation. CHARLES J. KENSLE and CHARLES BERRY (introduced by McKen Cattell) *Depts of Pharmacology and Anatomy, Cornell Univ Medical College, New York City*. A study of the action of DFP (diisopropyl fluorophosphate) on the rat phrenic nerve-diaphragm preparation (Bulbring, Brit J Pharmacol & Chemotherapy 1: 38, 1946) has shown that in low concentrations (5×10^{-5} M) it increased the response of the rat diaphragm to nerve stimulation at rates of 5 and 30/min, whereas higher concentrations (5×10^{-3} M) prevented the response. The high concentration of DFP caused failure within 2 minutes but this was readily reversed as long as one hour later. The bath temperature was 38°C. Measurement of the phrenic nerve action potential and the response of the diaphragm to direct stimulation localized this blocking action of DFP at the neuromuscular junc-

tion In the presence of low concentrations of DFP and after exposure to high concentrations, acetylcholine (1-2 micrograms/ml) 'curarized' the preparation The evidence obtained is inadequate to differentiate between the two most likely explanations for the reversibility of this block a) that the neuromuscular block produced by DFP is due to acetylcholine accumulation and that at least a functionally adequate amount of cholinesterase recovers its activity or b) that DFP in high concentrations possesses an action affecting neuromuscular transmission other than cholinesterase inhibition

Adrenolytic activity of a series of N-(9-fluorenyl)-beta-chlorethylamines JAMES KERWIN (by invitation), GLENN E ULLYOT (by invitation), EDWIN J FELLOWS, EDWARD MACKO (by invitation) *Research Div Smith, Kline and French Labs, Philadelphia, Penna, and Dept of Pharmacology, Temple Univ School of Medicine, Philadelphia, Penna* Substances with the following structure were compared with 'Dibenamine' for their effect after intravenous administration on the pressor action of epinephrine in cats anesthe-

R
|
R₁-N-CH₂-CH₂-Cl HCl

In all of the compounds R = the 9-fluorenyl radical Activity greater than that of 'Dibenamine' was noted only in the case of the compound in which R₁ was ethyl In those substances in which R₁ was methyl, propyl, isopropyl or secondary butyl, activity comparable with that of 'Dibenamine' was obtained The derivatives in which R₁ was n-butyl, isobutyl, n-amyl, isoamyl, n-heptyl, benzyl or phenylisopropyl were less active than 'Dibenamine'

Studies on radioactive digitoxin (*Digitals lanata*) T EUGENE KIMURA (by invitation) and E M K GELLING *Dept of Pharmacology, Univ of Chicago, Chicago, Ill* Radioactive digitoxin was biosynthesized by the incorporation of C¹⁴O₂ into *D lanata* by employing the method as reported by Geiling *et al* (Science 108 558, 1948) and by the concurrent use of a humidity-controller recently described (Science 108 560, 1948), while extraction of the glycoside was conducted in a manner substantially similar to that described by the authors Quantities of C¹⁴O₂, varying from a total of 600-1440 uc (300-180 uc/plant) were introduced in divided doses at suitable intervals into batches of *lanata* grown for 4-6 weeks Intensities of radioactivity from the crystallized glycosides in the order of 15,000 cpm/mg and 25,000 cpm/mg were registered from a 10-cm² surface, employing an internal Geiger counter (to be described elsewhere) when plants given 180 uc and 300 uc, respectively, were employed Preliminary studies on the perfused hearts of rats and guinea pigs indicate pres-

ence of higher levels of radioactivity localized in the ventricles than in the atria, the rat heart showing a greater degree of difference than the guinea pig Distribution studies have been carried out in the frog, guinea pig, and rat

Further studies on the pharmacology of adrenolysis THEODORE C KING (introduced by Theodore Koppányi) *Dept of Pharmacology, Georgetown Univ School of Medicine, Washington, D C* The hemodynamic responses of nembutalized dogs to epinephrine, DL-nor-epinephrine, and DL-N-isopropylarterenol (isuprel) (5-10 gamma/kg) were studied following administration of gradually increasing doses of yohimbine, benzylimidazoline (priscoll), dibenamine, and ergotamine Epinephrine effects are progressively decreased until complete abolition of the pressor response is obtained at the following dosage levels (mg/kg) yohimbine-0.75, priscoll-2.0, dibenamine-1.0 Reversal of epinephrine pressor effects does not occur until larger doses of these compounds are given (mg/kg) yohimbine-2.0, priscoll-10.0, dibenamine-10.0 Diminution but never complete inhibition or reversal of nor-epinephrine responses was observed with all doses cited Fifty per cent diminution of response to nor-epinephrine was observed with yohimbine-0.2, priscoll-0.4, dibenamine-3.0 (mg/kg) Isuprel effects showed 50% inhibition only following yohimbine (0.5 mg/kg) and priscoll (1.0 mg/kg) Isuprel effects were exaggerated by dibenamine Ergotamine tartrate (0.05 mg/kg or more) produced well over 100% potentiation of epinephrine and nor-epinephrine pressor effects Similar doses produced complete reversal of isuprel effects with pressor responses over 50 mm Hg Epinephrine responses potentiated by ergotamine are reversed only by larger than the minimum effective doses of priscoll or dibenamine

Pharmacological actions of two aliphatic amines THEODORE O KING (introduced by Theodore Koppányi) *Dept of Pharmacology, Georgetown Univ School of Medicine, Washington, D C* Cocaine HCl (5-10 mg/kg) administered intramuscularly completely abolishes or greatly inhibits pressor responses elicited by 2-methyl amino-6-hydroxy-8-methyl heptane (aranthol, Bilhuber-Knoll) (1.0-5.0 mg/kg) and by 2-methyl amino heptane (oenethyl, Bilhuber-Knoll) (0.02-0.10 mg/kg) In similar experiments in which dibenamine HCl (20 mg/kg) was used, complete abolition or marked (65-95%) inhibition of the pressor responses to aranthol and oenethyl were observed Cocaine was generally more effective than dibenamine in this respect so that when cocaine was used following dibenamine, further depression of the reduced responses were obtained In experiments in which dibenamine was used following cocaine, small pressor responses were

obtained with aranthol after dibenamine administration, even in cases where there had been complete abolition of pressor responses by cocaine. The effectiveness of aranthol in antagonizing hypotension due to spinal anesthesia (procaine) was tested in laminectomized dogs. Aranthol HCl (5 mg/kg) was administered after the mean arterial blood pressure fell to about 80 mm Hg. The injection of this amine produced pressor effects up to 50 mm Hg. The blood pressure then gradually fell to a level still 10 mm above the hypotensive minimum and remained at this level for 1½ hours. Aranthol was also effective in dilating the pilocarpine-constricted bronchial musculature. No tachypylaxis was observed when aranthol HCl (25 mg/kg) was injected intravenously at 5-minute intervals.

Heart sounds—electrostephone and recording machine (motion picture). HARRY KIRSCHBAUM (introduced by F. F. Yonkman) *Detroit, Mich.* This presentation deals with amplification and registration of cardiac sounds. It includes a description of the electrostephone and recording device. This device amplifies human fetal heart sounds and a permanent recording of the fetal and adult heart sounds can be made on wire, tape or film. By means of this instrument the response can be determined of both fetal and adult hearts to various pharmacologic agents. The machine has been used successfully in experimental research as well as in clinical surgery.

Cholinesterase as a cholinergic blocking agent. THEODORE KOPPANYI and ALEXANDER G. KARCZMAR (by invitation) *Dept. of Pharmacology, Georgetown Univ. School of Medicine, Washington, D. C.* Among the cholinergic blocking agents, atropine-like and curare-like drugs owe their activity to specific effects on acetylcholine receptors. They appear to act independently of the acetylcholine-cholinesterase chain reaction; in fact, some show an antiesterase rather than an anti-acetylcholine effect. The employment of a new purified, injectable, true cholinesterase (Special Chemicals Division, Winthrop-Stearns, Inc.) added a third type of cholinergic blocking agent to pharmacological technique (*Anat. Rec.* 101:36, 1948). This substance, upon intravenous injection, antagonizes both the muscarinic and nicotinic effects of injected acetylcholine in the intact mammal. The injection of cholinesterase into the turtle heart accelerates the rate and reverses other cholinergic effects obtained following the intracardiac injection of acetylcholine or of physostigmine. Cholinesterase injections, however, failed to prevent the cardiac effects of the faradic stimulation of the vagus. In cases where bradycardia produced by acetylcholine or physostigmine was abolished by cholinesterase, the vagus could not be paralyzed even by the intracardiac administration of massive

doses of this enzyme. The effect of cholinesterase on injected acetylcholine, and the comparative inefficiency of the enzyme in relation to vagus stimulation were observed also in dogs and cats. Unless it can be demonstrated that the enzyme does not penetrate to the site of acetylcholine liberation in the neuromuscular effector system, the above observation is of distinct theoretical significance.

Mechanism of action of anticholinesterase. THEODORE KOPPANYI *Dept. of Pharmacology, Georgetown Univ. School of Medicine, Washington, D. C.* Anticholinesterases potentiate the muscarinic and nicotinic effects of acetylcholine, the ganglionic effects of nicotine and the responses to cholinergic (including preganglionic) nerve stimulation. In larger doses they paralyze the sympathetic ganglia. This phenomenon is possibly independent of acetylcholine accumulation, because a) paralytic and antiesterase concentrations of individual drugs differ, b) nicotine pressor effects and pressor effects obtained on preganglionic stimulation are likewise abolished by paralytic doses of anticholinesterases, c) in some instances the paralytic effect of one antiesterase may be reversed by another, or even by the same anticholinesterase (*Science* 106:492, 1947). This latter effect may be related to the reversal by anticholinesterases of nicotine paralysis of autonomic (not solely sympathetic) ganglia. Antiesterases, however, differ in several respects from each other: a) Those of the physostigmine or neostigmine type prevent the pharmacological effects of DFP (intact animal). Acetylcholine pressor effects in atropinized and physostigminized animals are not further increased following administration of various doses of DFP. On the other hand, DFP and other phosphate esters have no such inhibitory effects on physostigmine or neostigmine, or on each other. b) The antiesterases differ as to potency, ganglionic paralytic dose, duration of action, and as to shape of curves, expressing the relationship between their concentration and the magnitude of the pressor effect of acetylcholine in atropinized animals. c) Some antiesterases appear to inhibit pseudo-cholinesterase but not true esterase. The assay of anticholinesterases used in this laboratory did not confirm this specificity. Their action is better explained in terms of the concept of 'essential cholinesterase' (*Milit. Res. Developm. Board* 4:271, 1948; *Bull. Johns Hopkins Hosp.* 83:532, 1948).

Pharmacologic and toxicologic data concerning diethylaminoethyl ester of 1-phenyl-cyclopentane-1-carboxylic acid hydrochloride (Parpanit). CHARLES P. KRAATZ (by invitation), CHARLES M. GRUBER, JR., HUBERT L. SHIELDS (by invitation) and CHARLES M. GRUBER *Dept. of Pharmacology,*

Jefferson Medical College, Philadelphia, Penna
 The LD₅₀ of parpanit has been determined as follows rabbits intravenously, 24.5 mg/kg, white mice intravenously, 45.1 mg/kg white mice intraperitoneally, 222.3 mg/kg, white rats intraperitoneally, 209 mg/kg. Toxic doses of the drug in these animals produce symptoms indicative of strong stimulation of the central nervous system, with death resulting ultimately from respiratory paralysis. In relatively non-toxic doses, parpanit has been found to have the following actions in experimental animals: a transitory vasodilatation independent of the central nervous system, a block of the terminal receptors of the parasympathetic-innervated structures rendering them insensitive both to nerve stimulation and applied acetylcholine, inhibition of activity of the intact intestine and depression of intestinal musculature, depression of the frog ventricle and excitation of the mammalian uterus. Comparative experiments have established that parpanit is similar qualitatively to traseratin which it resembles chemically, but is somewhat more active in inhibiting the intestine and in its parasympatholytic effect. Parpanit in the latter action is much less potent than atropine.

Effect of intravenous 'Tween' solutions in various animals and man JOHN C. KRANTZ, JR. and JOSEPH G. BIRD *Dept. of Pharmacology, Univ. of Maryland School of Medicine, Baltimore, Md*
 Polyoxyethylene derivatives of sorbitan esterified with fatty acids such as oleic, lauric and stearic, are used as dispersing agents for fatty substances under the name of 'Tweens'. We have observed that these substances when injected intravenously into dogs (in dilute solution) and other species of the canine family, produce a prolonged fall in blood pressure, which presumably is due to the release of histamine or some histamine-like substance. In other species of common laboratory animals this condition does not obtain, nor has it occurred in a limited number of tests in man.

Uptake of bromsulphthalein by the liver of the rat II. Studies with radioactive bromsulphthalein (BSP) JOHN KREBS (by invitation) and RALPH W. BRAUER *Dept. of Pharmacology and Experimental Therapeutics, School of Medicine, Louisiana State Univ., New Orleans, La*
 Bromsulphthalein containing S³⁵ has been prepared by the reaction of phenoltetrabromphthalein with H₂SO₄ containing S³⁵. The material has been purified by chromatographic adsorption, one milligram of the end product contains 0.1 millicurie of S³⁵. The rate of exchange with inorganic sulfate of the —S³⁵O₃ attached to the dye in aqueous solution, in tissue slice experiments, and *in vivo* is unimportant quantitatively if the experiments are completed within 3 hours or less, the exchange rate under these various conditions being of the order of 5% per hour or less. Radioactive BSP has been used in

a study of the release of BSP taken up by liver slices against very low concentrations of BSP in the circumambient fluid. Also the exchange of tagged BSP taken up by liver slices against various BSP concentrations in the medium has been studied. Results to-date indicate that the BSP taken up by liver slices falls into two fractions, one of which is released or exchanged at a rapid rate, while the second fraction, larger in amount, is released at a constant rate independent of the intracellular concentration. The dye was administered intravenously to a series of rats whose abdomen was prepared for quick laparotomy. Five minutes after the administration of the dye the livers were frozen by liquid air, transferred to a freezing microtome, and sections cut for radioautographic studies in a cryostat at -20°C. A stripping film technique was used for the preparation of the radioautographs. Results to-date confirm early conclusions that the uptake of BSP does not involve the Kupffer cells but proceeds directly from the plasma into the hepatic cells.

Quiescence preceding peristaltic constriction in the ileum HUGO KRUEGER *Dept. of Zoology, Oregon State College, Corvallis, Ore*
 Subcutaneous injection of morphine and its derivatives in adequate doses usually initiates peristalsis in Thiry-Vella loops of the ileum in dogs. Activity of the lower ileum was recorded once weekly from a Brodie bellows connected to a tandem, double-chambered, water-filled balloon inserted into the lumen of the loop. The pressure used to distend the chambers of the balloon was 31 cm. of water. Drugs were administered after a 100' control period. The advancing constriction of peristalsis, as described by Bayliss and Starling, is preceded by an advancing area of inhibition. In our experiments, the inhibition usually involved both a relaxation of the ileal muscle and a complete cessation of rhythmic contractions. Occasionally rhythmic contractions did not cease but were reduced in magnitude. The duration of the period of complete quiescence was 10 seconds or less in 731 out of 3057 measurements obtained from 2012 waves recorded during 536 experiments on 33 animals, 1453 waves had a period of complete quiescence between 10 and 30 seconds, 744 between 30 and 60 seconds and 129 were over one minute in duration. The longest period of quiescence measured was 290 seconds. Thus a quiescent period was questionable or negligible in 24% but was easily appreciable in 76% of the observations. The quiescence usually developed while the ileal muscle relaxed prior to the arrival of the advancing area of constriction but quiescence, prior to the arrival of the constricting ring, often occurred without relaxation of the ileal muscle, especially if the ileum was already nearly maximally relaxed.

***In vivo* action of morphine, urethane and pheno-**

barbital on the glycogen synthesis from glucose in the rat liver ERNEST KUN and L G ABOOD (introduced by E M K Geiling) *Dept of Pharmacology, Univ of Chicago, Chicago, Ill* Starved rats were injected with varying doses of morphine, urethane and phenobarbital one hour before glucose administration by stomach tube, and the liver glycogen and carbohydrate intermediates were measured 5 hours later. While normal starved rats synthesized an average of 36 mg glycogen/gm of liver in 5 hours after glucose administration, 100 mg/kg morphine or 1 mg/kg urethane completely inhibited glycogen deposition. Phenobarbital in 100 mg/kg doses produced 58% inhibition. The degree of inhibition was a function of the dose. Morphine proved to be the most potent inhibitor, 10 mg/kg causing 90% inhibition. Analyses for phosphorylated carbohydrate intermediates in drug-treated rat livers revealed a complete inhibition of esterification of inorganic P. Inorganic P was completely esterified in normal animals receiving glucose while none was esterified in the drug-treated ones. The quantitative distribution of the intermediates varied depending on the drug studied. A marked decrease in nucleoprotein phosphorus (0.1-0.2 mg/gm compared with the normal of 1.5 mg/gm) and phospholipid phosphorus (0.075-0.150 mg/gm compared with normal values of 0.9-1.1 mg/gm) was observed in the drug-treated animals.

Guanidine the agent responsible for the convulsive response to intracisternally injected streptomycin S KUNA (by invitation), F CUCHE (by invitation) and H MOLITOR *Merck Inst for Therapeutic Research, Rahway, N J* In an attempt to determine whether the convulsive response caused by streptomycin on intracisternal injection in rabbits was due to an intrinsic property of the streptomycin molecule, or to an impurity, the following investigation was undertaken. Testing individual components of this molecule indicated that guanidine was itself capable of causing this response. It was further demonstrated that streptomycin, streptidine or acetamidine caused like degrees of convulsive response when administered on the basis of their guanidine content. It was thus evident that the convulsive response produced by intracisternal administration is due to the guanidine content in the molecule. Impure streptomycin administered intracisternally on a unit basis caused more severe convulsions than the same amount of pure streptomycin similarly administered. Analyses of several lots of streptomycin of varying purity showed that on a unit basis, impure streptomycin contained a larger amount of guanidine than relatively pure streptomycin. When these lots were administered intracisternally in rabbits on the basis of their guanidine content, comparable convulsive responses were obtained. It is thus

evident that the extra guanidine present as impurity or as degradation products in low potency streptomycin is responsible for the greater convulsive response observed following intracisternal injection of such material.

Storage of DDT in the fat of the rat FRIEDA KUNZF (by invitation), A A NELSON, O G FITZHUGH and EDWIN P LAUG *Div of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D C* Answers to the following questions were sought: 1) Is there a level of DDT intake in the diet at which no storage in the fat occurs? 2) What is the relationship between length of exposure to DDT and storage in the fat? 3) How rapidly does DDT disappear from the fat after exposure is terminated? 4) What is the relation between age and rate of DDT accumulation in fat? 5) What is the lowest level of intake of DDT at which pathological changes may be seen in the liver? Answers to these questions are as follows: 1) Accumulation of DDT in the fat occurs at every level of intake down to and including 1 ppm. 2) With time, there is a progressive rise in storage, reaching a maximum at 23 weeks. This period of time necessary to reach maximum storage is independent of the level of intake within the range of 1-10 ppm. 3) Fifty per cent of the DDT store still remains after one month on a DDT-free diet, 25% after 3 months. 4) Comparison between groups of weanling and 1-month-old rats revealed identical rates of DDT accumulation, in spite of the fact that the consumption of DDT on a per kg body weight basis is considerably greater in the younger animals. 5) Evidence of liver injury has been noted in rats consuming diets containing 5 ppm DDT for 4-6 months.

Beryllium poisoning II One-year studies by intratracheal injection C W LABELLE, P MORROW, C BOOTH, F WESCOTT, G. MELVILLE (introduced by H C Hodge) *Dept of Radiation Biology, Univ of Rochester, Rochester, N Y* A study has been made on a total of 325 rats and 18 rabbits exposed to beryllium, with suitable controls, plus smaller numbers of mice, guinea pigs, monkeys and dogs. Each of these animals received beryllium by intratracheal injection, usually as an insoluble compound, at levels ranging from 5 to 100 mg Be/kg. Data are presented showing the resulting growth, mortality, hematology (10 variables), urine chemistry (15 variables), gross organ changes, organ weights (5 organs) and histological changes, and lung beryllium content over a period of one year after the injection. A chronic type of lesion developed in the lungs of most of the exposed animals which was consistent within a given species and for a given compound, although in no case identical with the human chronic lesion. During the same period, most of the commonly measured variables in the blood and serum remained normal.

The outstanding changes were increases in blood platelets and the absolute polymorphonuclear content of the circulating blood

Bronchodilator action of butanol derivatives

A M LANDS, O H SIEGMUND (by invitation) and ESTELLE ANANENKO (by invitation) *Biology Div, Sterling-Winthrop Research Inst, Rensselaer, N Y* Bronchodilator action was determined in isolated perfused guinea pig lungs. The doses, in micrograms, required to abolish histamine constriction were Butaneprine, 1-(3,4-dihydroxy-phenyl)-2-amino-1-butanol-120, isopropyl analog, 1-(3,4-dihydroxyphenyl)-2-isopropylamino-1-butanol HCl-50, cyclopentyl analog, 1-(3,4-dihydroxyphenyl)-2-cyclopentylamino-1-butanol HCl-10 epinephrine-5, Isuprel-15. Results obtained in guinea pigs with nebulized histamine are shown below

Drug	Dose mg/kg	Effect %
Butaneprine	0.5	78
Isopropyl analog	0.02	108
Cyclopentyl analog	0.05	116
Isuprel	0.01	118
Epinephrine	0.02	147

The cardiovascular action of these butanol derivatives is much weaker than that of Isuprel. The Isuprel ratios for action on heart rate were Butaneprine—300, isopropyl analog—30, cyclopentyl analog—60. If the bronchodilator action is compared with the effect on the heart rate, the greatest therapeutic margin is obtained with the cyclopentyl derivative and with the isopropyl derivative > Butaneprine. The most effective bronchodilator derivatives are effective spasmolytic agents for the intestine and uterus. Acute toxicity was determined by intravenous injection into albino mice. The LD₅₀ values in mg/kg were Butaneprine—117, isopropyl analog—57, cyclopentyl analog—84, epinephrine—27, Isuprel—77.

Alteration of the pH of the blood as a means of improving experimental cardiac anoxia KURT LANGE, VICTOR TCHERTKOFF, FRANK GRAIG and DAVID WEINER (introduced by M G Mulinos) *Dept of Medicine, New York Medical College, New York City* The anoxic pattern found in E K G's of dogs and rabbits following exposure to lowered oxygen tension in the inspired air or to cold environment can be reversed by the administration of acids lowering the pH. Moreover a shift of the blood pH to the acid side prevents the appearance of the anoxic pattern of the E K G of animals in an anoxia test. These effects are due to an increased oxygen dissociation with subsequent increase in oxygen available to the tissues. Normal animals do not show a change in their E K G pattern with lowering of the pH of the blood within reasonable limits. They do show an anoxic pattern in the

E K G when their blood is shifted to the alkaline side. Calcium, phosphorous, sodium and potassium do not play a role in the reversal of the anoxic E K G. The pH shifts required to produce beneficial effects need only be slight and appear to be well within the range of safety.

Electroshock seizures in the hamster and their modification by antiepileptic drugs EDWARD H LANPHIER (introduced by Carl C Pfeiffer) *Dept of Pharmacology, Univ of Illinois College of Medicine, Chicago, Ill* Application of electrical stimuli (60 cycle, 0.3 sec) via electrodes placed in the buccal pouches of the hamster proved rapid and advantageous in producing convulsions. Individual mean thresholds ranged from 25.0 to 50.5 ma with a mean of 36.5 ma. The average standard deviation in a series of daily determinations amounted to 6.3% of the average mean. The coefficient of correlation between weight and threshold proved to be +0.754, and useful predictions of threshold on the basis of weight could be made. Mortality in the hamster was 0.44% compared to 19% in the mouse. Clonic activity in maximal and sub-maximal seizures is much less prominent than in the mouse and rat, but modification of threshold and seizure pattern by antiepileptic drugs does not differ significantly. The hamster is thus a useful and convenient experimental animal for electroshock studies.

Effect of chemical configuration on the edema-producing potency (EPP) of acids, aldehydes, ketones and alcohols P S LARSON, J K FINNEGAN and H B HAAG *Dept of Pharmacology, Medical College of Virginia, Richmond, Va* The method used for measuring edema production is a modification of one that we have previously described (J Pharmacol 89:115, 1947). Morphinized male albino rabbits are tied in a supine position. A pocket is made of the right eye upper lid and kept filled with a solution of the irritant for 3 minutes. One hour later the rabbit is killed, the upper palpebral conjunctiva of each eye excised, weighed, dried and reweighed. The ratio of moisture to dry weight for each membrane is calculated and the difference between the ratios represents the degree of edema production. For each irritant studied, the molar concentration needed to produce a 2.5 unit increase in moisture to dry weight is determined. With monobasic organic acids, excepting formic, EPP increases with increasing molecular weight. Corresponding dibasic acids may have a lower EPP while unsaturation may increase EPP. HCl has no greater EPP than the more weakly ionized monobasic organic acids. With saturated aldehydes, excepting formaldehyde, increasing molecular weight results in increasing EPP. Unsaturation may markedly increase the EPP but this effect decreases with increasing molecular weight. Insofar as solubility permitted testing, it

appears that with ketones the EPP increases with increasing molecular weight. Among primary alcohols, EPP increases markedly with increasing molecular weight, decreases with unsaturation, and appears to be unaffected by isomerism. In passing from primary to secondary to tertiary alcohols, the EPP progressively declines.

Species variation in the protective action of anesthesia against the acutely lethal effect of nicotine. P. S. LARSON, J. K. FINNEGAN, J. CONWAY BIBB (by invitation) and H. B. HAAG, *Dept of Pharmacology, Medical College of Virginia, Richmond, Va.* The protective action of anesthetics against death from acute intravenous administration of nicotine was first noted by Franke and Thomas (*J Pharmacol* 48:199, 1933), who found that the smallest dose that was generally fatal for etherized dogs was 5 mg/kg as compared to 3 mg/kg for unanesthetized dogs. Subsequently Gold and Brown (*J Pharmacol* 54:463, 1935) found that after barbital slightly more nicotine is required to cause death in cats than in the normal animal, but that the increment is less than two-fold. In the present report, we have extended these observations to include other species. Male albino rabbits, rats and mice were used. Two intravenous LD_{50} determinations for nicotine were made on each species, one on unanesthetized and one on anesthetized animals. At least 3 groups of from 6 to 25 animals were used in each determination. The rabbits were anesthetized with sodium amytal (45 mg/kg, intravenously), the rats and mice with dial (60 and 100 mg/kg respectively, intraperitoneally). The results are summarized in the

(SY28) have been studied in rabbits. The drugs were applied to rectangular areas (1.25 x 12.5 cm) of the skin of the abdomen and repelled through the skin by a current density of 0.33 milliamperes/cm² applied for 5 minutes. SY 28 was applied in total doses of 0.02-0.4 mg, whereas Dibenamine was used in doses of 0.4-1.6 mg so as to produce equivalent blocking. The intensity of vasoconstriction following the intracutaneous administration of epinephrine (1:1000-1:40,000) was used as the indicator mechanism to reveal adrenergic blocking activity. The penetration of both SY-28 and Dibenamine was expedited by ion transfer and resulted in adrenergic blocking action at significantly lower dosage levels. SY-28 was found to be 10 to 20 times as potent as Dibenamine under the conditions of these experiments. Evidence of the epinephrine-reversal phenomenon has been noted in the rabbit skin following the local or intravenous administration of both SY-28 (0.1-4.0 mg/kg) and Dibenamine (4-32 mg/kg). These observations reveal the presence of vasodilator fibers in the skin of the rabbit.

Chronic toxicity of phenylmercuric acetate and mercuric acetate in the rat. EDWIN P. LAUG, O. G. FITZHUGH, FRIEDA KUNZE (by invitation), and A. A. NELSON, *Div of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.* At the end of a 2-year feeding experiment, in which phenylmercuric acetate and mercuric acetate were fed to rats at levels of 0.1 ppm to 160 ppm mercury in the diet, the following observations were made: 1) Phenylmercuric acetate is considerably more toxic than mercuric acetate on the basis of mortality and growth effect. Thus reduction in growth was noted on 10 ppm phenylmercuric acetate, but only at 160 ppm mercuric acetate. 2) Diets containing phenylmercuric acetate in the range of 0.5 to 10 ppm mercury, support approximately 20 times the storage of mercury in the kidney than comparable levels of mercuric acetate. 3) Phenylmercuric acetate produced gross effects on the kidneys, such as pitting and hypertrophy. These effects were evident at dietary levels as low as 2.5 ppm. 4) Injury to the kidney tubules, more pronounced in the females than the males, could be demonstrated at a level of 0.5 ppm for phenylmercuric acetate. This was $\frac{1}{10}$ of the level of mercury intake at which comparable damage could be demonstrated with mercuric acetate. 5) Urinary excretion studies show that on diets containing phenylmercuric acetate the excretion of mercury is 10 to 20 times greater than that on diets containing mercuric acetate. Conversely, the fecal excretion with mercuric acetate is 3 times as great as with phenylmercuric acetate. These facts are taken to indicate that the increased toxicity of phenylmercuric acetate arises from its superior absorption into the organism.

Species	$LD_{50} \pm S.E.$ for nicotine (mg/kg)	
	Unanesthetized	Anesthetized
Rabbit	5.9 \pm 0.6	9.4 \pm 0.2
Rat	2.8 \pm 0.3	27.5 \pm 1.7
Mouse	0.55 \pm 0.01	7.1 \pm 0.7

accompanying table. In rabbits, anesthesia raised the LD_{50} value for nicotine about 60%, which is in keeping with the slight increase reported for dogs and cats. However, in rats and mice, anesthesia elevated the LD_{50} values 10- and 13-fold respectively. Thus a marked species variation exists in the protective action of anesthesia on the acutely lethal effect of nicotine.

Cutaneous administration of adrenergic blocking agents by ion transfer. JULES H. LAST, ARTHUR A. RODRIGUEZ (by invitation), and ISADORE PITESKY (by invitation), *Depts of Pharmacology and Physical Medicine, Univ of Illinois College of Medicine, Chicago, Ill.* The local adrenergic-blocking effects of N,N-dibenzyl- β -chloroethylamine (Dibenamine) and N-ethyl-N-(1-naphthylmethyl)- β -bromoethylamine hydrobromide

A practical classification system for drugs C D LEAKE *Pharmacology Laby, Univ of Texas Medical Branch, Galveston, Texas* Aristotle emphasized the importance of classifying diverse concepts as a necessary prerequisite to their systematic consideration. Classification is accomplished logically by abstraction and analogy. Neither of these has been encouraged in the properly skeptical and critical development of modern experimental biology and medicine. With the tremendous accumulation of diverse factual concepts in such a science as pharmacology, a working classification is becoming increasingly necessary if the science is to be successfully taught or further explored. A classification of drugs on a chemical basis is not at present practical because the relationship between chemical constitution and biological activity is not yet satisfactorily developed, and does not broadly relate to the primary purposes of drugs. Such a classification of drugs as might be based on localization of biological activity, as proposed by H H Meyer, and later by Lamson, is also impractical because it is overly complicated, repetitive, and confusing in clinical practice. Indeed, the most satisfactory practical drug classification is one based on their chief use as a result of direct cumulative clinical experience. Such a classification is pedagogically helpful as has been well shown wherever it has been tried. It affords a systematic arrangement which aids the clinician and the researcher who may be trying to develop new drugs of clinical value or to improve on those already in use. Such a classification would conveniently follow clinical concepts in 1) drugs used mostly for diagnosis of disease, such as halogenated phenolphthaleins for liver function tests, 2) those directed most commonly to the prevention of disease, as vitamins, antiseptics, insecticides and rodenticides, 3) those employed primarily for the cure of disease ('chemotherapy'), as quinine in malaria and penicillin in syphilis, and 4) those drugs ordinarily found helpful in the alleviation of the symptoms of disease. For further convenience the latter may be subdivided into groups on the basis of the chief systems of the body involved with respect usually to stimulation or depression. Within these general groups further classification may advantageously be made on the basis of the mechanism of the biological action of the drugs concerned.

A warm wire analgesimeter R E LEE (by invitation), H L WILLIAMS (by invitation), and CARL C PFEIFFER *Dept of Pharmacology, Univ of Illinois College of Medicine, Chicago, Ill*. A simple apparatus to produce graded painful stimuli has been constructed. It consists of a stimulating point made of a 7 cm length of 26-gauge nichrome wire, bent in half to form a sharp-angled tip, connected in series to a dry cell and a potentiometer of 2 ohms resistance and a D C ammeter graduated

in 0.02 amperes reading up to 1.5 amperes. The temperature of the tip of the wire at ammeter readings of 0.4 to 1 has been calibrated with a thermocouple. Increases of 0.05 amperes result in an increase in temperature of 3 to 4°C. A temperature of 50°C constitutes a painful stimulus for most individuals. By use of the stimulator on the forehead it has been possible to determine the rise in pain thresholds in human subjects treated with analgesic drugs. The instrument has also been used successfully to determine the degree of analgesia produced by morphine sulfate (5 mg/kg) in mice. More quantitative results are obtained by this device than by the usual tail-pinch method. The device is also of teaching value to demonstrate delayed pain and to test for the duration of action of local anesthetics.

Pharmacology of thiomerin ROBERT A LEHMAN and E E KING (by invitation) *Dept of Therapeutics, New York University College of Medicine, New York City*. Thiomerin (di-sodium salt of N-[gamma-carboxymethylmercaptomercuri, beta-methoxy] propylcamphoric acid) is a mercurial diuretic obtained by replacement of the theophylline in mercuzanthin (Mercuriophylline, U S P XIII) by sodium mercapto-acetate to give a compound of the $-\text{CH}_2-\text{Hg}-\text{S}-$ type rather than $-\text{CH}_2-\text{Hg}-\text{N} <$. It was previously shown that in thiomerin (MT6) the acute cardiac toxicity characteristic of the conventional theophylline-bearing diuretics has been virtually eliminated at least with respect to the anesthetized cat (Proc Soc Exper Biol & Med 64 428, 1947). The present report concerns other pharmacologic properties of thiomerin as contrasted with presently used drugs. Intravenous lethal doses in the mouse have been estimated for thiomerin, mercuzanthin, mercurhydrin and salyrgan-theophylline and were found to be of the same order of magnitude when observed over a 4-day period. However, within the first half hour after injection, thiomerin is approximately $\frac{1}{10}$ as toxic as the other 3 drugs. Similar ratios were obtained using the Langendorff isolated cat heart. Absorption was estimated by analysis of injected muscle in rabbits and the absorption curve for thiomerin appears to resemble closely the curves previously reported for mercuzanthin and salyrgantheophylline (J Pharmacol & Exper Therap 62 26, 1938). The rate and completeness of excretion of mercury after administration of the diuretics was studied in cardiac patients. No striking differences were found. Storage of mercury in the kidney and liver after chronic administration in experimental animals has also been investigated. Data will be included on the fate of the sulfhydryl group as well as that of the mercury and a mechanism will be suggested to explain the pharmacologic activity of a mercurial diuretic in which

dissociation has been suppressed by mercaptide formation

Relative merits of commonly used sulfonamide drugs as components of mixtures DAVID LEHR *Dept of Pharmacology, New York Medical College, Flower and Fifth Avenue Hospitals, New York City* At a daily dosage of less than 4 gm, even the 'worst offender' sulfathiazole did not usually produce sensitization (D Lehr, Brit Med J 2 543, 1948) It follows that the renal protection of sulfonamide mixtures could be enhanced without the danger of increasing the incidence of allergic reactions, by using additional components in proportionately smaller amounts It can be assumed as established that, at present, sulfadiazine and sulfamerazine qualify for first and second place, respectively, as mixture components Sulfathiazole, sulfapyrazine, sulfamethazine and sulfacetimide deserve attention as possible 3d or 4th members Sulfathiazole was eliminated from the U S P because of its high clinical toxicity However, mixtures containing partial amounts of this drug demonstrated a low overall toxicity Sulfapyrazine, most insoluble of all compounds, induces frequent renal and allergic reactions and therefore offers no advantages over sulfathiazole Sulfamethazine possesses adequate solubility and low toxicity but is subject to substantial conjugation and plasma binding which diminish its therapeutic efficiency Sulfacetimide retains the high solubility of sulfanilamide and shares with the parent compound the improbability of renal concretum formation and the ready diffusibility into all tissues It shows low figures for conjugation, plasma binding and sensitization Recommended primarily against ocular and urinary infections, this drug demonstrates satisfactory bacteriostatic activity against a variety of human pathogens and gives good blood levels in man In experimental animals, sulfacetimide proved about 5 times less toxic than sulfadiazine The mixture sulfadiazine-sulfamerazine-sulfacetimide (equal amounts) revealed low toxicity in animals as well as excellent bacteriostatic activity *in vitro* and in experimental infections of mice After oral administration, blood and spinal fluid levels were similar to those from equal doses of sulfadiazine or sulfamerazine Preliminary clinical trials including several cases of pneumonia and two cases of penicillin resistant septicemia gave highly satisfactory results

Sodium phthalyl sulfacetimide A new gastrointestinal antiseptic DAVID LEHR *Dept of Pharmacology, The New York Medical College, Flower and Fifth Avenue Hospitals, New York City* Sodium phthalyl sulfacetimide (di-sodium N¹ acetyl N¹ phthalyl sulfanilamide di-hydrate) possesses good bacteriostatic activity against coliform organisms *in vitro* and in man In addition it displays satisfactory activity against many bacterial patho-

gens of the intestinal tract, especially the Shigella group (H Seneca and E Henderson, personal communication) The compound is readily water soluble and stable in the alkaline range of the body Spontaneous hydrolysis at the N¹ group remains negligible down to a pH of 6.0 A 20% aqueous solution of the drug has a pH of 7.30 After oral administration in rabbits, sodium phthalylsulfacetimide was present in aqueous solutions of 1500 to 3000 mg % strength in the small and large intestines, whereas the equimolar amount of sodium sulfadiazine, as representative of absorbable sulfonamides, was dissolved in the enteric contents only to the extent of 70 to 400 mg % Yet absorption of sodium phthalylsulfacetimide into the blood stream was minimal although it diffused readily into the deeper strata of the intestinal wall and produced at this site concentrations more than twice as high as the equimolar dose of sodium sulfadiazine From parenteral routes of administration in rabbits and rats sodium phthalylsulfacetimide readily entered the blood stream and was rapidly eliminated by the kidneys, causing high urinary concentrations The intraperitoneal toxicity was surprisingly low (approximate LD₅₀ for albino rats being 10 gm/kg body weight) In man a single oral dose of 10 gm sodium phthalylsulfacetimide did not produce any demonstrable blood levels and only about 1% of the dose appeared in the urine within 24 hours Therapeutic trials in acute gastrointestinal infections seemed encouraging and deserve further exploration

Tolerance in rats to new synthetic analgesic drugs JOHN R. LEWIS (introduced by A. M. Lands) *Biology Div, Sterling-Winthrop Research Inst, Rensselaer, N. Y.* The development of tolerance to the analgesic action of morphine and several compounds chemically related to Demerol or methadone was followed in rats The compounds investigated and respective doses used were morphine sulfate (10 mg/kg), 1-methadone hydrochloride (2 mg/kg), 1-isomethadone hydrochloride (3 mg/kg), ketobemidone or 1-methyl-4-(3-hydroxy-phenyl)-4-piperidyl ethyl ketone hydrochloride (3 mg/kg) and a sulfone derivative, 1-ethyl 1,1-diphenyl-3-dimethylaminobutyl sulfone hydrochloride (3 mg/kg) Data from a group of 20 rats were obtained for each compound These rats were given daily subcutaneous injections for 6 weeks and their analgesic reactions and body weights determined Tolerance developed most rapidly to the sulfone derivative and least to 1-isomethadone The development of tolerance to all the other compounds was quite similar and at the end of 6 weeks of daily administration the analgesic response was found to be only about 40% of the initial value As tolerance developed there was a decrease in duration of action which was probably related to the decrease in maximum effect There

was a general change in the character of the side-effects from depression to excitation following drug injection during the development of tolerance. After withdrawal, the only symptom observed was hyperexcitability which reached its maximum in about 48 hours. Recovery from the tolerance was least with ketobemidone and the sulfone derivative. With the other compounds, responses of about 90% of normal were obtained at 3 weeks after withdrawal. The daily administration of these compounds caused a decreased rate of growth which was probably due to a decrease in food consumption. Morphine caused the greatest and ketobemidone the least retardation of growth.

Screening test for radiopaque drugs. A. LIGHT (by invitation), P. B. DEWS (by invitation), J. TORNABEN (by invitation), E. H. LANG (by invitation), STATA NORTON (by invitation), and EDWIN J. DE BEER. *The Wellcome Research Labs., Tuckahoe, N. Y.* The methods currently used for the screening of compounds as possible cholecystographic agents usually require comparatively elaborate experiments on such animals as rabbits or dogs. Consequently, only limited numbers of animals may be studied. A convenient test employing the albino mouse has been developed. This test permits the use of large numbers of animals and the technique is simple because the mouse gall-bladder is not embedded in the liver and may be removed easily. Furthermore, only small amounts of drug are required. In performing the test, groups of mice are dosed orally with the drug being studied and, after a suitable time interval, are anesthetized with Evipal and killed. The gall-bladders are removed, pooled and dissolved in alkali, and the iodine is determined chemically. Dosage-response curves with standard drugs were obtained and the test has been employed for routine assays. The method has been adapted to other problems such as the screening of iodinated compounds for possible pyelographic properties.

A method for rapid graphic solution of time-percentage effect curves. JOHN T. LITCHFIELD, JR. *Chemotherapy Div., Stamford Research Labs., American Cyanamid Company, Stamford, Conn.* A method for rapid graphic solution of time-percentage effect curves has been developed. The method is derived from the provisional graphic solution described by C. I. Bliss (*Annals Applied Biol.* 24: 815, 1937). In his method, times are converted to logarithms and cumulative percentage effects to probits. These data are plotted, a straight line fitted by eye, and the parameters and errors of the line obtained by means of formulae given. Although this method of solution is relatively easy in the case of a complete curve (all individuals reacting), it becomes much more difficult when the curve is incomplete due to failure of some individuals to react. In the new method, which uses the data in

original form, and thus avoids the log-probit transformation, the data are plotted on logarithmic-probability paper, a straight line is fitted and the parameters are obtained from this line. Confidence limits of the parameters for 19/20 probability are obtained without computation by means of nomographs. When the curve is complete, only one of the nomographs is needed but when the curve is truncated, two additional nomographs are used. A complete solution may be made in any case in less than 5 minutes, without the use of a slide rule or calculating machine. The results are equivalent in all respects to those obtained by the more complex basic method. The method and nomographs are presented with several examples of their use.

Comparative toxicities of five substituted glycerol ethers. PRUDENCE C. LOEB (introduced by Charles H. Hine). *Div. of Pharmacology and Exptl. Therapeutics, Univ. of California Medical School, San Francisco, Calif.* Systemic toxicity after intragastric, respiratory and percutaneous absorption and topical effects from application to the eye and skin, and sensitization of skin were studied. The acute, intragastric LD₅₀'s (ml or gm/kg) in inbred white mice were: α -Ethyl (I) 9.35 ± 0.134 , α -Isopropyl (II) 8.2 ± 0.135 , α -Allyl (III) 4.2 ± 0.084 , α -Phenyl (IV) 2.65 ± 0.025 , α, γ -Diisopropyl (V) 1.86 ± 0.058 . Loss of righting reflex followed by reversible muscular paralysis was noted with all the compounds. Non-paralyzing doses produced no sensory depression. Death followed depression of respiration, probably of central origin. The vapor pressures are extremely low, and exposure of mice to vapor-saturated air at room temperature 8 hours daily for 10 days produced neither irritation nor significant mortality. Mist exposure for one hour produced slight temporary respiratory irritation in hamsters. Only V produced systemic toxicity after cutaneous application. II produced mild primary irritation of intact shaved rabbit skin and moderately severe irritation of abraded skin, III and V caused mild primary irritation of abraded skin. Both alcohol and glycerine produced greater irritation. V caused mild erythema of rabbit skin after seven daily applications of 2 ml/kg. Twenty such applications of I, II, and III were not irritating. Similar applications of 10 ml/kg (5% aqueous solution) of IV discolored the skin of rats, but did not cause irritation. II and V each caused mild transient irritation when instilled into the rabbit's eye, III caused moderate irritation. Guinea pigs were not sensitized by any compounds. The acute toxicity of the glycerol ethers is greater than that of the glycols.

Comparison between individual sensitivities to histamine and to diphenhydramine. S. LOEWE, L. A. WOODBURY (by invitation) and SUSANNE LOEWE PUTTUCK (by invitation). *Depts. of Phar-*

macology and Physiology, Univ of Utah School of Medicine, Salt Lake City, Utah When male guinea pigs inhaled nebulized histamine under uniform conditions, individuals differed little in 'histamine time' (T_H = inhalation time to a set endpoint) In 3 populations totaling 228 guinea pigs, mean T_H s were 79, 80 and 83 seconds, with coefficients of variation (C V) of 12, 23 and 17% T_H did not vary consistently with body weight When, however, the I P D₅₀ of diphenhydramine [median individual preventive (intraperitoneal) dose prolonging T_H to 390 seconds in 50% of experiments] was determined, individuals varied greatly (mean I P D₅₀ of 75 animals 66 mg/kg, C V 68%) Variation with body weight was also great, with body weights increasing from 230 to 550 gm, mean I P D's rose from 2.6 to 8.9 mg/kg In contrast, each individual's dose-response curve approximated an all-or-none curve 1) Sensitivities to histamine and to the antihistaminic are incongruous, this challenges the assumption of the same receptor substance for agonist and antagonist 2) Each individual is constant, but different individuals vary greatly in diphenhydramine response This strongly advocates the use of intraindividual methods (Science 106:89, 1947) of antihistaminic potency evaluation, to avoid the large standard errors of the group P D₅₀ assays For example, in intraindividual assays, C V's of the potencies (diphenhydramine = 1.0) of tripeleminamine (5.18), C-5581 H (1.75) and Antistin (0.48) were 2.7, 5.4 and 4.8%, respectively

Circulatory and other actions of sparteine in mammals Go Lu (introduced by P J Hanzlik) *Dept of Pharmacology and Therapeutics, Stanford Univ School of Medicine, San Francisco, Calif* The predominant effects of sparteine sulfate (5-20 mg/kg) intravenously in anesthetized (pentobarbital or morphine ether) and atropinized dogs and cats were bradycardia, sustained fall of blood pressure (varying with dosage), absence of central vasomotor depression Doses of 1 mg/kg sometimes caused fleeting opposite cardiac and blood pressure effects Subcutaneous, intramuscular and gastric administrations caused typical circulatory effects but the doses were higher Accordingly, sparteine is a direct cardiac depressant in mammals as in amphibia Electrocardiographically in unanesthetized dogs, the changes were typically quinidine-like Typical antifibrillation was demonstrated mechanically and electrocardiographically in anesthetized dogs with 0.5-1.0 mg/kg sparteine intravenously in auricular fibrillation produced by applying methacholine to the sinus node and slightly pinching the right auricle, higher doses (3-5 mg/kg) were required to arrest or prevent electrical fibrillation Other effects observed were slight reduction in excitability of vag and no antagonism of acetylcholine or metha-

choline (sparteine 5-10 mg/kg), fleeting depression of phrenics, prompt respiratory failure, the cause of death after 30-50 mg/kg intravenously (anesthetized dogs) Sparteine seems to be disposed of in the liver, corresponding circulatory effects requiring 3 to 5 times the dosage in the splenic vein compared with saphenous vein injection Brodie's methyl orange method adapted to estimating sparteine in the blood (sensitivity 1 gamma/cc) showed presence of the alkaloid for 2½ hours in the blood of dogs receiving 20 mg/kg intravenously, thus suggesting a comparatively slow escape from the circulation

Color and crystal tests for the identification of methadon and isomethadon G H W Lucas *Dept of Pharmacology, Univ of Toronto, Toronto, Canada* Special reagents for the rapid colorimetric identification of methadon as the free base and as salts, and of isomethadon, have been prepared by combining 90% sulphuric acid solutions of some heavy metals (copper sulphite 1%, gold chloride 1%, palladium oxide 0.3%) and an 0.5% solution of ammonium vanadate in concentrated sulphuric acid These metal solutions, which keep well, are combined in proportions of 8 to 1 with ammonium vanadate solution, which also keep well, the test solutions are useful for several hours only A useful solution which will keep about a day may be made by mixing the ammonium vanadate solution and Marquis reagent in the ratio of 1 to 2 Powdered ferric sulphate added to the ammonium vanadate solution, when freshly made, is a useful reagent The maximum sensitivity is reached with the gold and palladium reagents as one µg of methadon may be detected Many other salts of alkaloids, glycosides, barbiturates, antipyretics and antihistamine substances have been studied to determine any interference A reagent containing 1 gm of $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ and 9.4 cc of 55-58% HI solution appears to be the most sensitive for giving characteristic crystals with methadon (1:16,000) A 5% mercuric chloride gives very good and characteristic crystals but not so sensitive (1:4,000)

Local anesthetic action of some thiazolidone derivatives F P LUDUENA and JAMES O HOPPE (by invitation) *Biology Div, Sterling-Winthrop Research Inst, Rensselaer, N Y* The local anesthetic activity of several substituted 2-phenyl-(3-alkylamino or 3-dialkylaminoalkyl)-4-thiazolidone compounds has been investigated by one or more of the following tests sciatic nerve block (guinea pig), infiltration around the external canthus (rabbit), corneal instillation (rabbit), intradermal wheal (man) and spinal anesthesia (rabbit) With the diethylaminoethyl side chain the 3,4-methyl-enedioxyphenyl substituted compound (WIN 2125) was the most active (external canthus test) in comparison with analogs possessing the following phenyl substitutions 4-methoxy,

3,4 dimethoxy, and 3,4,5-trimethoxy WIN 2125 was as active and as toxic as procaine. The p-amino-phenyl analog is inactive. In the alkylaminoalkyl series the activity is greater. Two of these compounds, WIN 2661 (2-(3,4-methylenedioxyphenyl)-3-isobutylaminopropyl-4-thiazolidone) and WIN 2663 (the cyclohexylaminoethyl analog) have been studied more extensively. These two compounds are 4-5 times more active than procaine in producing nerve block and spinal anesthesia. Intradermally in man they are more than 5 times more active than procaine. Topically, on the rabbit cornea, they are less active than cocaine. Tissue irritation of these compounds is low, intradermally in rabbits, WIN 2661 and WIN 2663 in a 1% concentration produced mild and moderate irritation respectively (trypan blue test). No signs of irritation were observed in man after the intradermal injection of 0.5% solutions of these compounds. The respective values (in mg/kg) of WIN 2661 and WIN 2663 are as follows: mice, intravenously— 40 ± 1 and 30 ± 1 , subcutaneously— 320 ± 30 and 190 ± 10 , guinea pigs, intravenously— 20 ± 1 and 17.5 ± 1 .

Thromboplastic properties of some antiluetic drugs DAVID I. MACHT *Dept of Pharmacology, Labys of Sinai Hospital, Baltimore, Md*. The writer has already called attention to the fact that certain drugs, widely employed in medical therapeutics possess thromboplastic properties which may precipitate thrombo-embolic accidents. Among those studied experimentally were digitals, mercurial diuretics and the antibiotics penicillin and streptomycin. The author wishes to report now a new group of drugs which in animal experiments were found to shorten coagulation time of whole blood as tested by the Lee and White method. These are the antiluetic drugs arsphenamine, neo-arsphenamine, mapharsen, bismarsen, bismuth salicylate and sodium-bismuth-tartrate. All of these were studied on rabbits and cats, with and without general anesthesia, and administered either by intravenous or intramuscular injection. They all exhibited marked thromboplastic effects. A remarkable exception is Thiobismol, which exerts very little influence on coagulation time. The organic mercury compounds, mercurochrome, merodicein, and merthiolate on intravenous injection also hastened coagulation, and even small doses of HgCl_2 had the same effect. So did tryparsamide, and the widely employed new antisiphilitic, penicillin. The above properties of antiluetics should be borne in mind, in analyzing the causes of occasional grave reactions occurring in the course of antisiphilitic therapy.

Observations on several cyclohexyl- and phenyl-alkyl-amines EDWARD MACKO (by invitation) and EDWIN J. FELLOWS *Dept of Pharmacology, Temple Univ School of Medicine,*

Philadelphia, Penna. In the present studies, the pharmacological actions of 1-Cyclohexyl-2-amino-propane (S-10) and 1-Cyclohexyl-2-methylamino-propane (S-25) the N-methyl modification of S-10, have been compared with their corresponding phenyl analogues. Observations in rats, rabbits and guinea-pigs disclosed the fact that the cyclohexyl derivatives were decidedly less active central nervous system stimulants than their corresponding phenyl modifications. The cardiovascular action of the present compounds has been analyzed and their relative acute toxicity determined in three species of animals. Subacute toxicity studies also were carried out on the cyclohexyl derivatives.

Fate of pentothal in man LESTER C. MARK (by invitation), PHILIP A. LIEF (by invitation), E. M. PAPPER (by invitation), E. A. ROVENSTINE, ELEANORE BERNSTEIN (by invitation), and BERNARD B. BRODIE *Depts of Anesthesia and Biochemistry, New York Univ College of Medicine, and New York Univ Research Service, Goldwater Memorial Hospital, New York City*. A method was devised for estimating pentothal in biological material. This method is specific in that it does not include metabolic products of the compound in the measurement. The physiological disposition of the drug was studied. Negligible amounts of pentothal are excreted unchanged indicating that it is almost entirely transformed in the body. The drug metabolizes more slowly (about 15% per hour) than hitherto thought. The rapid recovery from anesthesia after the administration of small doses represents a shift of pentothal from plasma to tissues, rather than a rapid breakdown of the drug. Pentothal is extensively localized in various tissues of the dog. The tissue reservoir of pentothal may account for the long-lasting anesthesia obtained after the termination of sustained administration. The plasma concentration of pentothal is a function of plasma pH. Decreasing the pH to 6.8 in dogs by carbon dioxide inhalation lowered the pentothal level by about 35%, while increasing the pH by NaHCO_3 infusion to 7.6 produced a less striking change in the opposite direction. These changes were reversed when the pH was restored to normal. Evidence was found in human subjects of an acute tolerance to pentothal. Subjects maintained at high plasma concentrations of the drug awakened at higher plasma levels than those maintained at low plasma concentrations. Two transformation products have been isolated from urine. One of these has been purified and tested for anesthetic activity in mice. It has at most a mild sedative action.

Synthetic curare compounds IV N-Methyl-berbamine iodide and N-methyl-isotetrandine iodide DAVID FIELDING MARSH and DONALD A. HERRING (by invitation) *Dept of Pharmacology, West Virginia Univ School of Medicine, Morgan-*

town, W Va Berbamine was isolated from *Berberis vulgaris*. The curariform activity of its derivatives, N-methyl-berbamine iodide (MB), and N-methyl-isotetrandine iodide (N-methyl-O-methyl-berbamine iodide or MI), was determined in comparison with the corresponding isomers, d-tubocurarine chloride pentahydrate (dT), and d-O-methyltubocurarine iodide trihydrate (MeOdT). Structurally the berbamine derivatives

	dT	MeOdT	MB	MI
	mg/kg of body weight			
Albino rats				
LD ₅₀	0.27	0.032	0.40	0.77
Rabbits				
Head drop 50	0.125	0.016	0.125	0.28
Holaday head drop	0.15	0.02	0.15	0.35
Cat gastrocnemius muscle				
Equivalent paralysis	0.125	0.02	0.125	0.30
Dog head drop (3')	0.16	0.02	0.17	0.48
Man head drop (3')	0.15	0.03	0.18	

differ from the d-tubocurarine compounds in that the tetrahydroisoquinoline rings are adjacent instead of being separated by the oxybenzyl groups. Although the berbamine derivatives are not as potent as the d-tubocurarine compounds, the lack of even the minor undesirable side effects possessed by d-tubocurarine make their clinical investigation seem reasonable.

Further data on the feeding of beryllium salts to laboratory animals. E. A. MAYNARD (by invitation), W. L. DOWNS (by invitation), J. K. SCOTT (by invitation) and H. C. HODGE, Dept. of Radiation Biology, Univ. of Rochester, Rochester, N. Y. Data obtained from larger groups of rats (10 males and 10 females) fed beryllium sulfate and beryllium carbonate at a dietary level of 5% confirmed data from small groups (2 males and 2 females) previously reported, i.e., complete inhibition of growth, some mortality, and a rickets-like condition which was accompanied by a bony plate formation near the head of the tibia. Return to a stock diet with no beryllium sulfate brought about healing of the rickets but the bony plate remained. Pathological studies disclosed injury to kidney, liver, gonads and bone by 5% BeSO₄, rachitic-like bone injury to all rats and liver injury to some rats by 5% beryllium carbonate. Some bone deposition of beryllium was demonstrated by spectrochemical analysis. In the diet of rats, 10% beryllium sulfate caused 100% mortality in 10 days. When fed to dogs, 1 gm/kg/day of beryllium sulfate and 5 gm/kg/day of beryllium oxides had no effect on growth and produced no pathological changes.

'Curare-like' action of synthetic quaternary derivatives of stilbene. BERNARD P. MCNAMARA and TERESA SILVERMAN (by invitation), Medical Div., Army Chemical Center, and Univ. of Maryland, School of Pharmacy, Baltimore, Md. Four synthetic quaternary derivatives of stilbene have been investigated for their effects on neuro-

muscular transmission. The compounds are 1) Stilbestryl bis (2-(4-morpholino-ethyl) ether, bis methiodide, 2) Stilbestryl bis (2-diethylamino-ethyl) ether, bis methiodide, 3) Stilbestryl bis (3-diethylaminopropyl) ether, bis methiodide, 4) Stilbestryl bis (3-(2-methyl-piperidino) propyl) ether, methiodide. All four compounds exert a 'curare-like' action in rabbits and frogs. The paralyzing dose in rabbits is 0.1 mg/kg or less for these ethers. Several differences between their actions and those of curare (Intocostin) have been observed. Following intravenous administration of the stilbene derivatives in rabbits there is a delay of 5-15 minutes in the onset of paralysis. Neither prostigmine nor physostigmine antagonizes the paralysis produced in rabbits by these compounds.

Acute and subacute toxicity of 1,2,3 trichloropropane in mice and rabbits. Wm. A. McOMIE and T. R. BARNES (introduced by Hamilton H. Anderson), Div. of Pharmacology and Experimental Therapeutics, Univ. of California Medical School, San Francisco, Calif. The approximate time required for 1,2,3 trichloropropane to kill (within 48 hours) 8 of 15 mice exposed to a vapor concentration of 30 mg/l, was 20 minutes (at 22°C). Subsequently, liver damage accounted for 4 additional deaths, 7-10 days later. Immediate depression of respiration was noted, believed due to activity on the respiratory center. Subacute exposures over 10 days for 10 minutes each, at 15 mg/l, resulted in 7 deaths among 10 mice. In rabbits, 1,2,3 trichloropropane was an intense skin irritant, due in part to its solvent action on lipoidal components. Sloughing and cracking followed development of irritation and erythema. Ten applications of 2 ml each over 100 sq cm during 15 days resulted in subdermal bleeding and a painful reaction. One of 7 animals died after repeated exposure. Congested lungs were apparent. The remaining rabbits survived and healed with return of hair growth within 6 weeks. Our findings with 1,2,3 trichloropropane confirmed previous reports on the toxicity of chlorinated hydrocarbons. Since 10-minute repeated exposures to saturated vapor-air mixtures caused delayed deaths in mice, prolonged observation after contact is indicated. Inhalation should be avoided, as well as skin exposure, since systemic pathologic changes may follow. Romensky (1872) provided experimental observations on response to 1,2,3 trichloropropane. Paralysis was observed in frogs. Sleep and lowering of body temperature were noted in rabbits. In man, 3 gm orally caused sleepiness, marked headache, unsteady gait, and pain in the lumbar regions. Meyer (1899) reported paralysis of the respiratory center and serious effects on the heart.

A new antiadrenergic agent, 2-[N-p'-tolyl-N-(m'-hydroxyphenyl)-aminomethyl]-imidazo-

line HCl (C-7337) ROLF MEIER (by invitation) and FREDERICK F YONKMAN *Research Depts of Ciba, Basle, Switzerland, and Summit, N J* This new antiadrenergic agent was developed by Marver and Miescher in the imidazoline series as reported by Meier (N Y Acad of Sciences Conference, 1947) It is adrenergic in small doses and sympatholytic in higher concentrations Its antiadrenergic properties have been demonstrated as follows: Protection of mice from lethal effects of epinephrine intravenously, reversals in version of epinephrine hypertension in several species, inhibition or nullification of sustained vasoconstriction by epinephrine perfusion in the rabbit, obliteration of epinephrine's contractile effect of the isolated uterus of the rabbit, inhibition of the contractile action of epinephrine on the seminal vesicle of the guinea pig, nullification of adrenergic salivation in the cat and inhibition of adrenergic retraction of the feline nictitating membrane The antisymphathetic activity of C-7337 seems to be greater than that of Priscol and Dibenamine Its general tolerability locally and systemically is of real potential advantage and in this sense resembles Priscol

Comparative effect of digitoxin and ouabain on auriculo-ventricular conduction in the dog heart RAFAEL MÉNDEZ and JOSE PISANTY (by invitation) *Dept of Physiology and Pharmacology, National Inst of Cardiology, Mexico, D F, Mexico* There has been considerable controversy as to whether differences, other than in speed of action or elimination, exist between the effects of different cardiac glycosides The action of digitoxin (Digitaline Nativelle) and ouabain (Arnaud) upon A-V conduction was studied in dogs with the heart exposed under chloralose anesthesia Electrodes were attached to the right auricle and the ventricles Measurements of A-V conduction were taken during stimulation of the auricle at a constant frequency The glycosides were injected at 30-minute intervals in doses calculated to kill the heart in about 3 hours The results are summarized in the table

Digitoxin vs Ouabain on A-V Conduction

% of Lethal Dose (% of time necessary to kill the heart)	A-V Conduction Rate in % of Normal	
	Digitoxin (9 experiments)	Ouabain (9 experiments)
10	98.1	100.8
20	95.3	101.0
30	89.8	99.9
40	81.0	95.9
50	64.5	87.9
60 (Block beyond this dose)	56.8	77.0

Within its therapeutic range (30-50% of LD) digitoxin causes a significantly more pronounced lengthening of the A-V interval than ouabain

This effect although apparently mediated by the vagi seems specific on conduction for no significant differences were found in the effects of the two glycosides upon the sinus rate or rhythm The effect is also independent of any action on the pacemaker as measurements were done under constant frequency stimulation

Potency and pharmacological actions of nerifolin, a glycoside of *Thevetia Nerifolia* KALMAN C MEZEY (introduced by M B Chenoweth), *Cesar Uribe-Piedrahita Research Labors, Bogota, Colombia* Nerifolin obtained from thevetia trees indigenous to Colombia has a cat unit of 0.20 ± 0.002 mg/kg The minimal emetic dose in pigeons is 0.050 mg/kg and in cats, 0.98 mg/kg The minimal systolic dose in *Hyla labialis* by the U S P XI frog method is 0.0035 mg/gm Nerifolin is partially absorbed from the gastro-intestinal tract and exerts unusually marked action on isolated intestine It is completely eliminated in about 72 hours Except for oral absorption nerifolin most closely resembles ouabain pharmacologically

Reaction of cholinesterase with diisopropyl fluorophosphate containing radioactive phosphorus HARRY O MICHEL (by invitation) and STEPHEN KROP *Biochemistry and Pharmacology Sections, Medical Div, Army Chemical Center, Md* The possibility of compound formation between cholinesterase and diisopropyl fluorophosphate (DFP) was investigated by reacting a cholinesterase preparation from the electric tissue of *Electrophorus electricus* with P^{32} labeled DFP The preparation had an enzymatic activity of 2.6 gm of acetylcholine hydrolyzed/hr/mg of protein, as determined in 0.015M acetylcholine, 0.0342M $MgCl_2$ and 0.025M $NaHCO_3$ at pH 7.4 and 38°C The radioactivity of the labeled DFP was 19 millicuries per gram The reaction between the enzyme and DFP was permitted to proceed for 4 to 24 hours The cholinesterase was then precipitated with 5% trichloroacetic acid (CCl_3COOH) Horse serum protein was added as a carrier The precipitate was washed with CCl_3COOH and ethanol and then suspended in 0.02N NaOH Aliquots were dried and counted directly Phosphorus derived from DFP was found bound to protein in amounts directly proportional to the degree of inhibition of cholinesterase The amount of phosphorus bound to the completely inhibited enzyme was equivalent to 0.100 μg of DFP/mg of cholinesterase When DFP was replaced by sodium diisopropyl phosphate containing P^{32} , no phosphorus was bound by the enzyme

Relative potencies of various cinchona alkaloids in patients with auricular fibrillation WALTER MODELL, SAMUEL J SHANE (by invitation), CONRADO DAYRIT (by invitation) and HARRY GOLD *Dept of Pharmacology, Cornell Univ Medical College, Cardiovascular Research Unit of the*

Beth Israel Hospital, and Cardiac Service of the Hospital for Joint Diseases, New York City Six alkaloids, quinine, natural quinidine, synthetic quinidine, dihydroquinidine, cinchonine and cinchonidine were compared in 18 patients with auricular fibrillation by means of their effect on the rate of the circus movement as revealed by the deflections in the electrocardiogram. After a control tracing a dose was given orally on an empty stomach, and tracings were taken at intervals of 2 hours to determine the maximum effect. This was repeated at intervals of several days with a series of doses from 0.1 to 2.0 gm. The percentage slowing was plotted against the log of the dose. The results of the 244 experiments show that natural quinidine (a mixture of quinidine and dihydroquinidine), synthetic quinidine, and dihydroquinidine are of equal potency, that quinine and cinchonidine are about one half as potent, and that cinchonine is the least active, about one third as potent. The discrepancy between these results and those stated in the literature may be explained by the fact that the latter were not based on dosage-response curves and the doses used were so large as to fall in the insensitive range of dosage-response.

A method for the estimation of chloroform in small quantities of blood. LUCIEN E. MORRIS and EVAN L. FREDERICKSON (introduced by O. Sidney Orth). *Depts. of Anesthesiology and Pharmacology, Univ. of Wisconsin Medical School, Madison, Wis.* An improved method is presented which utilizes ether extractions and the Fujiwara color reaction of alkaline pyridine for rapid, accurate, photoelectric determination of chloroform in 1 cc. quantities of blood. Estimations of chloroform in the blood of anesthetized dogs gave values of from 18 to 50 mg. %, which compares closely with values obtained by earlier workers using more laborious or less delicate methods. Values found during clinical anesthesia indicate a marked species variation. Estimations of chloroform in the blood of 50 patients during anesthesia for major surgical procedures with chloroform as the sole agent gave an average value of 10 mg. % during maintenance.

Effect of pentobarbital on response of the cardiovascular system of dogs to epinephrine, acetylcholine and tilting. JAMES L. MORRISON, HARRY A. WALKER and ARTHUR P. RICHARDSON. *Dept. of Pharmacology, Emory Univ. School of Medicine, Emory Univ., Ga.* It has long been recognized that anesthesia alters the response of the circulation to the administration of many drugs, however, a quantitative description of these changes is at present lacking. We have attempted to obtain this information for pentobarbital. Unanesthetized dogs were tied to a tilting operating table and prepared for recording of blood pressure and intravenous injection of drugs. The response

of the circulation to the injection of a standard dose of epinephrine and acetylcholine in the unanesthetized state was determined. In addition, the response of the circulation to a sudden change in position was determined by tilting the animal from a horizontal to vertical position. Following these procedures, graded doses of pentobarbital were administered and the response to the standard test procedures redetermined after each dose of anesthetic. The injections of pentobarbital were continued until death of the animal. In a series of 6 dogs, the average lethal dose was 55 mg./kg. Gradually increasing doses of pentobarbital produced the following results: a) In the resting state pentobarbital produced a gradual fall in blood pressure with a decrease in pulse pressure and a marked increase in pulse rate. b) There was an increase in sensitivity to epinephrine characterized by a progressively greater rise in blood pressure, accompanied by a decrease and finally abolition of reflex bradycardia. c) There was an increase in sensitivity to acetylcholine characterized by a more prolonged fall in blood pressure and accompanied by a progressive decrease and final loss of reflex tachycardia. d) Pentobarbital produced a moderate postural hypotension accompanied by a loss of reflex tachycardia on tilting. These results can best be explained as being due to a selective central depression of reflex control of the circulation.

Antihistaminic properties of the β -haloalkylamine (dibenamine) series of adrenergic blocking agents. MARK NICKERSON and FRANCES B. HARRIS (by invitation). *Dept. of Pharmacology, Univ. of Utah College of Medicine, Salt Lake City, Utah.* The antihistaminic activities of 75 congeners of Dibenamine were determined by testing their ability to prevent histamine-induced contraction of the guinea pig ileum. All drugs were also tested against methacholine on the same preparation, with a very few exceptions the antihistaminic properties were found to be independent of significant cholinergic blocking activity. Antihistaminic activity in this series is not parallel to adrenergic blocking activity, but no compounds devoid of adrenergic blocking activity are among the more active antihistaminics. The β -haloalkyl grouping is essential for high activity. Substitution of a β -dimethylaminoethyl radical which provides maximal activity in several series of antihistaminics leads to inactivation. Phenoxyethyl (particularly 2-substituted) and α -naphthylmethyl amines are the most active. Unsymmetrical N-phenoxy-ethyl-N-benzyl compounds are almost always more active than N-diphenoxy-ethyl or N-phenoxyethyl-N-ethyl congeners. Several compounds with activities more than 10 times that of Benadryl are included in the series. In contrast to Benadryl and Pyribenzamine which produce a readily reversible 'equilibrium' blockade, members of this series produce a very

persistent blockade similar to that observed in connection with their adrenergic blocking action. Development of this blockade appears to occur in two stages. During the first stage these agents exhibit a competitive equilibrium with histamine which is no longer present after completion of the blocking action. A competitive inhibition of the action of these agents by Benadryl indicates a common site of action.

Mechanism of the acute lethal action of epinephrine in rats. MARK NICKERSON, JOHN BERGHOUT (by invitation) and ROBERT N HAMMERSTROM (by invitation) *Dept of Pharmacology, Univ of Utah College of Medicine, Salt Lake City, Utah.* Protection against the lethal effect of epinephrine in rats and mice has recently been employed as a test for adrenergic blocking activity. However, lack of information regarding the mechanism of death allows no definite conclusions regarding the specificity of this test. Sprague-Dawley rats were injected intraperitoneally with lethal doses of epinephrine, and protection was then provided by various physical procedures. Pentobarbital and heparin, employed in some of the tests, were found not to alter significantly either mortality or 'epinephrine' content of heart muscle as determined colorimetrically. Artificial respiration provides complete protection against several multiples of the lethal dose of epinephrine, but does not alter the 'epinephrine' content of the myocardium, which may be several times as great as that of control animals dying from smaller doses. Systemic arterial pressure stabilization also provides marked protection. In none of the animals was significant pulmonary edema observed. It is concluded that the lethal effect of epinephrine in rats is directly related to the induced increase in systemic arterial pressure which in turn induces respiratory failure. The mechanism of the last step is not clear, but it is not the same as that involved in transient 'epinephrine apnea'. Dibenzamine protects against the lethal effects of epinephrine but does not prevent 'epinephrine apnea'. Epinephrine toxicity in rats appears to be unrelated to an accumulation of the agent (or related catechols) in the myocardium.

Timed intravenous infusion of metrazol or strychnine in anticonvulsant testing. MARSHALL J ORLOFF (by invitation), H L WILLIAMS (by invitation), and CARL C PFEIFFER *Dept of Pharmacology, Univ of Illinois College of Medicine, Chicago, Ill.* The timed intravenous infusion of metrazol (0.5%) and strychnine (0.01%) makes possible an accurate graded evaluation of anticonvulsant compounds. The solutions are injected at the rate of 0.05 cc every 10 seconds into the tail vein of the mouse. Three signs of reaction follow each other in sequence as the concentration of metrazol is increased in the blood. These have

been designated as the 'first twitch,' the 'pseudo-convulsion,' and the 'persistent convulsion'. 'Tri-dione', phenobarbital, 'Dilantin', 'Phenurone', and 'Myanesin' have been used as anticonvulsants to test the accuracy of the method and to compare it with the subcutaneous metrazol method in general use. The chief advantages of the method are 1) fewer animals are necessary in order to evaluate the anticonvulsant, 2) it is less time-consuming than the subcutaneous metrazol method, and 3) it exhibits a high degree of sensitivity.

Effects of excess carbon dioxide in clinical anesthetic atmospheres upon cardiac automaticity and liver function. O SIDNEY ORTH, RALPH M WATERS, LUCIEN E MORRIS (by invitation), J L SIMS (by invitation), and ROBERT T CAPPS (by invitation) *Depts of Pharmacology, Anesthesiology and Internal Medicine, Univ of Wisconsin Medical School, Madison, Wis.* In the administration of anesthetics clinically there frequently is an accumulation of carbon dioxide in the respired atmosphere and a resulting systemic hypercarbia occurs. To determine the effects which such a condition might produce on cardiac automaticity and hepatic function in the human subject, moderate accumulations of carbon dioxide were produced either by permitting definite re-breathing or by the continuous addition of small amounts of carbon dioxide to the anesthesia bag while chloroform or ether was used as the sole anesthetic agent. Electrocardiographic observations were made continuously with the use of a direct recording machine and tracings were taken whenever desired. Analysis of the respired atmosphere was made every 3-5 minutes to prove the continuous presence of oxygen at or usually above 30%, and to know the percentage of carbon dioxide in the mixture. Blood samples were obtained periodically for determinations of hemoglobin, oxygen and carbon dioxide content and carbon dioxide capacity. Five tests of liver function, which included icterus index, cephalin-cholesterol flocculation, prothrombin time, bromsulfalein retention, and thymol turbidity, were made before the operation, 24 hours postoperatively, and every second day thereafter for 4 times or until liver function tests returned to normal.

Influence of roentgen rays on potency of penicillin. MARCUS OSTRO (by invitation) and DAVID I MACHT *Depts of Pharmacology and Radiology, Sinai Hospital, Baltimore, Md.* Aqueous solutions both of crystalline penicillin (C S C) and amorphous penicillin were treated with x-rays in open glass beakers, and compared pharmacologically with control non-radiated solutions of the same preparations. The apparatus employed was a water-cooled therapy tube operated on 200 K V and 20 M A with a target distance of 50 cm. The rays

employed were passed through a filter of 1 mm aluminium and 2 mm copper in some experiments, and a filter of 1 mm aluminium and 0.5 mm copper in others, the dosage employed in each case being the same. Dosages ranging from 93r to 280r were given to the solutions. Three kinds of tests were used for pharmacological comparison: 1) Phytotoxic tests on root growth of lupinus seedlings in plant physiological solutions revealed a greater phytotoxicity after irradiation through the 2 mm Cu filter, ranging from 20 to 30%. 2) The thromboplastic properties of penicillin after irradiation were also markedly increased, often 50% or more. 3) Bacteriological tests were made through the cooperation of Drs. Lawrence and Jerome Martin of the CSC Research Laboratories. These revealed no difference in chemotherapeutic and bacteriostatic potencies between γ -rayed and control solutions. The difference in the phytotoxic and thromboplastic properties occurred only when solutions of penicillin were irradiated, but did not occur when solid penicillin was exposed to γ -rays. Irradiations of mice with above filtered γ -rays in doses from 63r to 93r did not render the animals more vulnerable to subsequent injections of large doses of normal penicillin.

Experimental safety of sorbitan monostearate and its polyoxyethylene derivatives SIDNEY G. PAGE, JR. (introduced by HARVEY B. HAAG) *Medical College of Virginia, Richmond, Va.* Three products were submitted for investigation, containing Sorbitan Monostearate (Span 60) and Polyoxyethylene Sorbitan Monostearates (Tween 60 and Tween 61). Weighed contents were suspended in chocolate syrup and administered 3 times daily for 28 days to a group of 60 subjects. These ranged in age from 20 to 49 and were physicians, technicians or junior medical students. Physical examinations, blood and urine studies were made before administration and after 14 and 28 days of continuous administration. Further examinations were made 14 days after discontinuing administration of these products. The clinical observations included body weight, blood pressure and pulse measurements, the urinalyses included color, reaction, specific gravity, sugar and albumin, in addition to a microscopic examination. The blood examinations included determinations of hemoglobin, erythrocytes, leukocytes and differential examination, chemical determinations were made of the plasma protein, NPN, sugar, calcium and cholesterol. No significant variations were observed in any of the physical, microscopic or other examinations during this entire study. These substances did not produce any untoward effects in this investigation.

Effects of inhalation of high concentrations of carbon dioxide THOMAS M. PARRY (by invitation), JOSEPH N. SPENCER (by invitation), WILLIAM

B. DRAPER, RICHARD W. WHITEHEAD and ROBERT L. ARENDS (by invitation) *Dept. of Physiology and Pharmacology, Univ. of Colorado Medical Center, Denver, Colo.* Observations on dogs in diffusion respiration indicate that there is a progressive rise in blood carbon dioxide and a concomitant fall in blood pH during respiratory arrest. To determine the effect of the concentrations of CO₂ upon the blood pH, dogs were administered 10, 20, 30 and 40 % of carbon dioxide in oxygen for a period of 45 minutes while under light thiopental anesthesia. Changes in heart rate, ECG, and blood pH were noted, as well as the respiratory, anesthetic and toxic effects of the carbon dioxide mixtures. The inhalation of the carbon dioxide mixtures produced no significant change in the heart rate. Likewise, the only alteration in the ECG was an increase in the amplitude of the T wave, which returned to normal with the return of the blood to normal. The venous blood pH fell rapidly during the first 15 minutes of carbon dioxide inhalation, but thereafter decreased only slightly. The pH fell from a control average of 7.42 to 7.24, 7.05, 6.83 and 6.79 during inhalation of 10, 20, 30 and 40% carbon dioxide, respectively. Upon removal of the animals from the carbon dioxide atmosphere the pH rose rapidly. With 30 and 40% carbon dioxide the pH rose to 7.28 within 30 minutes while with 10 and 20% carbon dioxide it had returned to normal.

Effects of pituitrin, pitressin and pitocin on blood pressure and cardiac automaticity as modified by various anesthetic agents CARLOS E. PARSLÖE (by invitation), LUCIEN E. MORRIS (by invitation) and O. SIDNEY ORTH *Depts. of Anesthesiology and Pharmacology, Univ. of Wisconsin Medical School, Madison, Wis.* Accidents which have occurred following the administration of pituitary preparations to anesthetized patients make it desirable to reevaluate the pharmacological action and therapeutic uses of these drugs and to study any possible incompatibility which they may have with various anesthetic agents. Eighty-five experiments were made with 26 dogs. Anesthetic agents employed were either cyclopropane, ether, chloroform, nembutal, chlorotone or procaine. Unanesthetized animals also were studied. Blood pressure determinations and continuous electrocardiographic records were made and upon analysis subsequent to intravenous injection of each of the pituitary fractions the results were found to confirm and extend previous observations. Blood pressure response showed marked variation dependent on the type of anesthetic used. Most striking was a characteristic, almost precipitous fall in pressure observed after either pituitrin or pitressin during cyclopropane anesthesia. There were no significant changes following the use of pitocin. Alterations of cardiac rhythm were ob-

served in each of 14 conscious animals and in 9 with spinal analgesia. One-fifth of the animals tested with pituitrin or pitressin during cyclopropane anesthesia exhibited some type of arrhythmiae which was thought to be correlated with injection of the drug and associated with coronary constriction and a resultant myocardial hypoxia predisposing to cardiac dysfunction in the presence of anesthesia. Side effects of pituitrin administration, such as modification of respiratory activity to reduce effective exchange or retching and vomiting which may mechanically obstruct respiration, lead to increased hypoxia and are additional factors increasing the hazard of accident. Clinical electrocardiographic studies are in progress.

Antiseptic properties of certain mercurial diuretics. S. ANDERSON PEOPLES and ANN MCCORMICK (by invitation) *Division of Veterinary Science, Univ. of California, Davis, Calif.* Mercurial diuretics are rapidly excreted in the urine after intravenous administration, and if the compounds should then break down to give a sufficient concentration of ionized mercury, they would act as urinary antiseptics. In order to test this possibility, the *in vitro* antibacterial action of Salyrgan (I), Mercuzanthin, (II) Mercuhydrin (III) and mercuric chloride were tested against *Streptococcus faecalis*, *Streptococcus pyogenes*, *Escherichia coli*, *Staphylococcus aureus*, and *Corynebacterium renalis*. The concentration of mercury furnished by the compound in micrograms/cc required to inhibit growth was determined turbidometrically and the bactericidal concentration by subculture in thioglycollate media. The apparent percentage of the mercury in the compound which is ionized can be calculated from the ratio of the mercury as mercuric chloride to that of the compound required to produce the same endpoint. II was the most active, inhibiting all the organisms at 3-12, indicating that 50% of the mercury was ionized. I and III caused inhibition at 6-200, indicating 3-24% ionization. The bactericidal concentrations were difficult to determine accurately and varied from 12-400. These results were not materially altered when blood serum was added to the media nor when urine of pH 6.7 was used as media. The activity of all the compounds was increased by changing the pH of the media to either 5.0 or 8.0. These bacteriostatic and bactericidal concentrations can easily be reached in the intact animal and tests of these compounds in experimental urinary infections are now underway.

Release of various proteins from incubated surviving liver slices. RITA L. PESSOTTI (by invitation) and RALPH W. BRAUER *Dept. of Pharmacology and Experimental Therapeutics, School of Medicine, Louisiana State Univ., New Orleans, La.* In an effort to elucidate the factors which control the distribution of various plasma proteins

between the hepatic cells and the blood plasma, liver slices from dogs and rats have been incubated in protein-free or protein-containing solutions under various conditions. Analytical procedures applied include total protein determinations, esterase activity determinations, cholinesterase activity determinations, and electrophoretic analysis at pH 7.8. Results indicate that the release of protein with respect to the quantity, quality, and time course does not differ markedly as between different lobes of a liver obtained from the same animal, on the other hand, wide differences are obtained in going from one animal to the next. On incubation with Krebs solution at pH 7.4, the release of cholinesterase activity and of 'alpha globulins' proceeds much more rapidly than that of total protein. The addition of bovine plasma albumin to the incubation solutions accelerates and increases the amount of cholinesterase activity and of 'alpha globulins' released. These results parallel observations previously made in intact dogs under conditions of plasmapheresis or albumin transfusions. If liver slices are repeatedly transferred to fresh samples of Krebs solution a reduction in the rate of release of protein and of enzyme activity is usually observed after 2 or 3 transfers under the conditions of these experiments.

Nephrotoxic action of a cyanine dye, 1'-ethyl-3,6-dimethyl-2-phenyl-4-pyrimido-2-cyanine chloride. LAWRENCE PETERS and AEME HIGASHI (by invitation) *Dept. of Pharmacology, School of Medicine, Western Reserve Univ., Cleveland, Ohio.* Distribution studies in dogs following I.V. administration of dye showed its concentration in the kidneys to be higher than that in other organs. However, recovery from the urine never exceeded 10% of the dose given. Microscopic examination of unstained frozen-dried sections revealed the presence of large amounts of dye in the cells of the convoluted tubules. Doses greater than 10 mg/kg had a pronounced effect on renal function. Transient albuminuria occurred. Nitrogen retention, reflected in markedly elevated BUN and plasma creatinine concentrations, usually reached a peak between 48 and 96 hours, returning to normal over a variable period, depending on magnitude of dosage and individual susceptibility. Renal clearance values for inulin, creatinine, PAH (low and high plasma levels), glucose and sulfamerazine were all depressed during azotemia. The clearance of PAH (CP_{PAH}) at low plasma levels (0.8-1.8 mg %) fell less than the inulin clearance, hence the filtration fraction (CI/CP_{PAH}) was decreased. During recovery the clearances returned to levels equal to or only slightly in excess of pre-injection values. CP_{PAH} was again exceptional in that it increased 200-300%, resulting in a filtration fraction $\frac{1}{2}$ to $\frac{3}{4}$ of that existing before dye infection.

Though the dye possessed vasodepressor properties, the effects were obtainable following dye infusion at rates producing little or no fall in femoral arterial pressure. The animals were killed for post-mortem examination. Microscopically, sections revealed nephrotic changes confined principally to the cytoplasm of the tubule cells. Nuclear changes existed in some cases to be discussed.

Actions of 2,6-diaminopurine in mice, rats, and dogs **FREDERICK S. PHILIPS and J. B. THIERSCH** (by invitation) *Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York City*. Recent reports finding 2,6-diaminopurine, 1) an antagonist of folic acid and purine metabolism in microorganisms (Hitchings, et al *J Biol Chem* 174: 765, 1948), 2) a precursor in the synthesis of guanine in rats (Bendich and Brown, *J Biol Chem* 176: 1471, 1948), and 3) therapeutically active against leukemia in mice (Burchenal, et al *Cancer*, in press) led to studies of its pharmacology. Its toxicity by parenteral administration was determined in mice, rats, and dogs. Acutely toxic doses were fatal within 48 hours and in dogs caused protracted emesis, hemorrhagic diarrhea, severe losses of fluid and electrolyte, hemoconcentration, and collapse. However, daily doses as great as $\frac{1}{2}$ to $\frac{1}{3}$ LD-50 were tolerated by rats and dogs for 1 to 2 weeks or longer. Signs of chronic intoxication included anorexia, progressive weight-loss, and in peripheral blood, reticulocytopenia, leucopenia, and moderate anemia. Bone marrow from rats sacrificed in terminal stages of chronic intoxication was semi-fluid exhibiting significant depletion of both myeloid and erythroid elements. In dogs receiving daily doses serial bone-marrow aspiration revealed progressive diminution of total nucleated cell counts involving greater decreases in erythroid than in myeloid cells. Terminally nucleated erythroid cells were almost absent while myeloid cells in all stages were present, but, in less than normal quantities. In its effects on marrow and intestinal epithelium 2,6-diaminopurine resembled 4-amino-pteroylglutamic acid with regard to site of action. However, the actions of the purine and the folic acid antagonist could be differentiated in so far as the former failed to elicit megaloblastic erythropoiesis in dogs while the latter appeared to be more uniformly toxic to both myelopoiesis and erythropoiesis.

Quantitative estimation of pyribenzamine in blood **ALBERT J. PLUMMER** (introduced by **GEORGE I. MAISON**) *Dept of Pharmacology, Boston Univ School of Medicine, Boston, Mass.* With the cooperation of the Ciba Pharmaceutical Company, a chemical method for the quantitative estimation of pyribenzamine in blood has been developed. While this method is not as sensitive as the fluorimetric method of R. Mizzoni it is simpler. The

method 20 ml of blood are added to 30 ml of 15% Trichloroacetic Acid. The precipitated protein is removed by filtration and the filtrate, about 35 ml, is rendered just alkaline with 10% sodium hydroxide. The filtrate is extracted with two 15 ml portions of ether and these are combined and evaporated to dryness over a warm water bath. The residue is dissolved in 0.5 ml of 0.1% sulfuric acid and 1 ml of water. After allowing 5 minutes for solution the liquid is transferred to a Klett colorimeter tube and diluted to 5 ml. Iodine solution, 0.25 N, 0.04 ml is added and after 10 minutes the turbidity is read in colorimeter using No. 50 filter. The amount of pyribenzamine may be estimated by comparison with the readings obtained by developing turbidity in solutions of pyribenzamine hydrochloride of known concentration. It has been possible to measure with an error of plus or minus 5% amounts as low as 0.05 mg pyribenzamine hydrochloride added to 100 cc of human blood. The method is also applicable to the determination of antistine in blood. Here however, at present, the lowest detected concentration has been 0.3 mg per 100 ml of blood. No property of normal blood has interfered with the application of the method.

Augmentor effect as postulated by the electromotive theory of drug action **HARRY J. PRATT** (by invitation) and **R. BEUTNER** *Hahnemann Medical College, Philadelphia, Pa.* According to the electromotive theory of drug action, the stimulating action of a drug is proportioned to the electromotive change which it brings about at a phase boundary of water and an 'oil'. If the stimulant drug is an alkaloid like pilocarpine, the electromotive effect on the phase boundary is a negative variation, like the negative variation brought about by stimulation of nerve. This electric effect of the drug is enhanced by addition of sodiumbenzoate or other sodium of organic acids, but only within certain limits, a larger or smaller addition of benzoate will decrease the electric effect, (Beutner, *J Pharm* 34: 29, 1928). The stimulating action of pilocarpine, as tested on the isolated intestine of rabbit, is likewise enhanced by addition of sodiumbenzoate, this also occurs only within certain limits of concentration as experiments show, but only two single measurements by Storm van Leeuwen and von György were available so far to test the theory. In order to obtain additional data, the following experiments were made. To a strip of rabbit duodenum, suspended in Tyrode solution, 0.03 mg of pilocarpine was added. They produced a contraction of 45 mm. This was reduced 43% by 0.03 mg of sodiumbenzoate, it was potentiated 30% by 0.165 mg, 71% by 0.33 mg, 20% by 1.65 mg, 33% by 3.3 mg, and again reduced 28% by 16.5 mg or 70% by 33 mg. These data show that the potentiation of the constricting action of pilo-

carpine occurs only within certain limits as does the electric effect

Inhibition of myocardial reactions to epinephrine and nor-epinephrine by nitroglycerine W RAAB and EUGENE LEPESCHKIN (introduced by F J SICHEL) *Division of Experimental Medicine, Univ of Vermont, College of Medicine, Burlington, Vt* In atropinized cats the blood pressure and electrocardiogram were recorded after intravenous injection of epinephrine and nor-epinephrine (probably identical with sympathin) and after faradic stimulation of the stellate ganglia. The injections were followed by elevation of the blood pressure, tachycardia and transient flattening or inversion of the T-wave in leads CR1 and CR4 (between the 15th and 50th seconds) and later (until about the 160th second) usually by elevation of T. The effects of epinephrine and nor-epinephrine were almost identical, and similar to those produced by stimulation of the stellate ganglia. If nitroglycerine was injected together with epinephrine or nor-epinephrine, the effect of the latter on blood pressure and tachycardia was weakened, the depression of the T-wave was either entirely abolished or markedly diminished. This effect was considerably greater than would correspond to the slight or moderate elevation of T, sometimes elicited by nitroglycerine alone. Since the depression of T had proven entirely independent of blood pressure and heart rate, the inhibiting effect of nitroglycerine is assumed to take place in the myocardium itself through interference with the metabolic action of the sympathomimetic amines. As epinephrine is believed to dilate the coronaries, it seems unlikely that the inhibition of the epinephrine-induced electrocardiographic changes through nitroglycerine should be due to a further coronary dilatation. The T-wave depression after stimulation of the cardiac sympathetic was likewise inhibited by nitroglycerine. It is probably due to discharges of nor-epinephrine into the myocardial cells.

Purification of the toxic principle of agene-treated gluten for pharmacological study JACK L RADOMSKI, GEOFFREY WOODARD, and HENRY N FUYAT (introduced by ARNOLD J LEHMAN) *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D C* To facilitate the study of the pharmacology of canine hysteria it was desirable to purify the responsible etiological agent. The starting material was prepared by the hydrolysis of agene (NCl_3)-treated gluten with pancreatin. The pH of a batch of toxic hydrolysate was depressed stepwise to progressively lower values by the addition of a cationic exchange resin (Ion X) in its H^+ form. The resin was filtered off each time a desired pH was reached. This process was repeated until the pH was depressed beyond the range of interest.

Thus, the peptides and amino acids of this hydrolysate were fractionated according to the pH range in which they become adsorbable. Each of the batches of resin was separately eluted with dilute ammonia and the toxicity of the eluate determined by oral administration to dogs. It was found that the toxic principle was adsorbed exclusively between pH 3.35 and pH 3.95. It is felt that this method is generally applicable for the separation of peptides from protein hydrolysates and constitutes an improvement on previously published techniques. Further concentration was effected by washing the resin with dilute hydrochloric acid before the toxic principle was eluted with ammonia. The eluate may be dried to a white water-soluble powder with an acute toxicity of 500 mg/kg to dogs. This represents a 10-fold purification over the starting material. Still further purification was achieved by extraction with organic solvents. The pharmacological behavior of the partially purified toxic principle in dogs, monkeys, rabbits, and rats was investigated.

Metabolic factors contributing to the course of pentothal anesthesia CLAUDIA M RATLIFF (by invitation), A H MALONEY, JAMES R TUREMAN (by invitation) and WALTER M BOOKER *Dept of Pharmacology, School of Medicine, Howard Univ Washington, D C* Previous work reported from this laboratory has called attention to hyperglycemia and glycogenolysis under prolonged pentothal anesthesia. An extension of that work pointed out the indication for insulin during pentothal anesthesia. In the present work experiments are reported in which specific units of insulin are administered at regular intervals (intramuscularly) or by constant drip (intravenously) sufficient to keep the blood sugar in normal range during three hours of pentothal anesthesia. It has been found that insulin-treated animals require greater quantities and more frequent administration of pentothal sodium to keep them in surgical anesthesia as compared with untreated controls. Insulin treated animals have slightly higher liver glycogen values than untreated animals at the end of 3 hours of anesthesia. There is some evidence that the glycogen storage at the liver influences the destruction of pentothal, since in animals on high carbohydrate and on high protein diets, where the liver glycogen is high, accumulation of pentothal at the liver from hour to hour is less than in animals on normal diets. Taking liver samples every hour for pentothal and micro glycogen determinations, it has been found that the more rapidly the glycogen falls, the greater is the build up of pentothal at the liver. Furthermore, in animals on normal diet the liver is efficient the first hour in destroying pentothal, but as anesthesia is pushed into the second and third hours more and more pentothal can be demonstrated at the liver.

Induction of idioventricular rhythms by 1-1-trichlorethane and epinephrine BARBARA R. RENICK (by invitation), S. DONALD MALTON (by invitation), G. K. MOE and M. H. SEEVERS *Dept of Pharmacology, Univ of Michigan, Ann Arbor, Mich* Methyl chloroform was recommended as an anesthetic agent 60 years ago, but has never had clinical trial. In this laboratory attempts to induce anesthesia in 2 dogs without premedication led to sudden death, presumably cardiac in origin, in both animals. In 5 dogs under barbital anesthesia epinephrine was injected after administration of repeated small doses of methyl chloroform. Ventricular extrasystoles and ventricular tachycardia were produced in all animals. Maximum sensitization of the heart to epinephrine occurred after the administration of 0.25 to 0.4 cc/kg of methyl chloroform. Further administration of the agent raised the threshold dose of epinephrine, in part because of the resultant severe hypotension. Ventricular arrhythmias can be more regularly induced with methyl chloroform than with chloroform, but the sensitivity of the heart appears to be less than with cyclopropane.

Competitive inhibition of convulsions in guinea pigs R. K. RICHARDS and K. E. KUETER (by invitation) *Dept of Pharmacology, Abbott Research Laboratories, North Chicago, Ill* The authors have shown that para aminobenzoic acid (PABA) and diethylaminoethanol (DEAE), the split products of procaine, can either singly or, more efficiently, as a mixture inhibit the convulsions caused by 1 m injection of procaine in guinea pigs when administered i p prior to this drug. This work has been extended by using the dibutylamino-propyl (DBAP) ester butyn, a more potent local anesthetic of the PABA series. 30 mg/kg of butyn sulfate 1 m caused convulsions in 90% of the guinea pigs. This incidence could be reduced to about $\frac{1}{3}$ by pretreatment with 400 to 600 mg/kg PABA. DBAP and similar long-chain amino alcohols were too toxic for testing their possible antagonistic effect against butyn as it was demonstrated with DEAE in the case of procaine. Metycaine was studied because it is an ester of benzoic acid rather than of PABA. 45 mg/kg of metycaine HCl was convulsive in 82% of the animals. The incidence of convulsions was reduced to about 55% by premedication with comparable doses of either benzoic acid or PABA. As with butyn the metycaine side chain, 2-methylpiperidinopropyl, was relatively too toxic to investigate its possible antagonistic effect. These findings will be discussed from the viewpoint of the theory of competitive inhibition on the basis of structural similarity and the changes in pharmacologic properties which occur if the tertiary amino group of the amino alcohol is converted into a quaternary

ammonium base which deprives the esters of the local anesthetic properties.

Action of caffeine and aminophylline as respiratory stimulants in man GLENN H. RICHMOND (introduced by C. L. GEMMILL) *Dept of Pharmacology, Univ of Virginia, Medical School, Charlottesville, Va* The respiratory stimulating effect of CO₂ was used as a functional test to study the mechanism of action of caffeine and aminophylline as respiratory stimulants. Ventilation minute volumes were obtained with the subject breathing atmospheric air, 3% CO₂ in O₂, and 5% CO₂ in O₂ before and after the administration of 0.25 gm of caffeine subcutaneously and before and after the administration of 0.25 gm of aminophylline subcutaneously. The average increase in ventilation minute volume, of 6 subjects, when breathing 3% CO₂ was found to be 23% greater after caffeine than before. When breathing 5% CO₂ the increase was 51.7% greater after caffeine than before. It was concluded from this study that caffeine acts on the respiratory center by increasing its sensitivity to CO₂. Aminophylline was found to have no significant effect on the response of respiration to CO₂.

Lethal and electrocardiographic changes produced by quinine dihydrochloride in monkeys infected with *P. knowlesi* R. H. RIGDON (introduced by CHAUNCEY D. LEAKE) *Laby of Experimental Pathology and Heart Station, Univ of Texas, Medical Branch, Galveston, Texas* During our studies on malaria, quinine dihydrochloride was given to 31 monkeys. Some of those with a severe infection died immediately following an intravenous injection of quinine. Since other animals of equal weight survived a similar dose, experiments were designed to study this variation. To determine the effect of quinine on the electrocardiogram, 20 monkeys were used, 9 of which were normal and 11 infected with *P. knowlesi*. Limb leads and an apical chest lead were obtained and immediately thereafter quinine dihydrochloride was given intravenously and a second group of tracings was made during an interval of 10 minutes. A series of tracings were made on 4 monkeys given quinine both before and after their infection. In some experiments the dosage of quinine was varied to study its effect, while in other experiments the dose was kept constant to determine the variations accompanying the changes in the degree of parasitemia and the anemia. The lethal doses of quinine were 56 and 77 mg/kg for two normal monkeys. In contrast, the following doses, 28, 28, 28, 31, 37.5 and 47 mg/kg, were lethal for malarial infected monkeys who had red blood cell counts between 1.9 and 3.24 million. Malarial infection, per se, although severe, did not appear to affect the electrocardiogram in the majority of cases. In 3 monkeys the deleterious conduction and other electrocardiographic effects of quinine

were found to be greater when infected than when normal. In malarial infected monkeys the electrocardiographic changes following quinine appeared to parallel the degree of anemia rather than the degree of parasitemia.

Renal clearance of iodide in the dog DOUGLAS SHEPARD RIGGS *Dept of Pharmacology, Harvard Medical School, Boston, Mass*. Simultaneous clearances of iodide, chloride and creatinine have been determined in dogs maintained on a low-chloride, high-protein diet. The average glomerular filtration rate measured by the creatinine clearance was about 100 cc/minute. When plasma iodide concentrations were 1.6 mEq/l or less, the clearance of iodide was remarkably low, ranging from 0.05 to 0.79 cc/minute. With higher plasma iodide levels the clearance increased to as much as 8 cc/minute at 11 mEq/l of plasma. When plasma iodide was maintained at about 0.4 mEq/l and chloride excretion was elevated by the oral administration of sodium chloride, the clearance of iodide increased markedly to as much as 17.7 cc/minute. The iodide clearance was always somewhat greater than the chloride clearance, the difference being most marked when the chloride excretion was moderate. When chloride excretion was very high, the kidney seemed less able to differentiate between the two halides. This dependence of iodide excretion on chloride excretion resulted in prolonged retention of iodide in dogs whose kidneys were conserving salt. For example, a plasma iodide level of 1.2 mEq/l was maintained without appreciable change for 24 hours after a single subcutaneous injection of sodium iodide in a fasting animal. Administration of sodium chloride occasioned a prompt and profound fall in plasma iodide. These experiments indicate that the tubular reabsorption of iodide in the dog may be almost complete when total halide excretion is minimal.

Effects of 3-acetoxy phenoxy trimethylammonium methylsulphate on neuromuscular function WALTER F. RIEKER and W. CLARKE WESCOE (by invitation) *Dept of Pharmacology, Cornell Univ Medical College, New York City*. In the gastrocnemius-soleus preparation of the intact cat the close intra-arterial injection of 3-acetoxy phenoxy trimethylammonium methylsulphate (Nu 2017, Hoffmann-LaRoche, Inc.) produces an immediate maximal contraction of the muscles followed by intense local fasciculations. The fasciculations disappear completely within one minute. If the dose is sufficiently large, the fasciculations become generalized. The close intra-arterial injection of 5 μ g/kg potentiates the response of the muscles supra-maximal nerve stimuli. The duration of the potentiation is brief in contrast to that produced by physostigmine, neostigmine, and DFP. Varying degrees of depression of the muscular response to supra-maximal nerve stimuli are produced by large

doses of Nu 2017. The recovery from depression is rapid and the return to normal from a completely refractory state occurs within 20 minutes. After inactivation of cholinesterase by DFP, the close intra-arterial injection of a large dose of Nu 2017 produces a profound depression from which recovery is prolonged. The depression of the response to indirect stimuli produced by d-tubocurarine is antagonized by Nu 2017.

Preparation and pharmacological properties of the acid succinate of myanesin RICHARD F. RILEY and F. M. BERGER (introduced by HAROLD C. HODGE) *Depts of Pharmacology and Pediatrics, Univ of Rochester, Rochester, N. Y.* Observations on the metabolic fate of myanesin led to the assumption that myanesin acid succinate would represent a soluble, relatively non-toxic derivative of myanesin with a less intense and longer duration of action. In the present investigation, the succinate was prepared and tested and found to behave in accordance with this expectation. The succinate, prepared by reacting myanesin with succinic anhydride in pyridine, was obtained as straw-colored, extremely viscous liquid which gives a highly water soluble sodium salt. In the mouse, the paralytic and lethal doses of sodium myanesin succinate are found to be larger than those of myanesin. The antagonism and duration of action of these two drugs to metrazole convulsions was studied by the injection of these compounds before and after the administration of an LD₉₉ of metrazole. On a molar basis, the succinate is less effective in preventing convulsions when administered jointly with metrazole. The succinate is, however, equally effective when administered prior to the convulsant. The succinate in small doses abolishes tremors and hyperexcitability due to strychnine without abolishing the knee jerk in the cat.

Failure of alpha tocopherol to influence chest pain in patients with heart disease SEYMOUR H. RINZLER (by invitation), HYMAN BAKST (by invitation), ZACHARY H. BENJAMIN (by invitation), AUDRIE L. BOBB (by invitation), and JANET TRAVELL *Cardiovascular Research Unit of Beth Israel Hospital and the Dept of Pharmacology, Cornell Univ Medical College, New York City*. A stringent blind-test method, in which neither patients nor examiners knew whether alpha tocopherol or a matching placebo was being administered, was used to investigate effects of alpha tocopherol on long standing chest pain in 38 ambulatory clinic patients with heart disease (chiefly arteriosclerotic and hypertensive). For allotment to control and treated groups, patients were paired with respect to sex, age, cardiovascular status, duration and type of chest pain, and other pertinent factors. One of each pair was assigned to either control (placebo) or treated (vitamin) groups. Types

of chest pain encountered were effort angina, constant chest pain, and intermittent chest pain unlike effort angina. We regard the first group as primarily of cardiac origin, while the other two are considered chiefly somatic. The dosage plan was 200 mg of synthetic alpha tocopherol (Ephynal Acetate 'Roche') by mouth daily for 2 weeks and thereafter 300 mg daily. The average duration of vitamin administration was 16 weeks (10-20 weeks), and of the placebo 16.6 weeks (10-20 weeks). Response to medication was similar for both groups. No improvement was noted in 12 treated subjects (63%) and 14 controls (73%). Subjective improvement was reported by 7 treated patients (37%) and 5 controls (27%). Serial electrocardiograms, exercise tolerance tests, repeated blood pressure readings and measurements of skeletal muscle function did not reveal significant differences between treated and control groups. Our results fail to confirm the reported benefits of alpha tocopherol in cardiac pain.

Pharmacologic and sensitizing properties of SY-28 when administered by ion transfer. ARTHUR A RODRIGUEZ (by invitation), JULES H. LAST, and ADOLPH ROSTENBERG JR (by invitation). *Depts of Physical Medicine, Pharmacology, Dermatology and Allergy Unit, Univ of Illinois College of Medicine, Chicago, Ill.* The pharmacologic activity of SY-28 (N-ethyl-N-(1-naphthylmethyl)- β -bromoethylamine hydrobromide) was studied following administration by ion transfer to the normal skin of human subjects. Three-tenths of a milliamperere of anodal current was applied to 10 cm² areas of the skin. A total amount of 0.3 cc of a 10 mg % solution of SY-28 in propylene glycol was used. It was observed that SY-28 when administered in the above manner was a strong eczematous sensitizer. Twelve out of 46 patients (26%) became sensitized to the drug. Adrenergic blocking activity, lasting at least 24 hours, was demonstrated in 53 patients. Antihistaminic activity was also noted following administration of SY-28. There was clearly no interference with the pharmacologic action of SY-28 in those individuals who became sensitized.

Interaction between hematin and serum albumin. MORRIS ROSENFELD and DOUGLAS M. SURCION (by invitation). *Dept of Pharmacology and Experimental Therapeutics, Johns Hopkins School of Medicine and Dept of Physical Chemistry, Harvard Medical School Boston, Mass.* The reaction between hematin and human serum albumin to form methemalbumin has been studied by spectrophotometric procedures. Titration of hematin with albumin reveals a one to one molar ratio at the endpoint. Other interactions involving simple stoichiometric relationships are indicated by titration curves. Little interaction is observed spectrophotometrically with other plasma protein frac-

tions including fibrinogen and the alpha, beta and gamma globulins. A direct colorimetric procedure for the estimation of serum albumin has been based on the hematin reaction. The colorimetric measurements compare favorably with values obtained by the low temperature methanol fractionation method of Pillemer and Hutchinson.

Thiomerin toxicity and diuretic effects. A. RUSKIN, J. E. JOHNSON, W. N. RODDY (introduced by CHAUNCEY D. LEAKE). *Heart Station, Univ of Texas Medical Branch, Galveston.* The disodium salt of N-(γ -carboxymethylmercaptomercuri-B-methoxy) propyl camphoramic acid (thiomerin) in isolated rabbit hearts causes A-V and I-V block and asystole in doses 20 times those of meralluride (mercuhydrin), and about 50 times those of mersalyl-theophylline and mercuzanthin. In 50 clinical cases the diuretic effects of 2 cc doses of thiomerin subcutaneously are approximately equivalent to those of 2 cc doses of mercuhydrin intravenously, both containing the same amount of mercury (80 mg) in organic combination. Cases in which mercuhydrin produced untoward effects (nausea, vomiting, muscle cramps, toxic psychoses), tolerated thiomerin well. Rare local soreness and nodulation from thiomerin is now being investigated by similar comparative methods.

Adrenergic blocking agents and the vasopressor response to epinephrine. DAVID RUSSELL (by invitation) and GRAHAM CHEN. *Research Labs, Parke, Davis & Company, Detroit, Mich.* Quantitative results on 'epinephrine reversal' of blood pressure in the dog have been obtained with two adrenergic blocking agents: N-(2-bromoethyl)-N-ethyl-1-naphthalenemethylamine HBr and yohimbine. The data were found to conform to either Langmuir's adsorption isotherm or Gaddum's equation of competitive inhibition based on the law of mass action. A linear relationship is obtained when the degree of 'epinephrine reversal' is expressed in terms of the percentage of inhibition of epinephrine in probit units and the doses of the adrenergic blocking agent in logarithms.

Action of cholinesterase inhibitors on the isolated rabbit ileum. P. R. SALERNO (by invitation) and J. M. COON. *Dept of Pharmacology, Univ of Chicago, Chicago, Ill.* It has been reported by us (*Fed Proc* 7:212, 1948) that physostigmine, neostigmine, TEPP, and DFP, in molar concentrations of approximately 10⁻⁵, change the pendular movements of the isolated rabbit ileum to a peristaltic rhythm. The appearance of moving constriction rings in the muscle strip characterizes this change. The peristaltic rhythm can be reversed to a pendular rhythm by atropine and thiamine, or by the ganglionic blocking agents procaine, nicotine, tetraethyl ammonium and magnesium. Effective concentrations of the latter

blocking agents have no effect upon the sensitivity of the gut to acetyl choline added to the bath. The change in rhythm cannot be induced in the untreated muscle by acetylcholine itself. Gut preparations kept at 1°C for 2 days exhibit a typical pendular activity when placed in oxygenated Ringer-Locke at 37.5°C and respond to the esterase inhibitors by an increase in tone but not by the usual change in rhythm. After four days the gut is totally insensitive to the cholinesterase inhibitors and nicotine but responds to acetylcholine, pilocarpine, barium, and epinephrine. The cholinesterase activity of rabbit ileum was found to be stable at 1°C for at least 25 days. It is believed that the conversion from pendular to peristaltic rhythm induced by the cholinesterase inhibitors in the isolated gut depends upon the endogenous synthesis of acetylcholine and the integrity of the intrinsic ganglion cells. The loss of sensitivity in the old intestinal strips might be caused by a degeneration of the ganglion cells and a cessation of acetylcholine synthesis.

Inotropic synergism between digitalis bodies and serum calcium WILLIAM T. SALTER, JOHN GEMMEL (by invitation) and LOUIS SCIARINI (by invitation) *Dept of Pharmacology, Yale Univ School of Medicine, New Haven, Conn.* The hypocalcemic frog heart can be employed with a few ml of human serum or Locke's solution to test the inotropic potency of a cardiac glycoside. By adjustment of the concentration of Ca^{++} ions, graded stages of hypodynamic contraction can be produced serially. Such preparations at pH 7.3 follow the law $R/(100 - R) = [\text{Ca}^{++}] - (A - k[G])$, where R is the percentile contractile response of the heart, $[\text{Ca}^{++}]$ the concentration of calcium ions, A the individual 'heart constant', k the potency of the cardiac glycoside and [G] its concentration. Each preparation is calibrated in standard mammalian Ringer's solution to determine the 'heart constant' A. Then the $[\text{Ca}^{++}]$ is adjusted and the response R measured when the test heart has been equilibrated with the dissolved preparation or serum under test. Finally the value $k[G]$ is calculated. When $R = 50\%$, $k[G] = A - [\text{Ca}^{++}]$. In terms of ouabain, it is difficult to avoid toxic effects at concentrations higher than 5 micrograms %, even when the $[\text{Ca}^{++}]$ is low.

Isopropyl alcohol as an anticonvulsant RALPH W. SCHAFFARZICK (by invitation) and P. J. HANZLIK *Dept of Pharmacology and Therapeutics, Stanford Univ School of Medicine, San Francisco 15, Calif.* The minimum effective blood acetone for anticonvulsant action (cortical threshold by electrical stimulation) in rats receiving acetone gastrically was found to be about 50 mg % which could be nearly doubled and the threshold raised 100% with excessive doses, without ataxia. The minimum effective anticonvulsant blood concen-

tration of alcohol, after isopropyl alcohol gastrically in rats, was markedly irregular. After 500 mg and 1250 mg/kg doses of isopropyl alcohol gastrically, the increase in cortical threshold paralleled more closely the blood alcohol than the blood acetone. When the higher dose of the alcohol was given in 3 divided doses in 12 hours, the acetone was cumulative with peak increases in cortical threshold in 1 hour, recovery at end of 12 hours, and suggestions of tachyphylaxis to the cortical stimulus (rats and rabbits). Generally the acetone outlasted the anticonvulsant action (6-hour observations). Only about 3% (average) of the higher dose of the alcohol was excreted in 24 hours in the urine of rabbits, the ratio of excreted alcohol to acetone being about 2.3 to 1. The share of alcoholemia and acetone in the anticonvulsant action of isopropyl alcohol remains undetermined. Surely convulsant but generally not fatal doses of metrazol (50-70 mg/kg) and picrotoxin (2-3 mg/kg) subcutaneously were partially antagonized by the higher dose of isopropyl alcohol gastrically in the great majority of rats.

Effect of some vasodilators and sedatives on gastric secretion ROBERT B. SCHLESINGER (by invitation), F. STEIGMANN and LEO L. HARDT (by invitation) *Hektoen Inst for Medical Research of Cook County Hospital and the Dept of Therapeutics of Cook County Hospital and the Depts of Internal Medicine, Univ of Illinois, College of Medicine and Stritch School of Medicine, Loyola Univ, Chicago, Ill.* Because some patients have epigastric discomfort after the ingestion of some vasodilator and sedative drugs, the effect of such substances on gastric secretion was studied on 32 patients (21 with duodenal ulcer and 11 controls). The gastric contents were aspirated every 15 minutes and returned to the stomach except 10 cc which were used for titration for gastric acidity. Following the fourth aspiration one of the drugs to be tested was administered. The following drugs were tested, 1) aminophylline, gr VI enteric coated, 2) aminophylline, gr III plain, 3) sodium nitrite, gr I, 4) glucophylline, 4.68 gr, 5) nicotinic acid, 100 mg, 6) butisol tablets, gr ISS (sodium 5-ethyl-5-sec-butyl barbiturate), 7) elixir of butisol fl oz 1 (gr III) given orally, 8) aminophylline, gr 72 and 9) glucophylline 36 gm administered intravenously. Aminophylline and glucophylline intravenously and tablets and elixir of butisol increased the free acid, the total HCl and the volume. Nicotinic acid and aminophylline tablets (gr VI) increased the volume and total HCl. Glucophylline, aminophylline (gr III), and sodium nitrite decreased the free acid, the volume and the total HCl in all but 3 patients. Since aminophylline and glucophylline orally did not increase acidity, increased free acid following intravenous aminophylline (average 21 units) and

glucophylline (average 10 units) may result from central stimulation. The epigastric distress in some individuals is probably due to other causes than increased gastric acidity, although results of single gastric analyses must be cautiously interpreted.

Fluorimetric vs biologic assays (human and animal) of fractions of digitalis purpurea. LOUIS SCIARINI (by invitation) and WILLIAM T. SALTER, *Dept of Pharmacology, Yale Univ School of Medicine, New Haven, Conn.* Tincture of digitalis has been fractionated into 5 arbitrary extracts, based largely on differential solubility in chloroform and rather concentrated solutions of sulfuric acid. To each of these a fluorimetric procedure has been applied and the value compared with Harry Gold's human oral assay and/or with well-substantiated assays by the intravenous cat or pigeon methods. The relative significance of each fluorimetric value, measured against crystalline digitoxin as a standard, is given by the conversion factor F , when $F \times$ the fluorimetric value = biological activity. Even so, a correction must be made for digitoxin. The oral human activity resides almost exclusively in fraction V. The data suggest that the oral potency of digitalis purpurea is due solely to its digitoxin content. For our fraction V, a series of Harry Gold's preparations (in terms of digitoxin per gram of powdered leaf) gave: A) by Gold's human assay, a) 1.14, b) 1.15, c) 1.08, d) 1.43 and e) 1.02, respectively; B) by fluorimetry a) 1.14, b) 88.6, c) 1.09, d) 1.43 and e) 1.07, respectively. Much of the biological activity after intravenous injection of lethal doses into cats or pigeons occurs in fractions II and III. For example, in one preparation the percentile lethal activity (pigeons) in various fractions was: I) 36%, II) 21%, III) 14%, IV) 7% and V) 21%. For such values, a series of conversion factors (based on intravenous, animal, lethal effects) can be determined.

Distribution and excretion of beryllium sulphate using Be^{10} as a tracer. JAMES K. SCOTT and WILLIAM NEUMAN (introduced by H. C. HODGE), *Dept of Radiation Biology, Univ of Rochester, Rochester, N. Y.* Beryllium sulphate was administered intravenously to rats and rabbits; the rate of blood clearance, the urinary and fecal excretion and the distribution to organs was determined by counting techniques. In some cases nonradioactive beryllium sulphate was added to the isotopic beryllium sulphate. More than 95% of the beryllium was cleared from rabbits' blood during the first 5 hours after administration; the rate of clearance was more rapid when isotope alone was administered than when carrier was added. The urinary excretion of beryllium was greatest during the first 6 hours after administration; thereafter small amounts less than 1% were excreted daily. In rabbits and rats a higher % of the isotope was

excreted when isotope alone was given than when carrier was added: 28% and 11%, and 35% and 25% respectively. The total fecal excretion over a 7-day period was approximately 10% of the dose in the rats and 2% in the rabbits. When carrier was administered with the isotope approximately 35% was found in the skeleton of the rat and 51% in the skeleton of the rabbit; when isotope alone was administered the skeletal recovery was 48% and 54%. The amount of isotope recovered from the liver and spleen of the rat was insignificant when isotope alone was administered and amounted to 12% and 2.5% respectively when carrier was added. Significant amounts of beryllium were present in the bone marrow when carrier was administered with the isotope. The kidneys contained less than 1% of the dose.

Tachyphylaxis of sympathomimetic drugs. LLOYD D. SEAGER and WILLIAM WEISS (by invitation), *Depts of Pharmacology and Anesthesiology, Woman's Medical College of Pennsylvania, Philadelphia, Penna.* A comparison was made of a number of sympathomimetic drugs as to their property of showing tachyphylaxis. Blood pressure response in vagotomized dogs and cats was the function studied. Of the drugs studied, the phenomenon was found to occur most readily with desoxyephedrine. In some experiments, complete tachyphylaxis with reversal was found following an initial dose of 1 mg/kg. Oenethyl, propadrine, benzedrine, ephedrine and paredrine were next in order of susceptibility. As tachyphylaxis developed to any of these agents, the animal was found to show diminished or no response to the others of the group. A reversal of blood pressure after repeated dosage occurred most readily with desoxyephedrine and oenethyl but could be produced with large doses of the other compounds. Reversal was usually associated with some slowing of the pulse. Some diminution of the response to neosynephrine, and epinephrine was found after massive doses of ephedrine, desoxyephedrine, benzedrine, oenethyl, and propadrine. Nicotine was usually found to give some increase in blood pressure after complete tachyphylaxis had developed to one of the sympathomimetic agents.

Epinephrine and dibenamine in the alarm reaction. JOSEPH SEIFTER, WILLIAM E. EHRLICH, ALBERT J. BEGANY (by invitation), and GEORGE M. HUDYMA (by invitation), *Wyeth Inst of Applied Biochemistry, and the Graduate School of Medicine of the Univ of Pennsylvania, Philadelphia, Penna.* Colchicine is considered the most potent stimulus available for provoking the alarm reaction, characterized by involution of lymphoid tissue and shock. Since discharge of epinephrine is thought to be the first consequence of exposure to an alarming stimulus, it was of interest to determine whether the administration of epinephrine en-

hances the toxicity of colchicine and whether the administration of an adrenolytic drug decreases the toxicity of colchicine. Results, 1) epinephrine is a less intense alarming stimulus than colchicine, 2) dibenamine (dibenzyl- β -chloroethylamine HCl) produced a mild alarm reaction, which subsided in 24 hours, 3) non-lethal doses of epinephrine combined with non-lethal doses of colchicine resulted in fatality, with extreme alarm reaction, 4) dibenamine protected against the lethal effects of either colchicine or epinephrine, but not against the combination of colchicine and epinephrine, 5) the histological changes of the alarm reaction in the thymus and the adrenals provoked by epinephrine were reversed by dibenamine, but those provoked by colchicine were not.

Effects of certain central nervous system depressants and stimulants on intermediary metabolism of brain. F. E. SHIDEMAN, *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor, Mich.* The results presented here are preliminary data obtained from experiments designed to determine differences in biochemical behavior of 'stimulants' (convulsants) and 'depressants' (anesthetics) as judged by their *in vitro* actions on isolated central nervous system tissue. The effects of a series of central depressants and stimulants on no-substrate oxygen consumption of rat cerebral mince were determined. In no case was there obtained any significant effect other than inhibition. Excellent correlation between relative *in vitro* and *in vivo* potency was obtained in the depressant series of drugs but was not apparent in the stimulant series. A representative member of each group (chloral hydrate and metrazol) was employed for further studies in concentrations which had produced equal inhibitions of the Q_{O_2} of rat cerebral mince. The degree of inhibition of the no-substrate Q_{O_2} of brain mince with both compounds paralleled a) the degree of specialization of the tissue and b) the phylogenetic development of the animal species studied. With glucose, lactate, pyruvate, α -ketoglutarate and fumarate, Δ -substrate oxygen consumptions of rat cerebral cortex mince were equally well inhibited by both chloral hydrate and metrazol. When succinate was added as a substrate the 2 drugs showed a quantitative difference in their effects. Both compounds had the same qualitative effect on anaerobic glycolysis of rat cerebral mince but differed quantitatively. Whereas metrazol increased anaerobic glycolysis 25%, chloral hydrate effected a 100% increase in the $Q_G^{N_2}$.

Production of ventricular extrasystoles in the dog by a polypropylene glycol. F. E. SHIDEMAN and G. K. MOE, *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor, Mich.* A polypropylene glycol with an average molecular weight of 750 produced ventricular extrasystoles when it was administered intravenously in the anesthetized

dog. The total amount required to produce the extrasystoles varied (10 mg/kg to 90 mg/kg) from animal to animal but the duration appeared to depend on the total amount of polyglycol administered rather than on the size of a single dose. In no experiment was it possible to produce ventricular fibrillation either by further administration of polyglycol or by epinephrine, once extrasystoles had been established. Bilateral vagotomy in no way influenced the extrasystoles. Epinephrine or tetraethylammonium chloride in some cases caused transient disappearance of the cardiac irregularity, but not in others. Administered in divided doses in the heart-lung preparation, the polyglycol produced increasing degrees of decompensation and finally complete failure. In this preparation, and in artificially respired animals with the heart exposed, extrasystoles were never produced by the polyglycol alone or combined with peripheral vagal or cardiac sympathetic stimulation. However, when in the open chest preparation, the respiratory volume was reduced to a point where the animal displayed respiratory efforts, extrasystoles were readily induced and could be abolished by increasing the respiratory volume. Such findings would suggest that the sensitivity of the heart to this polypropylene glycol is dependent in some way on a relative anoxia.

Distribution effect and fate of oil aerosol particles after retention in the lungs of mice. MILTON SHOSHKES, WILLIAM G. BANFIELD, JR., and S. JOSEPH ROSENBAUM (introduced by AMEDEO S. MARRAZZI), *Toxicology, Pathology, and Aerosol Sections, Medical Division, Army Chemical Center, Md.* Mice were exposed to mists of corn, peanut, cod liver, mineral, and motor oils, with an average mass median diameter of 2.5μ in dynamic chamber-type experiments. The general dispersion of the retained oil droplets in the lung was found to be equal from apex to base and from hilus to pleura, and the highest concentration was noted in and around the terminal bronchioles and alveolar ducts. In a statistical analysis of the distribution and size of corn oil particles retained in the lung, it was noted that 80% of all the particles retained in all areas of the lung were 2.5μ or less in diameter. The initial concentrations of retained oil droplets in the lung after exposure to all the oil mists were similar. However, during a 96-hour period of observation, the concentration of the edible oils decreased progressively, while the concentration of the paraffin oil droplets remained essentially unchanged. Except for the appearance of scattered macrophages, no acute inflammatory changes were seen after a 2-hour exposure to these oil mists within a 96-hour observation period. After heavy exposures, mineral and motor oil droplet retention resulted in localized foreign body reactions of moderate severity as well as infrequently occurring

patches of lipid pneumonia. Cod liver oil droplets caused a moderate foreign body reaction and only negligible reactions to corn and peanut oil were seen.

Effect of renal dysfunction on the urinary excretion of fluoride in the rabbit F A SMITH and D E GARDNER (introduced by H C HODGE) *Dept of Radiation Biology, Univ of Rochester, Rochester, N Y*. The effect of uranium-produced nephritis on the urinary excretion of ingested fluoride has been studied in rabbits receiving 1) a subcutaneous injection of 0.3 mg U/kg as uranyl nitrate, 2) the same dosage of uranium in addition to 15 ppm fluoride in the drinking water, and, 3) 15 ppm fluoride in the water supply. Control urinary fluoride excretion was determined. Inspection of the analytical data shows that within 3 to 4 days after injection of the nitrate, the urinary excretion of fluoride decreased to a level only $\frac{1}{2}$ as great as obtained prior to the uranium injection. Twelve days after the injection, the urinary fluoride level was again normal in those rabbits which were not receiving fluoride in the drinking water, only 4 days were required for the return to normal levels of urinary excretion in those animals whose water supply contained added fluoride. Fluoride content of the tooth and bone indicated that the presence of uranium in the kidney may inhibit the deposition of ingested fluoride in the bone and thus offer a degree of protection against fluorosis. The fluoride contents of the tooth root, femoral epiphysis and jaw alveolar bone for rabbits receiving only added fluoride were significantly higher than in comparable tissues from rabbits treated with both uranium and fluoride, despite the greater quantity of fluoride consumed by these animals. Other supporting evidence for the protective effect of added fluoride on uranium poisoning was 1) a 2.5-fold lower blood N and, 2) lowered mortality.

Rate of removal of alcohol in acutely intoxicated chronic alcoholics H W SMITH and R G BELL (introduced by J K W FERGUSON) *Dept of Pharmacology, Univ of Toronto and Shadowbrook Health Foundation, Toronto, Canada*. The rate of removal of alcohol from the blood of 21 chronic alcoholics prior to treatment with intravenous insulin (40 U zinc insulin), glucose (50 ml of 50%) and 'Betalin' (Lilly, 2 ml) was compared to the rate during treatment. This rate was increased during treatment by 9.9% ($S^2 = 3.3\%$) when the rate was calculated on the basis of % of the amount of alcohol present. Seven untreated patients showed no change in the hourly rate of removal of alcohol from the blood during comparable periods of study.

Effects of tetraethylammonium chloride on pain thresholds in man RALPH R SONNENSCHN (by invitation), ELIZABETH H JENNY (by invita-

tion) and CARL C PFEIFFER *Depts of Pharmacology and Clinical Science, Univ of Illinois College of Medicine, Chicago 12, Ill*. The intravenous injection of 500 mg of tetraethylammonium chloride (9 trials on 4 subjects) raised significantly the pain threshold of the finger pad, lowered that of the nail bed, and left practically unaffected that of the tooth. Thresholds of the nail bed and pad of the 3rd finger were determined by a radiant heat device. Tooth thresholds were determined by electrical stimulation of the pulp through a metal filling, both the perceptive threshold (TI) and affective threshold (TII) were measured. Over a 30-minute period following injection, pad threshold showed an average maximum rise of 14% ($P < 0.001$), at this point, 10 minutes after injection, the nail threshold was decreased by 6% ($P < 0.02$). No consistent, significant change was seen in tooth thresholds. Preliminary experiments (Pfeiffer et al *Ann N Y Acad Sci* 51: 21, 1948) reveal that procaine block of the stellate ganglion, with Horner's syndrome, causes a rise in nail and fall in pad threshold. It therefore is unlikely that the above action of TEA is due to ganglionic blockade. Block of the median nerve with 0.5% procaine causes a rise in pad and fall in nail threshold. The effect of TEA is another example of this reciprocal relationship. The evidence indicates that pain of nail bed and pad is mediated by 2 separate mechanisms. The primary action of TEA in raising pad threshold may be through a selective peripheral or central effect on the underlying mechanism for pain from this area.

Clinical criteria in beryllium poisoning C J SPIEGL, F A SMITH, L J LAFRANCE, J W KEATING (introduced by H C HODGE) *Dept of Radiation Biology, Univ of Rochester, Rochester, N Y*. At present no reliable biochemical methods exist for the diagnosis of early or mild forms of beryllium poisoning. Accordingly a broad survey of clinical criteria was made in animals undergoing exposure. This included urinary protein and amylase, blood nonprotein nitrogen, urea nitrogen, serum protein, serum albumin-globulin ratio, fibrinogen, serum calcium, phosphorus, acid and alkaline phosphatase, arterial oxygen tension, 10 lipid fractions of the blood with special attention to phospholipid and free cholesterol concentrations in the red cells. Dogs and rabbits were studied that had been exposed to beryllium sulfate at concentrations ranging from 1 to 100 mg/m³ and beryllium oxide at from 10-85 mg/m³ for periods up to 100 days. Of the many criteria applied, arterial oxygen tension and red cell concentrations of free cholesterol and phospholipid gave indication of potential value as clinical procedures in following the course of beryllium injury. Many of the other criteria failed at lower levels of injury.

Cocaine-barbiturate antagonism JOHN E

STEINHAUS (introduced by A L TATUM) *Dept of Pharmacology, Univ of Wisconsin Medical School, Madison, Wis* A quantitative study of cocaine-barbiturate antagonism was undertaken to evaluate the effectiveness of prophylactic doses of barbiturate for toxic reactions of local anesthetics A series of young, male, New Zealand, white rabbits (1.5 to 2.5 kg) were injected subcutaneously with doses of cocaine ranging from 100 to 300 mg/kg They were then treated intravenously with various doses of pentobarbital at different time intervals with respect to the cocaine injection It was found in these studies that higher doses of cocaine required correspondingly higher doses of pentobarbital With symptomatic treatment the average dose of pentobarbital required for rabbits receiving 300 mg/kg of cocaine was almost double that required for animals in the 100 mg/kg series The time of pentobarbital administration for maximal effectiveness was at the time of cocaine injection Treatment 45 minutes before or 15 minutes after this time was ineffective When 100 mg/kg (the generally accepted LD₅₀) of cocaine was given subcutaneously, 15 mg/kg of pentobarbital resulted in 100% survival, whereas 5 mg/kg gave no protection These data suggest that the protection afforded by barbiturate depends upon the degree of depression and that approximately $\frac{1}{2}$ of the anesthetic dose is required for adequate protection This would make it appear questionable that the commonly used 'prophylactic' dose (0.1 to 0.2 gm orally) gives adequate pharmacodynamic protection

Comparison of the acute toxicity of hydroquinone and some related di and trihydroxy benzenes JAMES H STERNER (by invitation), STANLEY AMES (by invitation) and DAVID W FASSETT *Eastman Kodak Company, Rochester, N Y* Recent developments in use of polyhydroxy benzenes as antioxidants in fats for human consumption have stimulated an interest in their relative toxicologic properties The acute oral and parenteral toxicity of hydroquinone (HQ), nordihydroguaiaretic acid (NDGA), n-propyl gallate (NPG) and butylated hydroxy anisole (BHA) reveals certain marked differences with respect to onset and character of symptoms, time of death, and a wide spread between the ratio of parenteral and oral LD₅₀ values In the white rat, the approximate LD₅₀ in mg/kg intraperitoneally were NDGA—70, HQ—168, BHA—250, NPG—250, orally, the values were HQ—370, NDGA—2400, NPG—2500, BHA—2500 The rapid onset of symptoms and the occurrence of death within 30 minutes with hydroquinone contrasted sharply with the delayed onset of symptoms and marked variability in time of death with the other antioxidants which varied from a few minutes to 7–10 days The apparent recovery of the hydroquinone survivors was very rapid, a matter

of an hour or two, compared with the prolonged inanition and debility of the survivors of the animals given NDGA, BHA, and NPG These results suggest a fundamental difference in excretion and metabolism of hydroquinone (a normal excretory product in urine) and the compounds of more complex structure

Pharmacologic action of chlordane E F STOHLMAN, W T S THORP and M I SMITH *Experimental Biology and Medicine Inst, National Insts of Health, Bethesda, Md* The acute toxicity, LD₅₀ of chlordane in rats was found to be approximately 250 mg/kg when given orally in olive oil or Tween 20 The LD₅₀ of DDT under similar conditions is 150 mg/kg On intraperitoneal injection chlordane in Tween 20 was slightly more toxic than when given in olive oil In rabbits the LD₅₀ of both compounds is approximately 300 mg/kg Death from chlordane poisoning may be delayed for many days Chronic toxicity of chlordane was considerably greater in both rats and rabbits than that of DDT under similar conditions, probably due to greater cumulative effects of chlordane Repeated administrations of chlordane orally produced in rabbits focal necrosis of the liver, degenerative changes in the proximal convoluted tubules of the kidneys, and pulmonary exudates in the alveoli and bronchioles of the lungs, whereas with large single doses the primary effect appeared to be on the lungs, producing focal hemorrhages Organically bound chlorine was excreted in the urine of rabbits to the extent of about 18% of the dose given, most of it during the first 4 days following administration Significant amounts of organic chlorine were excreted in the urine of rabbits following 25 mg/kg or more chlordane

Acute inhalation toxicity of beryllium sulfate in animals H E STOKINGER, G F SPRAGUE, R H HALL, J K SCOTT, F A SMITH, B H BROWN, and A PETTINGILL (introduced by H C HODGE) *Dept of Radiation Biology, Univ of Rochester, Rochester, N Y* The acute toxicity from inhalation of beryllium sulfate mist in animals was determined in large-scale experiments wherein the toxic limits of exposure and the characterization of the acute response were established A series of 4 concentrations (100, 50, 10 and 1 mg BeSO₄ 6H₂O/cu m of air) were tested in daily 6-hour exposures for periods of from 14–105 days Particle size of the mist was from 0.5 to 1.3 μ Marked species variation in response was noted At levels of 100 and 50 mg/m³, 80–100% mortality occurred during week 1–6 in the cat, dog, rat, goat and monkey, 60% (week 1–2) in the guinea pig, 10–50% (week 2–5) in the rabbit, mouse and hamster, all swine and fowl survived Over-all mortality, chiefly confined to the rat, at 10 mg/m³ was 14%, at 1 mg (0.04 mg Be/m³) no mortality was observed in any species and weight losses were recovered at the

end of 90 days' exposure although certain evidences of poisoning were still present. The monkey, dog, cat and rabbit showed acute pulmonary inflammatory response, resembling that in man, the rat gave an atypical response. Effects that were severe and rapid in onset at the 100 and 50 mg -levels were minimal and slower in developing at the 10 mg -level changing to moderate with continued exposure and, at the 1 mg-level, were nonexistent in certain species at the end of 105 days' exposure. Highest levels produced severe proteinuria and changes in blood protein values, not apparent at lower levels. At the lowest level, reduced blood oxygen tension and alteration in blood cell lipid ratios occurred. A hypochromic macrocytic anemia appeared in the dogs. Beryllium concentrations in 20 tissues were determined. A control study with NaHSO_4 must show that the lesions produced in BeSO_4 exposures were due to beryllium and not to the sulfate ion.

Protective effect of certain drugs on epinephrine-induced pulmonary edema. CLEMENT A. STONE (by invitation) and EARL R. LOEW, *Dept of Physiology, Boston Univ School of Medicine, Boston, Mass.* Studies were made with certain drugs to determine their ability to prevent the pulmonary edema in rabbits following an intravenous injection of 0.375 mg/kg of epinephrine as the base. Protective drugs were injected intravenously 10 minutes prior to the epinephrine. The indication of efficacy was the presence of a statistically significant reduction in lung weight and/or mortality from that of a series of concurrent control experiments. Previous reports (Luisada, *Arch f exper Path u Pharmacol*, 132: 313, 1928) indicate that hypnotics in general are effective in reducing mortality due to epinephrine. Pentobarbital sodium (25.0 mg/kg) in our experiments was ineffective in preventing the hemorrhage and edema of the lungs, which is the usual cause of death after large doses of epinephrine. Atropine sulfate (5.0 mg/kg) significantly diminished the increase of lung weight, but not mortality, as compared to controls, but was not as effective as N-(2-chloroethyl)-N-ethylbenzhydramine HCl (SY-2, 2.5 mg/kg) or N-(2-bromoethyl)-N-ethyl-1-naphthalenemethylamine HBr (SY-28, 0.25 mg/kg). These drugs are potent adrenergic blocking agents, SY-28 having, in addition, a marked ability to antagonize histamine. The complete protection afforded by SY-2 and SY-28 is probably related to their ability to annul the acute hypertensive action of epinephrine. The failure of Neoantergan (2.5 mg/kg), a potent and specific antihistaminic, to protect indicates that histamine does not play a prominent role in the edema formation. The protective effect of relatively large doses of the anti-histaminic, Phenergan or 3277 R.P. (*Acta allergologica*, 1: 97, 1948) may be related to other

properties, among which is an appreciable atropine-like action.

Hypotensive activity of fractions from green Hellebore. J. W. STUTZMAN, GEORGE L. MAISON and G. W. KUSSEROW (by invitation), *Dept of Pharmacology, Boston Univ School of Medicine, Boston, Mass.* and *Product Development Dept., Rezell Drug Company, Los Angeles, Calif.* Fifty fractions of green hellebore were tested for hypotensive activity in normotensive dogs which were unanesthetized or lightly anesthetized with pentobarbital sodium. On intravenous administration the fractions were found to fall into three groups on the basis of hypotensive action. One group did not alter mean arterial pressure, a second caused a gradual minimal fall, and a third an abrupt fall with slow recovery over several hours. The last group included both water soluble and organic solvent fractions. In this group hypotension was accompanied by bradycardia of short duration. Minimal effective intravenous dosage was 0.002 mg/kg from a fraction which represented 0.35% of the crude drug. Potent fractions were effective orally at a dosage of 0.2 mg/kg. Toxic effects of high doses included retching, emesis, apnea, hypertension and convulsions.

Comparative anticonvulsant potencies of some phenylhydantoins and their corresponding phenylacetylureas. EWART A. SWINYARD and JAMES E. P. TOMAN, *Depts of Pharmacy, Pharmacology and Physiology, Univ of Utah College of Medicine and College of Pharmacy, Salt Lake City, Utah.* Anticonvulsant potencies (ED_{50}) of 5-phenyl-5-R-hydantoins (h) and their corresponding phenyl-R-acetylureas (a) were determined by their ability to modify maximal electroshock seizure pattern and to prevent Metrazol convulsions in rats. Drugs were given orally in 10% neutral acacia suspension and tested at the previously determined time of peak drug effect. Minimal neurological toxicity (TD_{50}) was determined, and protective indices ($\text{P.I.} = \text{TD}_{50}/\text{ED}_{50}$) were calculated. Maximum potency occurred with alkyl substitutions. Unsubstituted phenylacetylurea (Phenurone) was superior to its hydantoin homolog, whereas diphenylacetylurea was inactive in contrast to diphenylhydantoin (Dilantin). Studies on the metabolic fate of these compounds might help to identify a common denominator as the effective product.

Purification of diamine oxidase (histaminase). HERBERT TABOR, *Experimental Biology and Medicine Inst., National Insts of Health, Bethesda, Md.* A highly purified, stable diamine oxidase has been obtained from an extract of hog kidney acetone powder with the aid of a new colorimetric procedure for the estimation of histamine (Rosenthal and Tabor, *J Pharm Exp Therap*, 92: 425, 1948). The acetone powder was extracted with

0.2M phosphate buffer pH 7.2, and fractionated with Na₂SO₄ (14.5-21%). This fraction was dialyzed, heated to 60°C for 20 minutes, adsorbed on alumina gel C₇, and eluted with 0.1M K₂HPO₄. The dialyzed eluate was fractionated with ethanol at 0°C, and dried *in vacuo*. This dry preparation is 500 times more active per mg protein than the original extract, and represents a yield of approximately 25% 0.006 mg of this purified powder destroys 0.03 mm of histamine in 5 minutes in an incubation mixture containing 0.1 mm of histamine and 1.3 mg of crystalline bovine albumin (as a protective protein) in 3 cc of 0.1 M phosphate buffer pH 7.2 (37°C). Further purification can be obtained by adsorption on calcium phosphate gel and elution with phosphate buffer. The action of diamine oxidase on histamine probably results in destruction of the imidazol ring, since the products give no color with diazotized p-nitro aniline. In the destruction of putrescine and cadaverine, neither succinic acid nor γ -amino valeric acid, respectively, could be detected. The mechanism of breakdown of these diamine compounds is being further investigated.

Comparative study of the local toxic action of thiomerin, mercuzanthin and mercurhydrin. HARRY TAUBE (by invitation), Robert A. LEHMAN and E. E. KING (by invitation) *Dept of Therapeutics, New York Univ College of Medicine, New York City*. The mercurial diuretic thiomerin, (di-sodium salt of N-[gamma-carboxymethyl-mercaptomercuri, beta-methoxy] propylcamphoramic acid), has been compared with mercuzanthin and mercurhydrin with respect to the gross pathology produced following subcutaneous injection in the abdomen of the mouse and the histopathology occurring after intramuscular injection in the Tibialis anterior muscle of the rat. It was found that thiomerin was tolerated by the mice without gross pathologic changes while the other 2 drugs gave rise to varying degrees of necrosis under the same conditions. Intramuscular injection in the rat was selected for the histologic studies because of the greater homogeneity of the tissue and accuracy in the administration of the drugs. Results were put on a semi-quantitative basis by scoring each muscle according to the type and extent of response. Serial sections were prepared in every instance and were examined throughout by the same pathologist without foreknowledge of the treatments given. All 3 drugs were found to elicit an early inflammatory response characterized by the appearance of a polymorphonuclear infiltrate. In the case of thiomerin this exudate was entirely resorbed within 96 hours without evidence of residual damage. Ninety-six hours after injection of mercuzanthin or mercurhydrin there was evidence of marked fibroblastic proliferation indicating the irreversible character of the initial reaction to these drugs.

The relationship of chemical constitution to local toxicity will be discussed.

Comparison of local anesthetic activity of certain aliphatic amines. HERBERT W. TAYLOR, JR (by invitation), EDWIN J. FELLOWS, EDWARD MACKO (by invitation) and GLENN E. ULLYOT (by invitation) *Research Division, Smith, Kline and French Labs, and Dept of Pharmacology, Temple Univ School of Medicine, Philadelphia, Penna*. The comparative local anesthetic activity and irritant properties of 32 volatile aliphatic amines have been determined in rabbits by topical application of solutions of the free bases and neutral salts.

Both anesthesia and irritation decreased as the molecular weight was decreased in homologous series and as the point of attachment of the nitrogen was moved toward the center of the carbon chain. The effect of alkylation of the nitrogen or of the carbon chain was irregular. Among these compounds 2-methylaminodecane and 2-amino-2-methyloctane were found to be very active anesthetic agents in concentrations which did not produce irritation.

Effect of tridione (3,3,5-trimethyl oxazolidine-2,4-dione) on the respiration of mouse brain. J. D. TAYLOR (by invitation), R. K. RICHARDS, and G. M. EVERETT (by invitation) *Dept of Pharmacology, Abbott Research Labs, North Chicago, Ill*. The effect of Tridione on the O₂ uptake of mouse cerebral cortex slices in Krebs-Ringer phosphate solutions containing different substrates was determined by means of the Warburg technique. With glucose, pyruvate, lactate, succinate, acetate, citrate and glutamate as substrates, only glucose showed a significant inhibition with 3.5×10^{-2} M Tridione. At 7×10^{-2} M concentration of Tridione, oxygen consumption was inhibited in all substrates. Inhibition in glucose was the greatest (32%) and acetate the least (17%). In 1.4×10^{-1} M Tridione, inhibition ranged from 62 to 76% depending on the substrate, except for succinate and citrate where inhibition was 33 and 54% respectively. The depression of oxygen uptake by 7×10^{-2} M Tridione in glucose was removed by washing the brain slices after 30 minutes exposure to the drug. The addition of p-phenylenediamine to the inhibited respiring brain slices partially restored the oxygen uptake but glutathione, adenosine triphosphate and creatine were ineffective. Tridione in concentrations up to 7×10^{-2} M did not inhibit anaerobic CO₂ production of mouse brain brei in Krebs Ringer-bicarbonate. With 1.4×10^{-1} M Tridione there was 26% inhibition. Statistically significant inhibition of O₂ uptake in glucose was also observed in brain slices taken from mice 30 minutes after intraperitoneal injection of 1 or 2 gm/kg of Tridione. Calculations show that the amounts of Tridione used in these Warburg studies are within the pharmacological range of those used *in vivo*.

Determination of diethylstilbestrol in urine

R S TEAGUE, CHRISTIAN WINGARD (by invitation), and ALBERT E BROWN (by invitation) *Dept of Pharmacology, Medical College of Alabama, Birmingham, Ala* A method for the extraction and determination of free and conjugated diethylstilbestrol in urine using the Dingemans antimony pentachloride reaction has been developed. The method consists of the following steps. Urine is acidified and extracted with ether. Free and conjugated diethylstilbestrol are separated by partitioning between ether and aqueous sodium bicarbonate. The free material is then purified by washing the ether solution with aqueous sodium carbonate, extraction with normal sodium hydroxide, neutralization to pH 11 with phosphoric acid, and extraction with ether. The ether is evaporated and the residue taken up in ethanol. Aliquots of the ethanol solution are evaporated, the residues dissolved in absolute anhydrous chloroform, antimony pentachloride is added and the density of the resulting color estimated photoelectrically. The bicarbonate solution containing conjugated diethylstilbestrol is acidified with phosphoric acid and extracted with ether. The ether is removed, the residue taken up in decinormal sodium hydroxide, and brought to pH 2.8 with phosphoric acid. Hydrolysis is accomplished by autoclaving aliquots of this solution at 180° for 30 minutes. The solutions are then extracted with ether and purified as for free diethylstilbestrol. Recoveries of diethylstilbestrol added to urine have been near 100% and of added diethylstilbestrol glucuronide 90%. In the case of a patient to whom a dose of 100 mg of crystalline diethylstilbestrol was given orally, the urinary excretion in four days was found to be 8.0 mg of free diethylstilbestrol, 6.1 mg conjugated, a total of 14.1 mg.

An attempt to systematize the actions of epinephrine substitutes on smooth muscle organs
C H THIENES, JACK LANLEY (by invitation) and WAITER MATTISON (by invitation) *School of Medicine, Univ of Southern California, Los Angeles, Calif* A series of compounds chemically related to epinephrine have been tested on isolated small intestine, colon, and uterus of dog, cat, rabbit, rat, and guinea pig and their actions compared with the effect of epinephrine. No consistent relations could be demonstrated, except with arterenol, cobefrine, epinine, metasynephrine, metahydroxy-propadrine, and parahydroxy-propadrine, all of which had actions quite similar to epinephrine on all tissues. The remainder of the compounds were inconsistent in that on some types of organ, or organs of a given animal, the actions were similar to those of epinephrine, on others dissimilar, some compounds were even inconsistent to the extent of

causing contraction of an organ at one time and relaxation at another.

Thiocyanate excretion JESSELENE THOMAS (introduced by MARK NICKERSON) *Dept of Pharmacology, Univ of Utah College of Medicine, Salt Lake City, Utah* The use of thiocyanate in hypertensive patients is frequently accompanied by toxic reactions which persist for some time after the drug is discontinued. However, little is known concerning the mechanism of, or methods of promoting, thiocyanate excretion. The fact that thiocyanate and halogen distribution in the body are essentially the same suggested that renal thiocyanate excretion might be related to chloride in the same manner as bromide. Sodium thiocyanate calculated to give extracellular fluid levels of 15 mg % was injected intravenously into dogs under pentobarbital anesthesia. Diuresis was established by means of water orally, or 5% glucose or 1.8% sodium chloride solution intravenously. Blood and urine samples were collected at intervals and analyzed for thiocyanate and chloride. Sixty thiocyanate clearances provided a range of values from 0.1 to 7.1 cc/minute. Chloride excretion ranged from 0.003 to 1.42 mEq/minute and urine flow from 0.8 to 9.9 cc/minute. Thiocyanate clearances were plotted against chloride excretion for each animal, and a straight-line relationship was obtained in every experiment. Partial coefficients of correlation were found to be +0.92 (P < 0.02) for the relationship of thiocyanate clearance to chloride excretion, and only +0.33 for the relationship of thiocyanate clearance to urine flow. It is concluded that renal excretion of thiocyanate is essentially the same as that of chloride. Thiocyanate excretion may be greatly increased by the administration of NaCl.

Cytological effects of alloxan in intact and thymectomized rats

THURLO B THOMAS (by invitation), PAUL L EWING and G A EMERSON *Dept of Zoological Sciences, Carleton College, Northfield, Minn, and Dept of Pharmacology, Univ of Texas Medical Branch, Galveston, Texas* Thymectomy has been shown to increase the susceptibility of rats to the diabetogenic effects of 0.188-0.25 mm/kg of alloxan given intravenously, neither thymectomized nor intact rats become hyperglycemic after 0.125 mm/kg (*Fed Proc*, 6:328, 1947). The cytology of the pancreas, liver, spleen, kidney, lung, adrenal, pituitary, lymph node, thymus and testis has been studied in the above thymectomized and intact rats. The principal cytological effect of alloxan in diabetogenic doses is marked and widespread reduction in nucleoprotein reserves. In thymectomized and intact rats fasted for 24 hr before and for 12 hr following alloxan injection, the cytological differences were slight, they involved a somewhat more severe loss of chromatin from the nuclei and ribonucleoprotein

from the cytoplasm of cells in the thymectomized animals. The cytological reaction in both of the above groups of rats was much more severe than in intact animals fasted for 40 hr before and only 4 hr after alloxan. Non-diabetogenic doses of alloxan had cytological effects in both intact and thymectomized rats differing only in degree from the above reactions. There appeared to be no direct correlation between the severity of alloxan diabetes, as measured by the blood sugar response, and the extent to which nucleoprotein reserves had been depleted.

Toxic effects of parenterally administered cetylpyridinium chloride. CHARLES R. THOMPSON (by invitation) and HAROLD W. WERNER, *Pharmacology Dept., Research Labs., Wm. S. Merrell Co., Cincinnati, Ohio*. Although quarternary ammonium salts are finding many types of applications clinically there are no data on possible toxic effects of repeated parenteral administrations to laboratory animals. Therefore, the effects of repeated parenteral administrations of one quarternary ammonium salt, cetylpyridinium chloride (Cepyrin Chloride) were investigated in rats and dogs. Daily subcutaneous and intraperitoneal administrations of cetylpyridinium chloride to rats for three weeks in doses of 2.5 mg/kg decreased the rate of growth. No gross or microscopic tissue alterations were observed which could be attributed to drug administration. The subcutaneous administration of cetylpyridinium chloride to dogs as a 2% aqueous pyrogen-free solution for 1 week in daily doses of 4 mg/kg caused local abscess formation, hematologic alterations, and toxic changes in the livers and kidneys. Dogs, in which perivascular damage followed the intravenous administration of 0.5% and 2% solutions in daily doses of 1 and 4 mg/kg for 3 weeks exhibited similar toxic changes. The intravenous perfusion of cetylpyridinium chloride in doses of 4 mg/kg as a non-irritating 0.1% solution caused no local tissue damage, hematologic alterations, or toxic liver and kidney changes. The systemic toxicity of parenterally administered cetylpyridinium chloride appears to be correlated with the local necrotizing action of the compound.

Comparison of the actions of chloramphenicol and penicillin G against experimental relapsing fever. PAUL E. THOMPSON and MARY C. DUNN (introduced by A. C. BRATTON, JR.), *Research Labs., Parke, Davis and Co., Detroit, Mich.* Chloramphenicol (chloromycetin, Parke, Davis & Co.) exhibited marked antispirochetal activity when tested against standardized infections of *Borrelia novyi* in mice (using slight modifications of the methods of Richardson, Walker, Loeb and Miller, *J. Pharmacol. and Exp. Therap.* 85 (1) 23, 1945). Evidence of specific activity was indicated by suppression of the blood infection and

reduction of mortality among infected animals. Following these observations in preliminary studies, the activity of chloramphenicol was compared on a weight basis with that of crystalline penicillin G in a series of parallel experiments. Chloramphenicol was approximately $\frac{1}{4}$ as active as penicillin G in suppressing blood infections. However, comparison of the protective action of the drugs against lethal doses of spirochetes revealed that chloramphenicol was approximately $2\frac{1}{2}$ times as active as penicillin G.

Pharmacological evaluation of Trimeton, 1-Phenyl-1-(2-pyridyl)-3-N,N-dimethylpropylamine, and Chlor-Trimeton, 1-(p-Chlorophenyl)-1-(2-pyridyl)-3-N,N-dimethylpropylamine. RICHARD TISLOW, ANNETTE LABELLE, ALOIS J. MAKOVSKY, MARY ANN GRIFFITH REED, MARGARET D. CUNNINGHAM, JANE F. EMELE, ARNOLD GRANDAGE and RUTH J. M. ROGGENHOFFER (introduced by H. B. HAAG), *Biological Research Labs., Schering Corporation, Bloomfield, N. J.* Chlortrimeton and Trimeton (*Abstr. 1948 Chicago Meeting, Am. Chem. Soc.*, p. 4K), Pyribenzamine and Benadryl were compared for antihistamine potency (preventing death from 1.1 mg/kg histamine hydrochloride injected intravenously 1 hour later) and toxicity in guinea pigs. In dogs, Chlor-Trimeton potentiated the epinephrine increase of blood pressure, inhibited the depression of histamine but not of arecoline. Lethal doses of the antihistamine compounds upon intravenous infusion at 25 mg/kg/hour were

Compound	No of Dogs	Lethal Dose (mg/Kg)	σ
Trimeton hydrochloride	4	89.0	± 12
Chlor-Trimeton maleate	3	97.6	± 2
Chlor-Trimeton hydrochloride	3	58.8	± 7
Pyribenzamine	4	48.8	± 14
Benadryl	2	91.7	± 10

Taking the therapeutic index of Pyribenzamine as 1, the value for Trimeton is 4 and Chlor-Trimeton maleate 50. The chlorination of Trimeton in the phenyl ring results in a twenty fold increase in activity without any appreciable change in toxicity. This is in contrast to Benadryl, Pyribenzamine and Thienylene, the halogenation products of which show no such marked increase in activity.

Effect of convulsant and anticonvulsant agents on the activity of carbonic anhydrase. CLARA TORDA and HAROLD G. WOLFF, *New York Hospital and the Depts. of Medicine (Neurology) and Psychiatry, Cornell Univ. Medical College, New York City*. The concentration of carbon dioxide (carbonic acid, bicarbonates) available for metabolic processes in the brain may be an essential factor in the induction and prevention of convulsive seizures according to Lennox and collaborators. The effect of certain convulsant and anticonvulsant

agents on the activity of carbonic anhydrase was investigated to ascertain whether these substances exert any effect on the carbon dioxide uptake and release as effected by this enzyme. The activity of carbonic anhydrase was inhibited in the presence of the convulsant agents used (acetylcholine, camphor, dichloro-diphenyl-trichloro-ethane, digitoxin, pentamethylene tetrazol, picrotoxin, scilliroside, strychnine, and ouabain) in concentrations of $1 \times 10^{-4}M$ and less, the greatest inhibition being, on the average, 54%. The activity of carbonic anhydrase increased in the presence of the anticonvulsant agents used (hydantoin, methyl-phenyl-ethyl hydantoin, and phenobarbital) in concentrations of $1 \times 10^{-4}M$ and less, the greatest increase of activity being, on the average, 50%. The results presented suggest that convulsant agents, by inhibiting the activity of carbonic anhydrase (from blood and probably from brain), decrease the removal of metabolic endproducts from brain. Furthermore, anticonvulsant agents, by increasing the activity of carbonic anhydrase (from blood and probably from brain), increase the removal of the above mentioned metabolic products from brain.

Determination of beryllium in biological material T. Y. TORIBARA, W. F. NEUMAN, A. L. UNDERWOOD and M. W. CUCCI (introduced by H. C. HODGE) *Dept of Radiation Biology of the Univ of Rochester, Rochester, N. Y.* Beryllium administered intravenously is very toxic, the LD_{50} is approximately 0.36 mg/kg. Thus for distribution and excretion experiments, not more than 0.2 mg/kg can be administered. At such a dose level, many tissues may be expected to contain 0.1 microg/g or less. Thus, any procedure, to be useful, must detect with accuracy much less than one microgram. Chemical methods are not as sensitive as the spectrographic method for the determination of beryllium, but they are adequate for many purposes. Existing methods are too sensitive to pH, salt concentration and the presence of other metals. Because of these reasons and also because of the small amounts being sought, the isolation of beryllium is just as important as the determination. This work seeks to improve both the separation and the method of determination. One fluorometric and 5 colorimetric methods of determination and procedures for isolation of small amounts of beryllium will be discussed.

Synergistic effect of sodium acetate and ethanol in antagonizing sodium fluoroacetate (1080) poisoning in mice W. W. TOURTELLOTT (by invitation) and J. M. COON. *Univ of Chicago Toxicity Lab and the Dept of Pharmacology, Univ of Chicago, Chicago, Ill.* Following the suggestive results of Hutchens et al (*J Pharmacol*, in press) Carworth farm mice weighing 18-22 gm were poisoned with 1080 subcutaneously and immedi-

ately treated intraperitoneally with ethanol, sodium acetate, or sodium acetate dissolved in ethanol. The LD_{50} of the 1080 powder used in these experiments was found to be 17.0 mg/kg. The most effective combination of ethanol and sodium acetate found for antagonizing 1080 poisoning was 2.0 gm (16% solution) to 3.0 gm (24% solution) of sodium acetate/kg injected with 1.6 gm of ethanol (20% by volume)/kg. This saved 90-100% of the mice poisoned with 10 LD_{50} of 1080. When poisoned mice were treated immediately with 1.6 gm/kg of ethanol or 3.0 gm/kg of sodium acetate alone, then the LD_{50} of 1080 was raised by a factor of about 3 (54.0 mg/kg) or a factor of about 4 (63.0 mg/kg), respectively. However, when poisoned mice were treated immediately with 3.0 gm/kg of sodium acetate dissolved in 1.6 gm/kg of ethanol, then the LD_{50} was raised by a factor of 12 (201.0 mg/kg). These data indicated that ethanol and sodium acetate acted synergistically in antagonizing 1080 poisoning. When treatment was given at 3, 7, 10, and 22 minutes after poisoning, the LD_{50} was raised by factors of approximately 8, 4, 3, 2, respectively. The effect of treating poisoned guinea pigs, rabbit, and dogs with ethanol-acetate solutions and barbiturates will be discussed.

Influence of ethyl chloride spray on deep pain and ischemic contraction of skeletal muscle J. TRAVELL, and S. H. RINZLER, (by invitation) *Dept of Pharmacology, Cornell Univ Medical College, and Cardiovascular Research Unit of Beth Israel Hospital, New York City.* Since painful skeletal muscle spasm may be relieved by ethyl chloride spray, we investigated its effects on deep pain and muscular endurance during ischemia produced by a cuff on the upper arm. Endurance was measured by the number of times the subject could close the fingers during ischemia. A constant rate of 30 isotonic contractions per minute was maintained by a metronome. 1) After a series of control measurements, one arm was sprayed with ethyl chloride for 5 minutes and endurance determined again on both arms. Tests were run 20 minutes apart, when determined at such intervals on the same day, endurance remains relatively constant. Amounts of ethyl chloride used did not cause frosting, cold pain, or loss of cutaneous sensibility to pinprick. Results indicate that one such application of spray may double muscular endurance during ischemia. 2) After endurance was determined for 1 arm, without releasing pressure in the cuff, the forearm was similarly sprayed with ethyl chloride for 1 minute and contractions resumed until pain again became intolerable. The procedure was repeated on the other arm, except that a rest period of 1 minute was substituted for spraying. Data show that ethyl chloride spray, applied during ischemia at the limit of endurance,

markedly lessens pain and enables the subject to perform muscular contractions markedly in excess of the increment possible after rest alone. These effects obviously cannot be attributed to circulatory changes in muscle. Similar effects were not secured by one minute immersion of the forearm in cold water (12–20°C) during ischemia. Results suggest that impulses from superficial structures play an important role in deep pain perception and skeletal muscle function.

Thiouracil administration and thyroidectomy on experimental polyarthritis of rats HELEN B. TRIPI, WILLIAM C. KUZELL, and GRACE M. GARDNER (introduced by P. J. HANZLIK) *Dept. of Pharmacology and Therapeutics, Stanford Univ. School of Medicine, San Francisco 15, Calif.* Albino rats administered 0.1% thiouracil in the diet for periods up to 233 days developed hyperplastic thyroids with nodular hyperplasia. When infected with the L-4 strain of pleuro-pneumonia-like organisms these animals developed a more severe degree of polyarthritis than did the controls, and showed a higher mortality. Thyroidectomized albino rats which had an average basal metabolic rate of only 58% of normal (the same as for the thiouracil-medicated rats) when infected with the same microbes developed an arthritis which was no worse than that in the controls. Other rats poisoned with agents chemically and physically different from thiouracil, namely, arsenic trioxide, isopropyl alcohol and phenobarbital, showed no increase in severity of polyarthritis due to these microbes. Thus, the increased severity of this experimental polyarthritis in thiouracil-medicated rats is believed to be due to some peculiar intrinsic action of thiouracil.

Algesimetry in the study of procaine anesthesia PAUL E. TULLAR (introduced by THEODORE KOPFANYI) *Dept. of Pharmacology, Georgetown Univ. School of Medicine, Washington, D. C.* It was possible to differentiate between the drug factor (D.F.) and the pressure factor (P.F.) in infiltration anesthesia by the combined use of several testing methods. Three types of stimuli (radiant heat, electric and mechanical) were employed to determine the degree, duration, and other features of procaine-infiltration analgesia in man. Pain sensation is measured a) in seconds required to produce a sharp burning pain and withdrawal of the finger with a modified Wolff-Hardy stimulator, b) in cm. distance between the primary and secondary coils of a Harvard Inductorium, and c) in units pressure required to produce sharp 'pricking' pain with a calibrated, spring driven aesthesiometer. At no time were stimuli in excess of 30 seconds for burning pain, 4 cm. for faradic shock, or 10 gm. needle pressure permitted, in order to avoid burn. It was found that stimuli of the above maximal intensities did not interfere with the repeated use

of the nail-base areas. After establishing normal responses, test solutions (0.2 to 0.25 cc.) were injected subcutaneously into the sensitive nail-base of eleven subjects for a total of 24 individual tests. The injection of procaine-HCl (1% and 2%) resulted in increased thresholds to all 3 types of stimuli. The anesthesia was of short duration and showed gradual return to normal except in the case of radiant heat where marked hyperalgesia preceded the ultimate return to normal as occurred in a majority of instances. Injection of normal saline resulted in marked anesthesia to mechanical and electrical stimuli, but showed no or little analgesia to radiant heat stimulation.

Changes in blood volume during prolonged pentothal anesthesia JAMES E. TUREMAN (by invitation), A. H. MALONEY, WALTER M. BOOKER and CLAUDIA M. RATLIFF (by invitation) *Dept. of Pharmacology, College of Medicine, Howard Univ., Washington, D. C.* In connection with our work on prolonged pentothal sodium anesthesia it has been observed that the blood picture follows a more or less definite pattern which tends to represent a hemodilution. An attempt is being made to determine if the hemodilution is real or relative and the possible mechanism. At this time 38 experiments have been completed, using 6 rabbits and 32 mongrel dogs. The dogs were divided into 3 groups, a group on normal diet, a group on high carbohydrate diet, and a group on high protein diet. Initial blood samples were taken, following which dogs were anesthetized with pentothal sodium and maintained in surgical anesthesia for an average of 3 hours. Hemoglobin and/or hematocrit was determined at $\frac{1}{2}$ hour intervals in some instances and in others at 1 hour intervals. Hemoglobin decreased an average of 1.7 gm. % during the first hour then tended to increase until the end of the experiment. The hematocrit decreased an average of 6% during the first hour and increased similarly as the hemoglobin. At the end of the experiments the values approached or equaled the initial values. There was no apparent difference in the groups. In an attempt to discover the mechanism and determine if these results were real or relative, tissue space by the thiocyanate method and plasma volume by the 1824T dye method were determined. In 6 experiments it appears that fluid is drawn from the tissue spaces.

Adrenolytic activity of a number of N-phenylisopropyl-beta-haloethylamines GLENN E. ULLYOT (by invitation), JAMES KERWIN (by invitation), EDWIN J. FELLOWS and EDWARD MACKO (by invitation) *Research Division, Smith, Kline and French Labs., and Dept. of Pharmacology, Temple Univ. School of Medicine, Philadelphia, Penna.* Substances with the following structure were compared with 'Dibenamine' for their effect after intravenous administration on the pressor

action of epinephrine in cats anesthetized with pentobarbital sodium $R-N-CH_2-CH_2-X$ Ac-



tivity comparable with that of 'Dibenamine' was noted when R = phenylisopropyl and R_1 = allyl or isobutyl but where R = allyl or isobutyl but where $R \times$ phenylisopropyl and R_1 = ethyl or isopropyl, the compounds were less active. In the case of the derivative in which R_1 = benzyl and R = unsubstituted phenylisopropyl, activity greater than that of 'Dibenamine' was obtained. The same was true when R_1 = benzyl and R = p-methoxy-, p-hydroxy-, 3,4-dimethoxy-, or 3,4-dihydroxy-phenylisopropyl.

Anticonvulsive properties of myanesin K UNNA and A KAPLAN (by invitation) *Dept of Pharmacology, Univ of Illinois College of Medicine, Chicago 12, Ill*. The anticonvulsive effect of myanesin was studied in 500 mice treated with either strychnine or metrazol, or subjected to maximal seizures induced by electroshock. Myanesin was administered subcutaneously in doses ranging from 100 to 500 mg/kg, 10 minutes before the convulsive agent. A dose of 500 mg/kg represents the paralyzing dose. The observation of Berger and Bradley that strychnine convulsions are effectively antagonized was confirmed. Both mortality and severity of the convulsions caused by strychnine (175 mg/kg i.p.) were markedly reduced. The mortality of the mice treated with metrazol (90 mg/kg i.p.) was less effectively reduced. The metrazol seizure of mice treated with 100 or 200 mg/kg of myanesin, however, was at least as severe as in the controls and often prolonged. The tonic extensor phase of metrazol convulsions, in which most of the control animals die, was eliminated by myanesin; death of the myanesin-treated mice was delayed. The electroshock (25 mA for 0.3 seconds by eye-electrode) caused typical seizures in 89% and a mortality of 33% of the control animals. None of the myanesin-treated animals died from electroshock. The pattern of the seizures was changed by myanesin (200 mg/kg) in that the tonic flexor-extensor phase was abolished, and only clonic convulsions of a much more violent nature than in the controls were observed.

Elimination of d-tubocurarine in the rat E F VAN MAANEN (introduced by OTTO KRAYER) *Dept of Pharmacology, Harvard Medical School, Boston, Mass*. Rats were anesthetized with urethane. Contractions of the gastrocnemius muscle, maximally stimulated through the sciatic nerve, were recorded. The total curarizing dose was measured at different rates of administration of d-tubocurarine. The amount of fluid injected per hour was 4.75 cc. It was found that, when d-tubocurarine was administered at a rate of 1000 γ /hr/kg rat or more approximately 70 γ /kg rat was neces-

sary for complete curarization of the gastrocnemius muscle. Decreasing the rate of administration resulted in an increase in the amount of d-tubocurarine necessary for total curarization. It was impossible to obtain complete curarization, when less than 80 γ /hr/kg rat was administered, even though the injection lasted for 5 hours or more. In nephrectomized rats the total curarizing dose differed very little from that of normal rats, when 150 γ /hr/kg rat or more was administered. However, when less than 150 γ /hr/kg rat was injected the total curarizing dose was less than that of normal rats. Complete curarization was obtained in nephrectomized rats when the rate of administration was as low as 60 γ /hr/kg rat.

Secretion of cortical hormone by the isolated adrenal MARTHE VOGT (introduced by H B VAN DYKE) *Pharmacological Lab, Univ New Buildings, Edinburgh, Scotland*. The isolated adrenal of a dog, perfused with blood by means of a Dale-Schuster pump, secretes cortical hormone in quantities detectable by biological assay on adrenalectomized rats exposed to cold. The rate of secretion is not much lower than that of the gland *in situ* during the course of an abdominal operation. It is not abolished by perfusing with blood from a hypophysectomized donor, and is immediately enhanced by the administration of corticotrophic hormone. The rate of secretion is unaffected by a number of metabolites (glucose, lactic acid, amino acids), but is augmented by adenosine-tri-phosphate and by creatine phosphate. Inorganic phosphate has no such action. These effects may be connected with the energy requirements of the synthetic processes continually going on in the gland. Another factor found to increase the output of cortical hormone is a raised concentration of potassium in the plasma. In order to obtain an acceleration of cortical secretion which can be detected by the assay employed, the plasma potassium has to be increased considerably, i.e., to values which may occur in adrenalectomized dogs, but not in normal animals. It is interesting that this effect is obtained in the absence of the pituitary, and it would be desirable to know whether smaller rises in the rate of secretion, such as cannot at present be established by direct biological assay of the hormone in blood, would result from more physiological variation in the potassium level of the plasma.

Therapeutic efficiency of various dosage schedules of oxophenarsine in experimental trypanosomiasis HARRY A WALKER, SUSANNE WILSON (by invitation), and A P RICHARDSON *Dept of Pharmacology, Emory Univ School of Medicine, Emory Univ, Georgia*. Recently, the relation of dosage schedule to the therapeutic effect of a number of chemotherapeutic agents in the treatment of experimental bacterial and protozoal infections

has been investigated in order to establish optimal dosage regimes. This report concerns the relative therapeutic efficiency of dosages of oxophenarsine given by single and multiple intraperitoneal injections against *Trypanosoma equiperdum* infections in the mouse. Previously it has been demonstrated with this infection in the rat that arsenoxide (oxophenarsine) produces a prompt trypanocidal effect as indicated by a decrease in parasite density of the peripheral blood within a period of 30 minutes. In our experiments mice were infected intraperitoneally with an inoculum of 100 million parasites/kg of body weight and remained untreated until 24 hours after inoculation. From stained thin smears made every 5 minutes after a single intraperitoneal injection of oxophenarsine it was found that within 10 minutes a decrease in parasite density occurred. Within 45-60 minutes the maximum effect was obtained. On the basis of the above data and the fact that the arsenic content of the blood after an intravenous injection of oxophenarsine falls abruptly to a low level by the end of 1 hour, the ED_{50} of oxophenarsine given intraperitoneally in 10 equal doses every 1.5 hours starting 24 hours after inoculation was determined and found to be 0.30 mg/kg. The ED_{50} of a single dose injected 24 hours after inoculation was 0.15 mg/kg. The data indicate that a single dose of oxophenarsine exerts a significantly greater therapeutic effect than that produced by the same dosage given in 10 equal doses at intervals of 1.5 hours.

Cardiac and calorogenic actions of dicumarol and dinitrophenol. R. P. WALTON, C. B. HANNA (by invitation), R. P. PRYSTOWSKY (by invitation), and J. S. LEARY (by invitation). *Dept of Pharmacology, Medical College of South Carolina, Charleston, S. C.* Calorogenic and cardiac actions of dicumarol at high dose levels have been described respectively by Wakim, Chen and Gatch (*S. G. & O.* 76:323, 1943) and Carlson and Seager (*Fed. Proc.* 7:210, 1948). The experiments reported here were aimed at defining the nature of the cardiac action. The contractile force of a section of the right ventricle was determined directly in open-chest dog preparations along with other observations of systemic changes. Dicumarol was administered in doses of 10 to 100 m.p.k. by i.v. infusion over periods of 30 min. Doses at the low extreme had no clear effect while those at the high extreme quickly produced terminal fibrillation with little evidence of contractile force stimulation. Intermediate doses in about 60% of the trials produced first slight to moderate depression followed by distinct to marked increases in contractile force, stroke amplitude, heart rate, arterial pressure, external respiratory movements and rectal temperature. The contractile force increases usually reached a maximum about 60 min after

the start of the infusion. For the most part, effects developed at parallel rates. In a few instances, however, the cardiotonic action clearly preceded the temperature rise and also was characteristically obtained in experiments in which the rectal temperature rise was prevented by the use of ice packs. Moderate increases in venous pressure were considered insufficient to account for all of the increases in contractile force. A closely similar group of responses were obtained with dinitrophenol in the dose range of 10 to 20 m.p.k. Distinct contractile force increases of the same order were obtained by the application of diathermy. Perfusion of isolated rabbit hearts with dicumarol or dinitrophenol produced marked depressant effects preceded, at times, by stimulation. In the open-chest experiments and in intact dogs receiving 250 to 400 m.p.k. dicumarol orally ECG records commonly showed T-wave reversal and deflection of the RS-T segment but did not usually show A-V dissociation until near the terminal stages.

Effect of various lactones and of veratrine on the mammalian heart. R. P. WALTON, M. DEV. COTTEN (by invitation), and O. J. BRODIE (by invitation). *Dept of Pharmacology, Medical College of South Carolina, Charleston, S. C.* Contractile force changes of a section of the right ventricle were determined in open-chest vagotomized dogs along with ECG and arterial pressure recordings. Four lactones, previously shown to have digitalis-like effects on the cold-blooded heart, had no stimulant effects on the mammalian heart *in situ*. Administered by i.v. infusion over periods of 30 min, the compounds were psi-santonin, 30 to 150 m.p.k., 1-ascorbic acid, 50 to 250 m.p.k., beta-gamma-angelica lactone, 400 to 800 m.p.k., di-lactone of pulvinic acid, saturated solution in saline, 20 cc/kg. The first 3 of these compounds produced significant depression suggestive of that obtained with alcohol. The di-lactone of pulvinic acid had no clear effect, doses up to 2 m.p.k. were injected in dioxane solution with, however, immediate precipitation.—The commercial *N.F.V.* mixture of alkaloids termed 'veratrine' was administered by infusion as above, also, by immediate injections, doses were alternated with epinephrine both before and after dibenamine. Pressor effects and cardiac stimulation with veratrine and epinephrine were generally similar although the effects of veratrine were more sustained. Dibenamine blocked effects of both drugs in a parallel manner. In contrast to epinephrine, veratrine, in small doses frequently produced temporary depression and, in larger doses, a greater tendency to fibrillation, also, stimulant effects more frequently diminished on repeated injection. Veratrine doses of 350 gamma/kg corresponded approximately to epinephrine doses of 5 gamma/kg. Cardiac stimulant effects of veratrine were accompanied by strong skeletal

muscle movements which were disturbing if not blocked by curare ECG effects consisted mainly in the R and S-wave voltage changes which usually occur with left axis deviation, frequent reversal of T-wave and, in the terminal stages, ventricular ectopic beats with frequently a bi-directional rhythm A-V dissociation did not commonly occur until near the terminal stages In isolated rabbit heart preparations, veratrine concentrations 10 to 20 times those of epinephrine produced generally similar effects and could be successively repeated several times

Pharmacological studies on 2-[N-p'-tolyl-N-(m'-hydroxyphenyl)-aminomethyl-]imidazoline hydrochloride (C-7337) M R WARREN, R A WOODBURY and J H TRAPOLD (by invitation) *Division of Pharmacology, Univ of Tennessee, Memphis, Tenn* 2-[N-p'-tolyl-N-(m'-hydroxyphenyl)-aminomethyl-]imidazoline hydrochloride (C-7337) has been shown to be an effective adrenergic blocking agent in dogs following oral and intravenous administration Its onset of action is more rapid than that reported for Dibenamine after intravenous injection but its duration is shorter The pressor response elicited by anoxia or the intravenous injection of carbaminoylcholine in atropinized dogs is converted into a depressor response Doses which cause 'epinephrine reversal' have no significant effect on the depressor action of acetylcholine and histamine Ephedrine sulfate given intravenously will combat the hypotensive action induced by rapid injection of C-7337 and decreases the duration of its adrenolytic effect Isolated uteri from estrogen-treated rabbits are stimulated by weak concentrations but only inhibition is obtained with greater concentrations Contractions elicited by histamine, epinephrine, pitocin and pitressin are likewise inhibited Spontaneous contractions of the isolated ileum of the rabbit, cat and rat are suppressed as are contractions induced by barium chloride, acetylcholine, and pitressin This inhibitory action on the intestine is not abolished by atropine Acute toxicity work in dogs indicates that approximately 100 mg/kg, administered twice daily, are required to kill 50% of the animals within a period of 1 week Single doses as large as 500 mg/kg failed to prove lethal Chronic oral administration of C-7337 in doses up to 50-60 mg/kg twice daily for three months caused no appreciable gross toxic manifestations with the possible exception in young or pregnant animals

Isomers of methadon and isomethadon and respiration of brain homogenates DANIEL T WATTS, (introduced by C L GEMMILL) *Dept of Pharmacology, Univ of Virginia Medical School, Charlottesville, Va* The effect of d-, l-, and d,l-methadon and d-, l-, and d,l-isomethadon on the oxidation of glucose, succinate and ascorbate by

fortified brain homogenates was investigated A Warburg apparatus was used to determine simultaneously in triplicate the oxygen uptake in control vessels and in vessels containing the d-, l- and d,l-methadon or isomethadon Oxygen uptake, expressed as percentage inhibition (O_2 uptake in control vessels, minus uptake in vessels with drug, divided by control uptake) was as follows

Substrate	Drug Conc. Molar	Methadon			Isomethadon		
		d,l	d-	l	d l	d	l-
Glucose	0 005	65	50	56	76	55	62
Succinate	0 003	—	—	—	57	57	60
Succinate	0 007	74	66	76	—	—	—
Ascorbate	0 005	53	53	76	84	82	86

The observed decrease in O_2 uptake may be due to a general enzymatic inhibition or to a specific block at the cytochrome-cytochrome oxidase level Inhibition of ascorbate oxidation which requires only the catalytic action of cytochrome-cytochrome oxidase indicates blockage occurs at this level

Effect of local anesthetics on respiration of brain homogenates DANIEL T WATTS, (introduced by C L GEMMILL), *Dept of Pharmacology, Univ of Virginia, Medical School, Charlottesville, Va* The effect of selected local anesthetics on the oxidation of glucose, succinate and ascorbate was investigated The homogenates were diluted to reduce endogenous activity to a minimum and then reinforced with as many factors as possible to obtain maximum oxygen uptake for the substrate under investigation Additional cytochrome c, calcium and aluminum ions were added for succinate and ascorbate oxidation Hexose diphosphate, adenosine triphosphate, diphosphopyridine nucleotide, cytochrome c, nicotinamide and magnesium ions were added for glucose oxidation Oxygen uptake was measured with Warburg manometers simultaneously in duplicate for controls and for vessels containing the drugs at a final concentration of 0 005 M Oxygen uptake expressed as per cent inhibition was as follows

	Glucose	Succinate	Ascorbate
Cocaine HCl	32	9	0
Procaine HCl	22	11	9
Metycaine HCl	46	8	4
Pontocaine HCl	64	44	36
Nupercaine HCl	74	95	68

In a further attempt to isolate the point of blockage by the local anesthetics it was found that nupercaine a) did not inhibit the reduction of methylene blue by succinate and homogenate, b) inhibited the oxidation of reduced cytochrome by homogenate in presence of oxygen, c) inhibited the reduction of oxidized cytochrome c by homogenate and succinate when the cytochrome oxidase

was inhibited by cyanide. These observations indicated that the cytochrome oxidase and the factors necessary for the reduction of cytochrome *c* are sensitive to the action of nupercaine.

Cardiac-active principles in osage orange. R A WAUD, C W GOWDER (by invitation) and J S LOYNES (by invitation) *Dept of Pharmacology, Univ of Western Ontario, London, Canada.* It has been shown previously by one of us (RAW) that Osage Orange contains a substance or substances which cause marked augmentation of the isolated hypodynamic frog's heart when perfused through the vena cava. Prolonged augmentation was also shown in a large percentage of isolated rabbit hearts perfused by the method of Langendorff. The augmentation was not accompanied by changes in heart rate nor by changes in conduction. Systolic standstill did not occur. These principles have been investigated further and we believe that a large part of the cardiac activity is due to a phytosterol which occurs in the non saponifiable fraction of the oil contained in the Osage Orange. This sterol has been isolated and obtained in crystalline form. Definite activity is shown by a concentration of 1/200,000 of this crystalline substance. From the work of Danilewsky in 1907 on the actions of cholesterol on the frog's heart one might expect the results we have described because of the close structural relationships between the plant sterols and cholesterol. We have repeated the work with cholesterol and in addition have tested the activity of several other sterols. Ergosterol has a similar but weaker action. Similar activity was found in the California Orange.

Absorption and distribution of isopropyl alcohol. JOAN WAX (by invitation), FRED W ELLIS, and ARNOLD J LEHMAN *Dept of Pharmacology, School of Medicine, Univ of North Carolina, Chapel Hill, N C.* As part of a systematic pharmacologic study of isopropyl alcohol (IPA) the gastrointestinal absorption and tissue distribution of this substance was investigated. In pentobarbitalized dogs *in situ* stomach and/or intestinal loops were isolated, and IPA absorption was studied as affected by site and extent of absorbing surface, concentration, time, successive doses, and systemic ethyl alcohol. Absorption of IPA occurred from all portions of the digestive tract, most rapidly in the jejunum and least in the stomach. During a 30-minute absorption period IPA was distributed to the spinal fluid, brain, liver, kidney and skeletal muscle. Spinal fluid and blood concentrations were approximately equal, but there was no constant relationship between tissue and blood concentrations. Increased absorption area resulted in increased tissue concentration, but did not influence total absorption. The percentage absorption was not markedly affected by the concentration of IPA although at the site of

highest absorption (jejunum), 10% strength was absorbed significantly better than 5%, 50%, and 99%. Previous absorption of IPA from intestinal loops did not influence subsequent absorption from adjacent loops. Ethyl alcohol administered intravenously markedly decreased the intestinal absorption of IPA.

Fate of certain antihistamines. E LEONG WAY, ROBERT E DAILEY (by invitation) and DONALD L HOWIE (by invitation) *Dept of Pharmacology, The George Washington Univ School of Medicine, Washington, D C.* Certain antihistamines have been successfully determined in biologic tissue by application of the Brodie methyl orange technique. A high degree of specificity was obtained when the method was modified for determining tripeleannamine (pyribenzamine) and phenindamine (thephorin). Tripeleannamine was used as the standard for comparison with other agents. In rats fasted for at least 18 hours, after a single oral dose of 100 mg/kg about 35% of the total dose was recovered in the gastro-intestinal tract after 2 hours and about 5% after 4 hours. After intraperitoneal administration of tripeleannamine to rats or guinea pigs high concentrations were obtained in the spleen, lung, liver and kidney. Concentrations in the brain were almost as high and low levels were found in muscle, heart and blood. Preliminary results with phenindamine indicate that its distribution follows somewhat the same pattern as that for tripeleannamine. After 3 daily doses of either tripeleannamine or phenindamine for several days, slight or no levels were detected in the tissues after 24 hours, indicating little or no storage of the compounds. Evidence that both compounds are rapidly metabolized is indicated by the fact that only slight amounts of either compound were recovered in excreta after parenteral administration. Preliminary *in vitro* results on tripeleannamine indicate that the detoxication site is the liver.

Fate of dl-methadon. E LEONG WAY, CHEN-YÜ SUNG (by invitation) and WILLIAM P MCKELWAY (by invitation) *Dept of Pharmacology, The George Washington Univ School of Medicine, Washington, D C.* The fate of dl-methadon was investigated in rats by application of Brodie's methyl orange technique. The method was modified to give a high degree of specificity for methadon in biological tissues. In fasted rats, about 25% of the total methadon dose given orally was recovered in the gastro-intestinal tract after 2 hours. The compound was found to be firmly bound by all tissues, especially muscle. After intraperitoneal administration of methadon highest concentrations were found in the lung, liver, kidney and spleen. Low levels were present in blood, brain, muscle and heart. After injections of methadon 2 or 3 times daily for several days, there was little

or no methadon in tissues removed 24 hours after the last dose, indicating that prolonged storage of methadon does not occur. Only a small fraction of the total administered dose was recovered in the urine as methyl orange reactant. It was also found that *in vitro*, liver and kidney slices metabolized methadon within 4 hours to substances which are not measured by the methyl orange technique.

Vasoconstrictor activity of epinephrine, ephedrine and a new sympathomimetic diamine 1-(*m*-hydroxyphenyl)-*N*²-methylethylene diamine (NU-1683) HOWARD WAYNE (by invitation), LEWIS FRANKLIN (by invitation), and HAROLD D. GRLEN, *Dept. of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C.* Lehmann and Randall recently described a series of sympathomimetic diamines (*J. Pharm. and Exp. Therap.* 93: 114, 1948). We compared the vasoconstrictor activity of one of these, NU-1683, with that of epinephrine and ephedrine by injections of each into the femoral artery of dogs while measuring the venous outflow with a modified Gaddum-type meter connected to the femoral vein. 1 μ gm of epinephrine reduced flow by 15-78% (av. 49%) in 35 injections in 6 dogs. Latency of response was 20-30 seconds, minimum flow occurred approximately 50 seconds later and lasted 10-50 seconds. Duration of response was 2.8 min (range 1.3-6.7 min). Tachyphylaxis did not occur. 1-3 mg of NU-1683 reduced flow 24-78% (av. 45%). Minimum flow lasted 20 seconds, duration of response was 4.2 minutes (range 1.3-8.5 mins) in 13 injections in 7 experiments. Tachyphylaxis did not occur. 1 mg of ephedrine reduced flow 25% (range 15-43%) in 6 injections in 3 experiments. Minimum flow lasted 13 seconds. Duration of response was 0.9 minutes (range 0.5-1.2 mins). Tachyphylaxis did occur. With each drug the latent period of response and times for occurrence of minimum flow were about the same. Sufficient data is not yet available to predict the dose response curves for these drugs. A detectable reduction of flow (10%) is usually produced by 0.1/gm of epinephrine, 0.1 mg of NU-1683 and 0.1 mg of ephedrine, and an almost complete cessation of flow (90% reduction) by 3-10 gm of epinephrine and 10 mg of NU-1683.

Separation by fluoroacetate of energy sources for smooth muscle contraction JAMES R. WEEKS (by invitation) and MAYNARD B. CHENOWETH, *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor, Mich.* Contraction of rabbit jejunum segments in oxygenated Krebs-Henseleit solution is maintained equally well by 0.005 M glucose or sodium acetate. Addition of sodium fluoroacetate (FA) decreases contraction amplitude. Communication with Dr. Alfred Farah during early phases of our study disclosed that muscles contracting in

glucose are more resistant to FA than in sodium acetate. This has also been found true for acetate in the form of monoacetin, although this is the only effective *in vivo* FA antagonist. By use of various concentrations of FA acting on jejunal segments in 0.005 M glucose it has been found that even at very high concentrations of FA reduction of contraction amplitude below about 35% of initial amplitude does not occur. In 0.005 M acetate FA in similar high concentrations reduces contraction almost to zero. Addition of glucose to 0.005 M returns contraction to the approximate level expected had glucose been present initially. Yet at low concentrations of FA acetate is a better antagonist than glucose. These findings may be harmonized by presuming that intestinal muscle obtains energy aerobically from breakdown of glucose to pyruvate and subsequently from syntheses in the tricarboxylic acid cycle involving acetate. When acetate as the sole available substrate is blocked from entry into the cycle by high concentrations of FA, contraction ceases, while low concentrations of FA are unable to produce a block in presence of excess acetate. In presence of glucose energy for partial contraction is available despite presence of FA block of acetate.

Mechanism of cardiac injury in experimental hypothermia DAVID WEINER, KURT LANGE and MICHAEL M. A. GOLD (introduced by M. G. MULINOS), *Dept. of Medicine, New York Medical College, New York City*. Rabbits suffering from lowering of body temperature show a reduction in heart rate directly proportional to the fall in body temperature. The P-R interval and the QRS complex show a prolongation roughly proportional to the fall in body temperature. The relative duration of electrical systole as expressed by Bazett's formula reveals a prolongation which becomes relatively greater as body temperature decreases. The very marked S-T segment and T wave changes under such conditions show individual differences in extent and localization in both rabbits and dogs. The changes in rate and conduction are exclusively the result of the direct effect of cold. The prolongation of electrical systole is partly the result of cold directly on the muscle fibres and partly the result of anoxia due to lowered oxygen dissociation. The T wave changes are exclusively the result of anoxia. The anoxic nature of the S-T segment and T wave changes as well as part of the prolongation of electrical systole is proved by the fact that increasing the oxygen dissociation of the blood by acidification reverses them to normal. Acidification of the blood of uncolled rabbits and dogs does not change the EKG. Anoxemia plays no role in the production of any of the changes seen in the heart with exposure to cold. We are dealing with anoxia without anoxemia.

Fate of dicoumarol in man MURRAY WEINER

(by invitation), JULIUS AXELROD (by invitation), SHEPARD SHAPIRO (by invitation) and BERNARD B BRODIE *Depts of Biochemistry and Medicine, New York Univ College of Medicine, and New York Univ Medical and Research Services, Goldwater Memorial Hospital, New York, N Y* A method for the estimation of dicoumarol in biological material has been devised. It is specific in that neither normal biological material nor metabolites of the drug are included in the measurement. With this method, those factors which affect the plasma concentration of the drug such as absorption, excretion, metabolism and tissue localization are being studied. Only traces of dicoumarol were found in the urine after its administration, indicating the almost complete metabolism of the drug in the body. The rate of metabolism is slow and varies with the dose. It varies from about 25% per day at the upper therapeutic plasma levels to over 75% per day at low plasma levels. There are wide variations in the rate of transformation in different individuals, even at similar plasma levels. The drug is highly localized on plasma proteins (about 99% plasma binding) and in various tissues. Its low concentration in plasma water explains in part, its low transformation rate. The rate of absorption of the drug from the gastrointestinal tract is slow and varies with the individual and with the dose. Thus in the same individual a dose of one mg/kg yielded a peak plasma concentration within 6 hours while a dose of 8 mg/kg did not yield a peak level until more than 24 hours. The relationship between dicoumarol concentration and prothrombin time elevation is being studied in the hope that safer dosage with the drug may be achieved by following plasma levels.

Influence of xanthopterin on the folic acid content of liver, *in vitro* ARNOLD D WELCH, EVELYN M NELSON (by invitation) and MARILYN F WILSON (by invitation) *Dept of Pharmacology, School of Medicine, Western Reserve Univ, Cleveland,*

The activity for *L. casei* or *S. faecalis* of rat liver homogenate or cell-free extract is increased by incubation at pH 7, 37°C, if added promptly, the increase is augmented by xanthopterin (X). The additional material formed behaves like pteroylglutamic acid (PGA), added PGA disappears but little, if at all. Homogenate from PGA-deficient rats is unaffected by X. The PGA content of pig liver homogenate also is increased by X, in a typical 18-hour incubation, the PGA content (in $\mu\text{gm/gm}$ of liver) rose, at pH 7, from 0.25 to 3.0 (4-hour figures somewhat lower), at pH 4.7, 0.65 without or with X, at pH 7 (4 hours) followed by pH 4.7 (14 hours), the values rose from 1.7 to 18.5. At pH 7, added pteroylhexaglutamylglutamate (PG_7) is partially converted to PGA, at pH 4.7 nearly completely, the conversion is unaffected by X. Apparently, the 'potential PGA' of pig liver is resistant,

at pH 4.7, to enzymic conversion to PGA, however, at pH 7 this form is converted enzymatically to a compound, presumably PG_7 , that is susceptible to enzymic conversion to PGA, particularly at pH 4.7. Conceivably, the precursor of PG_7 , at pH 7, is subjected to two reactions, a slower one leading to formation of PG_7 , the other rapidly yielding an inactive substance, X may inhibit this second reaction. Although X also may serve as a precursor, of PGA (through synthesis of PG_7 or its precursor), there is now but little evidence for this (no augmentation by reduced DPN, ATP, p-aminobenzoyleglutamate or its N-formyl derivative). Experiments with isotopically labeled compounds will be described.

Pharmacology of a new cholinergic agent W CLARKE WESCOE (by invitation) and WALTER F RIKER *Dept of Pharmacology, Cornell Univ Medical College, New York City* The compound, 3-acetoxy phenoxy trimethylammonium methylsulphate (Nu 2017, Hoffmann-LaRoche, Inc.), is of considerable pharmacologic and academic interest because of its close chemical relationship to acetylcholine and neostigmine. It possesses both nicotinic and muscarinic properties, injected into the intact cat it produces all the characteristic signs of acetylcholine action. Compared with acetylcholine its duration of action is longer, though still brief, and its potency is less. Like acetylcholine it is inactivated rapidly by the specific and non-specific cholinesterases of erythrocytes, brain, and serum, its affinity for the specific type esterase is lower than that of acetylcholine. It possesses the immediate and intense effects of neostigmine on skeletal muscle, providing thereby additional evidence for the direct action of neostigmine on this effector.

Pentobarbital tolerance in rabbits B A WESTFALL (introduced by J K W FERGUSON) *Dept of Physiology and Pharmacology, Medical School, Univ of Missouri, Columbia, Mo* Seventeen young albino rabbits (mean weight 2.31 kg), selected from a pool of several litters were injected intravenously with an anesthetic dose of pentobarbital sodium (40 mg/kg), and the sleeping-time noted for each animal. One week later the same 17 animals were injected with the same dose of pentobarbital sodium (40 mg/kg), the sleeping-time noted, and this routine was continued at weekly intervals until a total of five injections had been given. The data reveal a significant difference (about 30%) between the mean sleeping-time (121.1 min) after the initial dose as compared to the mean sleeping time (88.6 min) of the same animals after the same dose of pentobarbital one week later. However, the latter value (mean, 88.6 min) was almost identical with the mean sleeping time values observed after the third (mean, 87.7 min), fourth (mean, 87.6 min) and fifth (87.9 min) injections.

of pentobarbital. It might be pointed out that this study is the first reported in which the repeated injections of pentobarbital were as infrequent as one week apart and that the tolerance developed as a result of the initial dose, not only persisted at least a week, but was maintained by weekly injections of the pentobarbital.

Potentialization by means of CO₂ of the action of penicillin against pneumococcus infection. RICHARD W. WHITEHEAD, J. HOWARD WILLIAMSON (by invitation), WILLIAM E. CLAPPER (by invitation), JOSEPH N. SPENCER (by invitation) and WILLIAM B. DRAPER. *Depts. of Physiology and Pharmacology, and Bacteriology, Univ. of Colorado Medical Center, Denver, Colo.* *In vitro* experiments have shown penicillin to be most effective as an antibiotic in an acid medium. It was thought to be of interest to determine the action *in vivo* of induced carbon dioxide acidosis upon the effectiveness of penicillin using white mice as experimental animals. Type III pneumococcus was used as the test organism. One thousand lethal doses were given as the standard quantity for a 20-gm mouse. Preliminary studies showed that the maximum protection with a single dose of penicillin was obtained with 2500 U. Acidosis was induced and maintained for 45 minutes by inhalation of 20% CO₂. In control experiments it was shown that CO₂ alone had no effect upon the time of death of the animals infected with pneumococci. A series of 9 experiments was performed using a total of 72 animals, all of which were given 2500 U of potassium penicillin G at the time of inoculation. Thirty-six of these animals were treated for 45 minutes with CO₂ 2 hours after having been inoculated with the test organism and penicillin. The average life span for the animals treated with penicillin alone was 38.9 hours. The average survival time for the animals given penicillin and carbon dioxide was 47.1 hours. The difference of 8.2 hours in survival time was shown to be statistically significant.

Biopsy technic and analysis of variance applied to pentobarbital inhibition of brain oxidations. D. S. WILKINS (by invitation), R. M. FEATHERSTONE, C. E. GRAY (by invitation), J. T. SCHWIDDE (by invitation), and M. BROTMAN (by invitation). *Divisions of Anesthesiology and Neurosurgery, Depts. of Surgery and Pharmacology, College of Medicine, State Univ. of Iowa, Iowa City, Ia.* This experiment combines a method of dog brain biopsy with analysis of variance and standard manometric technic to ascertain a suitable pentobarbital concentration for studying that drug's effect on the oxygen consumption of various brain levels. The method allows dog brain tissue to be its own control in either *in vitro* or *in vivo* experiments upon drug effects. The dog's calvarium is removed under general anesthesia. Six days later the brain is

biopsied, using procaine in the scalp wound, and tissue slices uninfluenced by drugs are prepared. An attempt is here made to establish a pentobarbital concentration which significantly depresses cortical oxidations without completely inhibiting them. Cortex from each of 5 dogs was treated with 1) 0.00, 2) 0.04, 3) 0.08 and 4) 0.12% pentobarbital. Glucose was added at 90 minutes. Small sample statistical analysis of variance (Snedecor, *Statistical Methods*, 1946) was applied. Main variations were dogs, treatments, time.

Variation Source	Degrees of Freedom	F	Significant at 1% Level
Dogs	4	2.916	4.37
R _x (1) and (2)	1	68.681	8.02
(2) and (3)	1	11.849	8.02
(3) and (4)	1	0.000	8.02

(Error term: first order interaction, making summary applicable to general dog population)

CO ₂ d.w.	(1)	(2)	(3)	(4)
60-90 min	0.183	0.072	0.024	0.029
120-180 min	0.141	0.046	0.021	0.021

Pentobarbital 0.04% appears more satisfactory for the continuance of this study than 0.12% (Quastel) or 0.08% which gave essentially complete inhibition.

Immediate reactions of mice to large amounts of ouabain. W. LANE WILLIAMS and ROBERT TARAIL (introduced by R. N. BIETER). *Depts. of Anatomy and Medicine, Univ. of Minnesota, Minneapolis, Minn.* Mice (20-25 gm) tolerate as much as 2 mg of ouabain (subcutaneous or intraperitoneal) provided the glycoside is administered in several unit amounts of not more than 0.25 mg during an interval of 30-60 minutes. Such daily treatment can be continued for a considerable period (*Federation Proc.* 7:259, 1948). Mice thus treated eventually showed punctate myocardial necrosis and renal and hepatic lesions. Mice also tolerated simultaneous treatment with relatively large amounts of ouabain and calcium provided the total dose for the interval did not exceed that mentioned above. However, a majority of mice receiving a single injection of more than 0.4 mg of ouabain died within 15 to 28 minutes and showed massive pulmonary edema and hemorrhage. Pulmonary involvement was more extensive than that observed terminally in mice treated chronically with similar amounts of ouabain but in divided doses. Some of these mice received as much as 2 cc of saline as solvent or vehicle for the glycoside (Lilly preparation of *Strophanthus gratus*). Equivalent amounts of saline were well tolerated in daily single unit injections for 30 days. The lesions indicate that the acute effects of large amounts of ouabain are directly or indirectly pulmonary. Upon relatively long-term treatment

similar amounts (as to total daily dose) administered as several injections during 30-60 minutes usually produced myocardial damage without concomitant immediately lethal pulmonary lesions

Respiratory activity in vitro of cardiac muscle of ouabainized dogs ALBERT WOLLENBERGER (introduced by OTTO KRAYER) *Dept of Pharmacology, Harvard Medical School, Boston, Mass* The cardiac glycoside ouabain was administered to dogs by slow continuous intravenous infusion. The heart was excised at the onset of cardiac irregularities or of ventricular fibrillation. Slices of left ventricular muscle were prepared and incubated in glucose-phosphate-saline. During the first 30-60 minutes these slices respired at a somewhat higher rate than cardiac slices of control dogs. Subsequently, however, the respiratory rate declined well below the control level, particularly in the case of the slices of fibrillating hearts. Extent and rapidity of the decline were inversely proportional to the tissue concentration. At a tissue concentration of 1% or less, the slices of fibrillating hearts ceased to respire during the second hour of incubation.

Toxicity of hydroquinone for laboratory animals GEOFFREY WOODARD, E CONWAY HAGAN, and JACK L RADOMSKI (introduced by BERT J Vos) *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D C* Because of the interest in the proposed use of hydroquinone as an antioxidant for edible fats, experiments were undertaken to determine its toxicological properties as well as those of its oxidation products quinhydrone and quinone. Approximate acute oral LD₅₀'s in mg/kg of hydroquinone in 2% aqueous solutions are: rats, 320, mice, 400, guinea pigs, 550, pigeons, 300, cats, 70, dogs, 200, and in rats for quinhydrone, 225, and quinone, 130. The approximate intravenous LD₅₀'s in rats are: hydroquinone, 115, quinhydrone, 35, and quinone, 25. The acute toxicity of hydroquinone is reduced in the non-fasted rat and is influenced by the concentration administered. Symptoms of hydroquinone poisoning develop 30

to 90 minutes after oral administration and consist of hyperexcitability, tremors, convulsions and, in addition, salivation in dogs and cats, emesis in dogs and pigeons, and incoordination of the hind limbs of dogs. Deaths occur within a few hours. Quinhydrone and quinone, however, do not produce the same symptoms of poisoning and deaths may be delayed several days. Oral administration of hydroquinone at 100 mg/kg in the dog and at 70 mg/kg in the cat produces mild to severe swelling of the area around the eye, of the nictitating membrane, and of the upper lip. However, dogs receiving 25 and 50 mg/kg daily for 4 months have shown only slight eye involvement. Routine blood counts in cats and dogs acutely and subacutely poisoned with hydroquinone indicate increased activity of the cell-forming tissues.

Evaluation of drugs possessing anti-dysmenorrhoea properties R A WOODBURY and PAUL P C FENG (by invitation) *Division of Pharmacology, Univ of Tennessee, Memphis, Tenn* Vasopressin (0.05 to 0.3 vasopressor units of Pitressin) injected intravenously in humans immediately caused marked uterine activity and caused or intensified the distress characteristically like that of dysmenorrhoea (*Federation Proc* 5: 215, 1946). These observations suggest the hypothesis that drugs which are effective in relieving dysmenorrhoea symptoms by mechanisms other than through analgesia may well possess anti-vasopressin activity to one or more of the actions of vasopressin. Drugs useful in the treatment of dysmenorrhoea such as atropine, papaverine, Pava-trine, Trasentin, Nethamine, Isopropylphenephine, neostigmine, progesterone, testosterone and magnesium salts are found to have pronounced anti-vasopressin activity to one or more of its actions. In the search for additional therapeutic agents for the treatment of dysmenorrhoea, the above data indicate that the anti-vasopressin activity of compounds should be determined along with the anti-histaminic and the anti-cholinergic activity.

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(For possible corrections in any of the following abstracts see the June issue)

Blood pressure measurements and changes in peripheral vascular bed of unanesthetized mice

GLENN H. ALGIRE *National Cancer Inst., Bethesda, Md.* The transparent chamber technique as adapted to a skin flap in mice makes accessible for microscopic observation a layer of subcutaneous and muscular tissue approximately 0.5 mm thick, and having a surface area of 150 sq. mm. Microscopic observations, photographs and quantitative measurements may be made at magnifications up to 500X. An indirect method has been devised for blood pressure measurements of any vascular component within the chamber during direct microscopic examination by transmitted light. Measurements may be made on the same vessel intermittently throughout the day, and repeatedly for the duration of the preparation (approx. 30 days). The apparatus consists of a mercury sphygmomanometer system and air reservoir connected to a glass tube having a thin rubber membrane across the end. A micromanipulator is used to bring the membrane into contact with the under surface of the skin. Pressure applied using the sphygmomanometer bulb results in slight bulging of the membrane. As the entire field is visualized under the microscope one can obtain arterial systolic and diastolic pressure, and venous pressure. The pulse wave can be seen at maximum amplitude approximately midway between systolic and diastolic pressures. Parallel observations and measurements may be made of correlated vascular phenomena such as changes in caliber of vessels, rates of flow, vasomotion, and intravascular agglutination.

Radiation effects on tissues studied with C^{14} -labeled glycine KURT I. ALTMAN, GEORGE W. CASARETT, THOMAS R. NOONAN and KURT SALOMON (introduced by F. S. ROBSCHT-ROBBINS) *Dept. of Radiation Biology, Univ. of Rochester, Rochester, N. Y.* Young adult male rats were exposed to 300 or 600 R total-body Roentgen radiation. Glycine labeled with C^{14} in the α -carbon atom (specific activity of 1.83 μ c/mg) was administered by stomach tube or intravenously at varying intervals after irradiation. The most striking feature observed in rats given 2 μ c by stomach tube immediately after irradiation and killed 24 hours later was the high C^{14} -activity of the gastrointestinal tract contents. With a dose of 300 R 16.7% and with

600 R 32.8% of the total activity administered were found in the gastrointestinal tract contents, as compared with 0.82% in the control animal. In order to eliminate possible malabsorption, glycine (1 μ c) was injected intravenously and rats were sacrificed 5 hours later. It was found that the uptake of labeled glycine by muscle was definitely lowered in all irradiated rats, particularly when glycine was given immediately after irradiation. Definite changes were also observed in brain when glycine was injected 48 hours after irradiation. In this case the total C^{14} -activity of the brain was approximately 3 times that of the control. This increase was due to an increase in the C^{14} -activity of the phospholipid fraction. Radiation effects are also demonstrable in other organs, such as testes, kidney, and liver. When glycine was injected 48 hours after exposure to X-rays a striking increase in the C^{14} activity of the expired CO_2 was observed.

Ovarian hormones and uterine pigmentation in vitamin E-deficiency W. B. ATKINSON (by invitation), H. KAUNITZ and C. A. SLANETZ (by invitation) *Depts. of Anatomy, Pathology and Animal Care, College of Physicians and Surgeons, Columbia Univ., New York City.* One of the most constant findings in intact vitamin E-deficient rats is a chocolate brown discoloration of the uterus which is characterized histologically by the accumulation of acid-fast pigment granules in the cells of the myometrium and, to a lesser extent, the endometrial stroma. The role of the ovary in the development of this condition has not been elucidated. In the present experiments, rats were ovariectomized at 21 days of age and were maintained on a vitamin E-deficient diet which contained 10% commercial lard and which limited the tocopherol intake to about 30 micrograms daily. The animals were divided into 4 groups, the first receiving no further treatment, the remaining 3 being injected once weekly with 5 micrograms of estradiol (Roche-Organon), 4 mg of progesterone (Ciba) or both hormones together, respectively. The uteri were examined macroscopically and histologically after 5 to 11 months. Pigmentation was almost completely absent in the untreated ovariectomized animals. The animals receiving estrogen alone showed slight uterine pigmentation.

in the areas characteristic of the unspayed deficient rat. Concurrent treatment with progesterone seemed to intensify the response. Treatment with progesterone alone evoked slight pigment deposition limited almost entirely to the circular layer of the myometrium. These observations clearly demonstrate that the ovarian hormones are involved in the accumulation of pigment in the uterus of the vitamin E-deficient rat.

Electron microscope studies on virus-cell relationships F B BANG and G O GEY *Depts of Medicine and Surgery, Johns Hopkins Medical School, Baltimore, Md*. Newcastle and mumps viruses freshly prepared from allantoic fluid and saline show characteristic morphologies in the electron microscope. The shape of Newcastle virus when fixed by osmic acid is determined by the concentration of saline in which the virus is fixed. The absorption of both of these viruses on the ghosts of chicken red cells may be demonstrated by fixation with osmic acid in solution at intervals after exposure of the red cell ghosts to the virus, and drying the mixture on formvar screens. The effect of Newcastle virus on chick fibroblasts and macrophages and on rat fibroblasts has been studied by infecting the cells either in roller tube cultures before transfer to formvar coated coverslip preparations or at the time of transfer. A similar morphological effect has been demonstrated on all 3 cell types.

Cytochrome oxidase, succinoxidase and phosphatase activities of tissue of rats on protein-deficient diets E P BENDITT, C H STEFFEE (by invitation), T HILL (by invitation) and T L JOHNSTON (by invitation) *Dept of Pathology, Univ of Chicago, Chicago, Ill*. Protein-deficient but otherwise adequate diets when fed to animals result in loss of protein from many vital organs including the liver and kidneys. In an effort to elucidate the nature of this protein loss rats were sacrificed after varying periods on protein deficient diets. Cytochrome oxidase, succinoxidase and alkaline phosphatase were assayed in liver homogenates and alkaline phosphatase determined in kidney homogenates. Cytochrome oxidase and succinoxidase decreased progressively with time and somewhat faster than the liver protein. Liver phosphatase, on the other hand, increased in concentration as the liver shrank. The total liver phosphatase remained approximately constant. Kidney phosphatase after several months of protein depletion was reduced in proportion to the kidney protein. The difference in behavior of the liver phosphatase from the kidney enzyme and from the other liver enzymes studied is apparently related to the fact that the liver enzyme is not present to a significant extent in the hepatic parenchymal cells but is associated with the vascular bed and bile ducts. This work substantiates that

of others who assayed under similar conditions other enzymes including catalase, xanthine dehydrogenase, cathepsin and arginase (Miller, *Fed Proc* 7 174, 1948) d-amino acid oxidase (Seifter et al *Fed Proc* 7 187, 1948). It appears from the evidence that at least a part of the 'reserve' protein lost from vital organs such as the liver and kidney by animals on protein deficient diets is not inert material but is part of the actively functioning cytoplasmic protein constituents, i.e. enzymes.

Effect of acute x-radiation on distribution and excretion of radio sodium in the rat L R BENNETT, VERA C BENNETT and JOE W HOWLAND (introduced by F S Robschert-ROBBINS) *Dept of Radiation Biology, Univ of Rochester School of Medicine and Dentistry, Rochester, N Y*. Radiated rats have been shown to have an increased thiocyanate space for 2 weeks following a mid-lethal dose of x-radiation. A study of the sodium distribution and excretion of similar animals using radio sodium has been made. In rats during the first 4 days post radiation a marked retention of radio sodium occurs. Tissue distribution studies show a shift into the intestinal tract which may exceed that observed in control animals by as much as 2% of the total dose administered. Smaller shifts of radio sodium into the kidney and spleen are noted. There is an increased radio sodium content in the liver which appears to be definitely related to the increased serum level to which it holds a constant relationship. The serum sodium content increases, the maximum levels (up to 5-8 mEq) being observed at the end of 3-4 days after irradiation. Urinary radio sodium excretion may be reduced to one half that observed in control animals. Studies on dehydrated rats indicate that the urinary retention and elevated blood sodium level are not accounted for on the basis of dehydration alone.

Lipids in the lymph of rats J L BOLLMAN and E V FLOCK *Mayo Foundation, Rochester, Minn*. Lymph from the lymphatic draining the small intestine and lymph from the liver and lymph from the thoracic duct was obtained in different rats after recovery from the operation during which a small plastic tube was inserted into the respective lymphatic and brought through the abdominal wall. The concentration of neutral fat of the intestinal (and thoracic duct) lymph of the fasting rat was considerably greater than that of the plasma. The phospholipid but not the cholesterol content of this lymph was also higher than that of plasma. The feeding of a lipid free meal did not alter these values. Following the feeding of a meal containing fat but devoid of phospholipid or cholesterol there was a marked increase in the neutral fat and phospholipid but not the cholesterol content of the intestinal lymph. The feeding of phospholipid produced only the increase in the phos-

pholipid content of the lymph which would accompany the increased neutral fat (from hydrolyzed phospholipid) content of the lymph. The concentration of neutral fat, phospholipid and cholesterol in lymph from the liver of fasting rats was almost the same as the concentrations of those substances in the plasma and were not materially altered by feeding meals which did elevate the plasma content of these substances.

Blood supply of neoplasms in the liver CHARLES BREEDIS and GANG YOUNG (by invitation) *Dept of Pathology, Medical School, Univ of Pennsylvania, Philadelphia, Penna, and Pathology Research Inst, Medical College, National Sun Yat Sen Univ, Canton, China*. By means of injection experiments during life and after death, it was shown that malignant neoplasms growing in the liver tend to acquire an exclusively arterial blood supply. The neoplasms tested were as follows: V-2 carcinoma strain of rabbits, T-241 sarcoma strain of mice, metastases of spontaneous carcinoma of the kidney of the frog, and primary hepatomas and cholangiomas of rats induced by butter yellow. A variety of metastatic carcinomata found at autopsy in 12 human cases were similarly injected. In all instances the blood supply to the tumors was predominantly or exclusively arterial, in sharp contrast to the predominantly portal supply of liver tissue. It was found that the blood supply of the experimental rabbit tumors was arterial whether inoculations had been made into the portal vein, the hepatic artery or directly into the liver parenchyma. Only very small tumors (1-2 mm or less) tended to retain some portal vessels. With increase in size of the tumors the portal vessels became obliterated, and blood was supplied through the hepatic artery.

Cutaneous melanin production in the mouse by 5, 9, 10-trimethyl, 1-2-benzanthracene F H BURGOYNE, W E HESTON, J L HARTWELL (all by invitation) and H L STEWART *National Cancer Inst, Bethesda, Md*. By painting the skin at the base of the tail of F_1 hybrids of strains $C_{57}B1 \times dba$ twice weekly with 0.06% solution of 5,9,10-Trimethyl, 1-2 Benzanthracene in benzene, localized pigmented areas were produced. The pigmented areas appeared in the skin between the 5th and 15th months, often in association with areas of atrophy, hyperplasia, inflammation, ulceration, papilloma formation, squamous cell carcinoma and sarcoma. In histological preparations the pigment in these areas was blackened by Krajian's silver impregnation method, failed to give a positive Prussian Blue reaction and was bleached by strong oxidizing agents, thus identifying it as melanin. When the pigmented areas were bleached they were found to be composed of melanophores or macrophages with finely stippled cytoplasm and an eccentrically placed nucleus.

The 'dopa' reaction applied to skin recently excised from the painted area revealed large numbers of melanoblasts in the basal layer of the epidermis. Thus, painting the skin of these F_1 hybrid mice with 5,9,10-Trimethyl, 1-2 Benzanthracene resulted in an increase of melanoblasts, with deposition of melanin in macrophages in the derma and the subcutaneous tissue. No malignant melanomas were induced.

Effects of x-rays on the metabolism of nucleic acids in hematopoietic tissues C E CARTER (introduced by SHIELDS WARREN) *Biology Division, Oak Ridge National Lab, Oak Ridge, Tenn*. The alterations in the metabolism and content of nucleic acids in hematopoietic tissues have been studied through microchemical and isotope procedures employing P^{32} following varying doses of total body x-radiation. In bone marrow the desoxyribonucleic acid content falls within the first 24 hours following radiation. Similar changes were found in spleen and thymus where the desoxyribonucleic acid changes are marked but a decrease in ribonucleic acid content is also found. No immediate effects of radiation on the content of nucleic acid in these tissues were noted. Experiments employing P^{32} in bone marrow cell suspensions in homologous serum likewise showed no significant alteration of the rate of incorporation of P^{32} into nucleic acids following removal from rabbits immediately after exposure to 1000 r. Experiments conducted 24 hours following exposure show marked decrease of respiration in 500 r and 1000 r group with elevated ratios of ribonucleic acid to desoxyribonucleic acid specific activity indicating a depression of desoxyribonucleic acid synthesis. Of several enzymes concerned with nucleic acid and protein metabolism studied in spleens of irradiated rats, only ribonuclease shows significant deviation from normal and these changes are established in the first 24 hours following exposure. All of the aforementioned changes pose the problem of correlation of metabolic activity with cell type in a complex cellular system.

Experimental production of a nutritional macrocytic anemia in swine G E CARTWRIGHT (by invitation) and M M WINTROBE *Dept of Medicine, Univ of Utah College of Medicine, Salt Lake City, Utah*. A deficiency of pteroylglutamic acid has been produced in 32 swine fed a basal diet supplemented with sulfasuxidine, a folic acid antagonist (crude methyl folic acid) and all the known B vitamins except pteroylglutamic acid. The hematologic manifestations observed were 1) a severe macrocytic anemia, 2) leukopenia, due to a reduction in both polymorphonuclear and mononuclear cells, and 3) a slight thrombocytopenia. The bone marrows were hyperplastic with a relative increase in immature nucleated red cells. These cells closely resemble the megakaryoblasts seen

in the bone marrow of patients with pernicious anemia in relapse. The hematologic manifestations described above, appeared in the presence of 'extrinsic factor' in the diet and their development was not prevented by the daily intramuscular administration of 1 U S P U of purified liver extract from the beginning of the experiment. The feeding of a diet low in protein (10% casein) resulted in a more rapidly developing anemia but the degree of macrocytosis was less. Pteroylglutamic acid deficient pigs, either receiving 'extrinsic factor' (crude casein) in the diet or an 'extrinsic factor-free' diet (purified casein) responded rapidly and maximally to pteroylglutamic acid, pteroyldiglutamic acid, pteroyltriglutamic acid or to pteroylheptaglutamic acid. Thymine and xanthopterin had little or no activity. Tyrosine, adenine and uracil were inactive. Purified liver extracts as well as vitamin B₁₂ were found to possess some hemopoietic activity under the conditions employed but the activity was considerably less than that of pteroylglutamic acid compounds.

Biochemical and clinical effects of cationic exchange resin. THEODORE S. COBBEY, JR. (by invitation), ROBERT H. WILLIAMS, NORMA MACRAE (by invitation) and BEVERLY T. TOWER (by invitation) *Dept of Medicine, Univ of Washington, Seattle, Wash.* Several cationic exchange resins have been given to normal subjects and patients with edema. Investigations were made for changes in the clinical status and in the balance of H₂O, Na, K, Ca and Mg. The resins markedly increased the excretion in the stools of Na, K, Ca and Mg. Although there tended to be a decrease in the excretion of these elements in the urine, the response was variable. The increased excretion of the elements in the stools was not associated with a comparable decrease in the urine. The resins promoted a marked increase in ammonia and titratable acidity in the urine. In certain patients with edema associated with congestive heart failure it was noted that loss of weight and edema occurred simultaneously with a positive Na balance, conversely, gain in weight and edema occasionally was disproportionate to the quantity of Na retained. These observations suggest that the Na balance need not necessarily parallel the water balance in congestive heart failure. After a period of 3 days on the resin one non-cardiac subject had a marked diuresis followed by weakness, hemoconcentration, and a distaste for water. These symptoms were immediately relieved by intravenous saline. One cardiac patient developed the same picture after being on the resin for 21 days.

Determination of the blood iodine. A simplified method for use in the clinical laboratory. ARTHUR C. CONNOR (by invitation), ROY E. SWENSON (by invitation) and GEORGE M. CURTIS *Dept of Research Surgery, Ohio State Univ, Columbus, Ohio*

A shorter and simpler plan of determining the blood iodine has been attained by developing a method which requires no transfer of material during the entire procedure, and necessitates an apparatus of specially constructed glassware. Three ml of serum are fractionated in a 250 ml reagent flask by use of the Somogyi reagent. This flask is centrifuged in an International, size 2 centrifuge. The supernatant fluid containing the soluble 'inorganic' iodine is poured off and read in the colorimeter directly. The protein precipitate containing the physiologically significant protein-bound iodine remains in the 250 ml reagent flask and is oxidized by the chromic acid procedure. The same flask fits a distillation assembly. The iodine is then released by phosphorous acid and collected directly in a colorimeter tube which also fits the distillation assembly. The quantity of iodine is determined by its catalytic effect on the rate of reaction between ceric sulfate and arsenious acid. An ordinarily well-trained technician can make an analysis in less than 3 hours and about ten analyses in an eight-hour day. The average deviation is plus or minus 5%. Since the method is essentially a closed procedure contamination has been practically eliminated.

Kidney protein associated with systemic arteriolar necrosis. G. I. DESUTO-NAGY and L. L. WATERS (introduced by M. C. WINTERNITZ) *Dept of Pathology, Yale Univ School of Medicine, New Haven, Conn.* Previous experiments have demonstrated that when saline extracts of kidney tissue are injected into nephrectomized dogs, segmental necrotizing lesions of systemic arterioles result (Winternitz, *et al*, 1939). In continuance, acid extracts of fresh hog kidney were subjected to fractional acetone precipitation. The precipitates were redissolved, dialyzed, and reprecipitated with acetone. Three fractions resulting from these procedures have been examined for their vasomotor activity and for their association with the occurrence of acute arteriolar lesions. The dialysates of all the fractions contained vasodepressor substances of small molecular weight. When these were injected into nephrectomized test dogs no systemic vascular changes were encountered. A complex fraction, containing vasopressor (renin-like) substances, thromboplastic material, inert globulins and lipids was found to be associated with the frequent occurrence of vascular changes in the test animals. A small non-dialyzable fraction of the acid extract obtained on precipitation by 20 volumes of acetone was devoid of either vasopressor or vasodepressor activity. This protein-like material contained phosphorus. When injected into nephrectomized dogs it was found to be associated with widespread arteriolar necrosis. In some instances necrosis of arterial smooth muscle followed the injection of as little as 19.1 mg N.

Iron excretion in human subjects as measured by the isotope technique REUBENIA DUBACH (by invitation), CARL V MOORE and SHEILA CALLENDER (by invitation) *Washington Univ School of Medicine, St Louis, Mo* The excretion of iron by human subjects over a 140-day period was studied by the isotope technique. Observations were continued for a period of time greater than the length of life of the red cells into which labeled hemoglobin was incorporated in order that any unusual excretion of radioiron at the time of hemoglobin breakdown might be noted. Following intravenous injections of 5 to 20 mg of radioiron, 4 normal individuals excreted in the feces 0.03 to 0.07% of the dose per day for 4 to 24 days, later, after the isotope had reached a constant level in the blood (75 to 92%), an average of approximately 0.01% of the dose was excreted daily for 140 days. A woman with hypochromic anemia excreted an average of 0.028% of the dose daily for the first 5 days, later, when 100% of the isotope had appeared as hemoglobin, she excreted 0.002 to 0.007% of the dose daily for 140 days. A patient with sickle cell anemia excreted an average of 0.05 to 0.08% of the injected dose per day. Assuming complete mixing of isotopic iron with total hemoglobin iron, the total fecal excretion of iron derived from hemoglobin was calculated to be 0.2 to 0.9 mg/day for the normal subjects, 0.04 to 0.13 mg for the woman with hypochromic anemia, and 1.3 to 1.7 mg for the girl with sickle cell anemia. The effect of a hemolytic crisis on iron excretion was studied in 2 dogs. After a high concentration of radioiron had been built up in the red cells by repeated injections, severe hemolysis was induced with phenylhydrazine. The excretion of iron in the feces and urine increased significantly (0.1 to 0.12% of the dose per day, compared to 0.01 to 0.06% in control periods) but remained small in relation to the amounts of iron liberated by red cell destruction.

Effect of hydrogen-ion concentration on the cultivation of macrophages in vitro I N DUBIN and C K YEN (by invitation) *Division of Pathology and Bacteriology, Univ of Tennessee College of Medicine, Memphis, Tenn* Macrophages from chick embryo spleen were grown in vitro at 37°C for 4 to 6 days using chicken serum and salt mixtures containing bicarbonate buffers as nutrient. The pH values, which ranged from 6.4 to 9.0, were maintained at constant levels by continuous flow of gas-mixtures containing required concentrations of CO₂. The pH values were determined with a Beckman pH meter. The macrophages grew equally well between pH 6.8 to 8.4. Growth fell off sharply at pH 6.6 and ceased about pH 6.4, beyond pH 8.4 growth was poor, but cells in rare numbers were found up to pH 9.0. There was indirect evidence that the internal pH of the cells was affected, as

shown by the color reactions of the cells when stained with Giemsa, on the acid side the cells stained reddish while on the alkaline side bluish. On the acid side the cells were round, large, contained abundant cytoplasm and numerous fat vacuoles, there was also a tendency towards formation of multinucleated giant cells. On the alkaline side the cells had much less cytoplasm and fewer fat vacuoles and presented a stellate or elongated shape. This difference in shape may reflect a difference in irritability and mobility. The mechanism of action of the pH is not known but may operate through changing the concentration of available ions such as calcium and phosphate.

Acid phosphatase activity of the gastric contents of patients with carcinoma of the stomach CHARLES E DUNLAP and GEORGE W CHANGUS (by invitation) *Dept of Pathology, School of Medicine, Tulane Univ and Charity Hospital, New Orleans, La* In patients with carcinoma of the stomach histochemical studies have shown high acid phosphatase activity in the neoplasm and also in the surrounding gastric mucosa. Chemical determinations for acid phosphatase were done by the method of Gutman and Gutman on gastric contents, aspirated from a series of fasting patients with and without carcinoma of the stomach. It was found that aspirates having an initial pH of less than 3.5 seldom contained significant amounts of acid phosphatase and that the enzyme, when present in other samples, could be irreversibly inactivated, in vitro, by acidification to pH 3.5 or less. At a pH greater than 3.5 the enzyme was fairly well preserved for 48 hours at 4°C but rapid deterioration occurred at room temperature. Thus gastric aspirates containing 'free acid' (pH less than 3.5) as well as those that had stood for more than 2 hours without refrigeration were considered unsuitable for acid phosphatase determinations. In a great majority of the patients with carcinoma of the stomach no 'free acid' was present and the aspirates were found to contain more than 10 units of acid phosphatase per hundred cc. Most samples from patients without gastric carcinoma contained less than 10 units. The study, to date, has covered only a limited number and variety of gastric lesions and includes no cases of early carcinoma.

Blood dyscrasia in mice ingesting toluidine blue THELMA B DUNN *National Cancer Institute, Bethesda, Md* Inbred mice were given 0.1% or 0.2% solution of toluidine blue instead of drinking water. With an appropriate concentration of the dye the urine was deeply colored, but no severe toxic symptoms developed. After an interval of 10 days or longer the peripheral blood in some animals showed a reduction in the circulating red blood cells without a corresponding reduction in hemoglobin. In the blood smears the erythrocytes showed fragmentation, polychromasia, anisocytosis.

sis, macrocytosis, siderocytosis and normoblasts. The total and differential leukocyte counts were not significantly altered. On postmortem examination a pronounced siderosis of the spleen, liver, bone marrow, kidney and lymph nodes was found. The bone marrow showed hyperplasia of erythropoietic elements. When the toluidine blue was withdrawn, the peripheral blood picture rapidly returned to normal, and the siderosis of organs decreased. This method is now being used to produce siderosis in the tissues and hyperplasia of the bone marrow in mice. The possible effect on the incidence of neoplastic disease, particularly leukemia, is being investigated.

Incidence of neoplasms and pathological changes in rats as a result of prolonged choline deficiency CYRUS C. ERICKSON and WALTER GOEBBEL (by invitation) *Depts of Pathology and Surgery, Duke Medical School, Durham, N. C.* Neoplasms of one or more types were observed in 58% of choline-deficient rats by Copeland and Salmon (D. H. Copeland and W. P. Salmon, *Am J Path* 22: 1059, 1946). The purpose of these experiments was to test a similar choline-deficient diet for the production of neoplasms in the Osborne-Mendel strain of rats used in this laboratory, and to utilize such test animals for anterior chamber tumor transplant experiments. The diet consisted of 30% alcohol-extracted peanut meal, 6% alcohol-extracted casein, 40% sucrose, 20% pure lard, and 4% salt mixture. To each kg of diet, vitamins were added as in the Copeland-Salmon choline-deficient diet. The experimental diet was started on 149 rats at the age of 20-30 days and was given ad libitum. Choline supplement was administered to the choline-deficient group during the first 4 weeks. Twenty-four rats making up the control group received the same diet with 3 gms of choline chloride added to each kg of diet for the duration of the experiment. Sixty of the experimental group and 20 of the control animals were still living after 19 months. Cirrhosis of the liver was a consistent finding in the rats on the deficient diet. The high incidence of neoplasms previously reported as a result of this choline-deficient diet has not been observed in this experiment in the 65 choline-deficient animals expiring prior to 19 months.

Injury and recovery of mouse testes following whole-body exposure to x-radiation ALLEN ESCHENBRENNER, *National Cancer Institute, Bethesda, Md.* Groups of mice received 50 R, 100 R, 200 R, 300 R, or 400 R acute whole-body radiation at 3 months of age. Four mice of each dose group and four non-irradiated mice of the same age were killed at weekly intervals up to and including 12 weeks following exposure. The testes were dissected free and weighed on a torsion balance after which they were prepared for histologic examination. Minimum testes weights were observed from

3-4 weeks after irradiation and returned to normal 6 to 14 weeks after irradiation. The minimum weights of testes observed were dependent upon dose. The time required to effect the minimum weights as well as the time required for the weights to return to normal were functions of dose. Histologic examination of these testes indicates that the decrease of testes weights following acute irradiation is due to marked transient retardation in rate of production of spermatogonia. The progeny of spermatogonia are not appreciably destroyed nor prevented from maturation. After it was found that the weight of testes of mice exposed to 200 R had returned to normal by 12 weeks, an additional group of mice was given a whole-body exposure of 200 R at 3 months of age and again 12 weeks later. Following the second exposure the mice were killed and examined as before. The pattern of injury and recovery following the second dose, as reflected in fresh testes weights, was the same as observed following a single dose. These experiments on acute irradiation confirm conclusions drawn from earlier experiments on chronic irradiation, that the primary effect of radiation of the testes is on the spermatogonia.

Massive hepatic necrosis in protein-depleted, partially hepatectomized rats fed hypertonic diets ROBERT L. ESTRADA (by invitation), ZACHARY A. SIMPSON (by invitation), HARRY M. VARS and I. S. RAYDIN *Harrison Department of Surgical Research, Schools of Medicine, Univ of Pennsylvania, Philadelphia, Penna.* The effect of forced feeding with concentrated solutions of sucrose, gelatin and casein hydrolysate on normal partially hepatectomized and on protein-depleted, laparotomized or partially hepatectomized rats has been studied. Massive hepatic necrosis occurred in the rats receiving these diets. Gastric distention and increased intra-abdominal pressure were observed in the experimental groups and were simulated in others by attaching a balloon to the stomach at laparotomy. Pneumoperitoneum immediately after laparotomy demonstrated that increased intra-abdominal pressure per se was not sufficient to produce liver injury. The emptying time of the stomach following the administration of the various diets was determined by x-ray examination. No retention was present after 2 hours in the control group. In the groups receiving 50% sucrose and the 50% hydrolyzed casein diet, there was 50% and 90% gastric retention, respectively, at the end of 2 hours. The debilitated rat is very susceptible to liver injury. It is our opinion that the hepatic necrosis which occurred in rats receiving the 50% casein hydrolysate diet was not due to toxicity of the amino acids, since similar lesions were produced when other diets were fed, and that gastric distention per se was sufficient to slow the portal circulation and to lead to anoxia of the hepatic

parenchymal cells. The condition was further aggravated by pressure exerted directly upon the liver. This represents another mechanism which by interfering with hepatic blood supply leads to hepatic necrosis, the extent of the necrosis being further aggravated by the nutritional state of the animals.

Effect of lecithin in the formation of thrombin
JOSEPH E. FLYNN and EUGENE T. STANDLEY, (introduced by H. P. SMITH) *Dept. of Pathology, Columbia Univ., New York City*. In activating purified prothrombin, the amount of thrombin obtained depends in part upon the amount of Factor V added. With a suboptimal amount of Factor V, the yield of thrombin is low, and can be increased by the addition of lecithin. The lecithin is not identical with Factor V, but it does potentiate its effect.

Inorganic composition and phosphatase activity of human cartilage in relation to morphological differentiation
RICHARD H. FOLLIS, JR. *Dept. of Pathology, Johns Hopkins University, Baltimore, Md.* A method has been devised to compare the chemical composition and morphological characteristics of developing human cartilage. Segments of whole costochondral junctions removed at autopsy from children of varying ages are clamped in a freezing-type microtome by the shaft and first sliced longitudinally down through the cartilage just into the shaft. Transverse slices of desired thickness then are made completely through one of the two longitudinal halves and part-way through the other. Appropriate chemical analyses are made of the free slices, their fellow halves, held together by an intact outer layer of cartilage, are fixed and prepared for histological examination. This technique permits a comparison of chemical composition and cellular differentiation in various strata of developing cartilage, a unique tissue with such adaptability to studies of chemical embryology. Observations to be presented include the percentage composition of calcium, phosphorus and carbonate and phosphatase activity, all in direct relation to the degree of maturation of cartilage cell strata, from those containing small undifferentiated cells to those having large hypertrophic cells being invaded by capillaries. There is a characteristic quantitative increase in concentration of the inorganic elements which proceeds slowly and then suddenly mounts rapidly as the zone of hypertrophic cells is reached. Similar, though more marked differences, are found in phosphatase activity.

Nucleic acids and the production of antibody by plasma cells
CAROLYN FORMAN (by invitation), WILLIAM E. EHRLICH and DAVID L. DRABKIN *Depts. of Pathology and Biochemistry, Graduate School of Medicine, Univ. of Pennsylvania, and the Philadelphia General Hospital, Philadelphia,*

Pa. It has been suggested that protein synthesis is associated with nucleic acid metabolism. Deoxyribose nucleic acid (DNA) appears to be connected with the multiplication of chromosomes, whereas the production of cytoplasmic protein is thought to be conditioned by ribose nucleic acid (PNA). We have investigated the relationship of antibody formation and the production of nucleic acids in lymph nodes of rabbits, injected with typhoid vaccine. The increase in DNA was found to be parallel with the increase in weight of the nodes, suggesting that the latter was due largely to multiplication of cells. PNA, on the other hand, showed its greatest rise between the 4th and 6th day after vaccine injection when antibody formation is at the peak. Thereafter, PNA rapidly decreased. A histologic study of methyl green and pyronin stained sections of the nodes revealed that during the first 6 days of the experiment the cellular reaction was chiefly that of plasma cell proliferation, and that the plasma cells contained most of the PNA. The lymphocytes, on the other hand, showed their greatest activity only after PNA and antibody formation had passed their peaks. These results are interpreted as indicating that the plasma cell and not the lymphocyte is responsible for antibody formation.

Minimum daily requirements of indispensable amino acids for maintenance of adult rats
L. E. FRAZIER, R. L. WOOLRIDGE, C. H. STEFFEE, (all by invitation) and E. P. BENDITT *Dept. of Pathology, Univ. of Chicago, Chicago, Ill.* The quantitative requirements for the 9 amino acids indispensable for maintenance of appetite, weight and nitrogen equilibrium of adult, well-nourished, male, albino rats (Wissler *et al.* *J. Nutrition* 36:245, 1948) were measured. Mixtures of crystalline amino acids were used as the nitrogen source. The caloric intake was maintained at or slightly above 1200 Cal/m²/day and the nitrogen intake at or slightly above 5 gm/m²/day. Vitamin, mineral and fat intakes were adequate. The level of the amino acid under consideration was varied while other diet factors were kept constant. Weight changes and nitrogen balance were determined. Seven to 14 animals were used for each amino acid. The minimum quantity of amino acid necessary to maintain weight and nitrogen equilibrium was estimated from the curves relating amino acid intake and weight change or nitrogen equilibrium respectively. In all instances the quantity of amino acid necessary to maintain nitrogen equilibrium appeared to be somewhat lower than the amount necessary to maintain the weight. The requirements for each amino acid for nitrogen equilibrium and weight balance respectively were as follows: Tryptophane 1.8 and 2.2, histidine 2.1 and 2.2, phenylalanine 3.1 and 6.0, lysine 3.7 and 4.5, threonine 5.1 and 5.3, methionine 5.3 and 7.3, leucine

6.5 and 8.0, valine 7.0 and 10.0, isoleucine 10.8 and 13.7 mgm/100 cm² of body area/day. Furthermore, animals offered an amino acid mixture containing all the indispensable amino acids at the above levels were maintained in nitrogen equilibrium.

Fates of parenterally administered homologous serum protein and casein hydrolysate JOHN FULLER (by invitation), E. M. HUMPHREYS (by invitation), C. H. STEFFEE (by invitation), R. W. WISSELER (by invitation), and E. P. BENDITT *Dept of Pathology, Univ of Chicago, Chicago, Ill.* Groups of protein-depleted rats were fed a protein-free basal ration by gavage. One group was given homologous serum intravenously, a second, casein hydrolysate (Amigen) intravenously, and a third, Amigen orally. Each animal received 5 gms protein/kgm body wt/day for 9 days. Nitrogen balance, blood compartments, and extracellular fluid volume determinations were made. Carcasses, and livers were analyzed for protein, fat, water and ash. Percentages of nitrogen intake retained in 9 days were

Compartment	I V serum	I V hydrolysate	Oral hydrolysate
Plasma	6.9	2.6	1.1
Liver	8.9	4.9	4.6
Carcass	32.8	10.7	36.8
Total	48.6	18.2	42.8

Animals receiving oral hydrolysate had the smallest extra- and intra-cellular fluid and plasma volume increases. The largest plasma volume as well as plasma protein increases were associated with intravenous administration of serum. Although the animals receiving intravenous hydrolysate gained far less plasma protein than those receiving serum, their plasma volume increases were nearly as great. No increased nitrogen excretion was found in the serum-injected animals in the ten days following cessation of injection during which they received an adequate protein diet. It appears that these rats utilized injected serum protein for tissue synthesis. The rats appeared to utilize the serum protein more than twice as effectively as the injected casein hydrolysate. The poorer utilization of the injected hydrolysate may have been due to its content of polypeptides.

Prophylaxis of allergic acute disseminated encephalomyelitis in guinea pigs with salicylate and para-aminobenzoic acid ROBERT A. GOOD, BERRY CAMPBELL and T. A. GOOD (introduced by IRVINE McQUARRIE) *Univ of Minnesota, Minneapolis, Minn.* That acute diffuse central nervous system disease can be produced in experimental animals by injection of heterologous and homologous brain tissues was shown by Rivers, Sprunt, and Berry. Schwentker and Rivers, among others, demonstrated that this phenomenon was associated

with formation of organ-specific antibodies against brain tissue. Recent studies have resulted in the production of auto-allergic disease of the central nervous system of monkeys, rabbits, and guinea pigs by a single or few injections of homologous brain tissue with adjuvants. Recent studies have shown that 70 to 90% of guinea pigs develop symptoms of progressive encephalomyelitis 13 to 37 days after injection of homologous brain plus an adjuvant of heat killed acid fast organisms, Falba, and Bayol F. In an attempt to test the effect of para-aminobenzoic acid and sodium salicylate medication on the course of this disease, 124 guinea pigs were studied. Ninety % of control animals developed progressive encephalomyelitis between 13 and 20 days after a single injection of guinea pig brain with adjuvants. While neither subcutaneous administration of sodium salicylate nor oral administration of para-aminobenzoic acid in moderate dosages alone affected the course of the allergic encephalomyelitis, premedication and medication with a combination of salicylates and para-aminobenzoic acid reduced its incidence to 10%. Administration of sodium salicylate in larger dosages resulted in demonstrable but less effective protection in spite of salicylate toxicity. Neither agent alone nor combination of the two had any ameliorating effect when administered after the encephalomyelitis had developed.

Canine hemophilia the clotting anomaly and effectiveness of transfusions JOHN B. GRAHAM (by invitation), JOSEPH A. BUCKWALTER (by invitation), L. J. HARTLEY (by invitation) and K. M. BRINKHOUS *Dept of Pathology, Univ of North Carolina, Chapel Hill, N. C.* A sex-linked hemorrhagic disease of male dogs was described by Field et al. These animals have been studied in our laboratory for the past two years. The clotting defect appears to be identical with human hemophilia. Bleeding time from small puncture wounds is normal while bleeding from larger wounds is extensive. The clotting time is prolonged. Prothrombin is present in normal amounts, but its conversion to thrombin is extremely slow. The rate of prothrombin utilization can be increased by the addition *in vitro* of thromboplastin, normal dog plasma, or Fraction I (Armour, Squibb). Prothrombin conversion in whole hemophilic blood approximates the normal rate after addition of normal plasma. This corrective action appears to be dependent upon the presence of formed elements, particularly platelets. Mixtures of platelet-poor hemophilic plasma and platelet-poor normal plasma do not clot for hours, and almost no prothrombin is converted to thrombin. Normal blood and plasma transfusions correct the clotting defect. The corrective effect is most pronounced for approximately 24 hours, but a slight effect persists as long as 72-120 hours. Normal serum trans-

fusions have no effect Transfusions of fresh and aged hemophilic blood do not alter the clotting anomaly Untreated bleeder dogs usually die by the age of 3 months By the use of repeated plasma transfusions, the hemophilic dogs have been reared to maturity

Spontaneous adenomatous gastritis of mice of strain dba WILLIAM V HARE (by invitation) and HAROLD L STEWART *National Cancer Institute, Bethesda, Md* Inbred mice of strain dba develop a spontaneous lesion of the glandular stomach and duodenum, which is progressive and may be fatal Grossly it is characterized by a pebbled appearance of the gastric mucosa or by large hypertrophic rugae with deep furrows between them, or a combination of the two Microscopically the epithelial cells are frequently hyperchromatic and the glands are atypical and may penetrate the submucosa The submucosa usually shows an inflammatory reaction which may also be present in the forestomach as well In the duodenum single or multiple papillary adenomas develop having a broad base, and tending to fill the lumen, although they do not invade the muscularis No evidence of malignant degeneration in either the gastric or duodenal lesions has been found In some cases, however, there is obstruction of the pyloric canal

Effect of aging on the protein fractions of the normal human heart JOHN W HARMAN and JEAN H WEBSTER (introduced by D MURRAY ANGEVINE) *Dept of Pathology, Univ of Wisconsin Medical School, Madison, Wis* A series of 34 human hearts, of ages varying from birth to 80 years and normal as determined by gross and histological features, are analyzed for various protein fractions Representative samples of the principal chambers are ground finely, acetone dried and defatted with Skelly Solvent #2 Aliquots dried to constant weight are analyzed for total protein, alkali soluble protein, collagen and elastin by Conway's micro-kjeldahl technique At different ages the ventricles are unaltered in the proportions of these fractions Up to the age of 10 the 4 chambers are chemically similar, whereas beyond this age in certain hearts the atria contain a significantly increased quantity of collagen Differences between atria of the same organ are not observed, these chambers fluctuate concomitantly in their composition In all chambers, with the usual conversion factor of 6.25, only about 75-80% of the standard dried preparation is composed of protein Part of the remaining per cent is a fraction soluble in 30% KOH, precipitable with alcohol, which is Biuret negative and yields no reducing substances by acid hydrolysis The constant proportion of the protein fractions in aging normal hearts, except for certain atria, indicates that senility does not quantitatively affect the organ Collagenization of the atria may be of significance functionally, since

these chambers so frequently suffer rhythmic disorders

Nitrogen mustards as effective blood and plasma sterilizing agents FRANK W HARTMAN and GEORGE H MANGUN (by invitation) *Dept of Labys, Ford Hospital, Detroit, Mich* The authors recently demonstrated the ability of methyl-bis(beta-chloroethyl) amine hydrochloride (HN2) to sterilize blood and plasma with relatively slight changes in their components with the exception of an increase in prothrombin time Other mustards (tris(beta-chloroethyl)amine and bis(beta-chloroethyl) sulfide) are approximately equal in effectiveness The virucidal and bactericidal action of HN2 is dependent to some degree upon the medium, the pH, and the presence of substances competing for the agent In citrated plasma the sterilizing dosages of HN2 are 1) New Jersey vesicular stomatitis, 300 mg/l, 2) lymphocytic choriomeningitis, 400 mg/l, 3) St Louis encephalitis, 300 mg/l, 4) *E coli*, 450 mg/l, 5) Hemolytic streptococcus, 450 mg/l, 6) *Staphylococcus aureus*, 800 mg/l, 7) *Bacillus subtilis*, 150 mg/l In the absence of plasma HN2 is still more effective, illustrating the protective or competitive effect of the plasma In saline, *E coli*, for example, is completely killed at 100 mg/l In ACD citrated whole blood, 450 mg/l are required for the complete destruction of N J vesicular stomatitis virus Studies carried out on ACD citrated whole blood treated with 500 mg/l of HN2 show 1) a very slight increase in fragility to hypotonic saline, 2) no immediate hemolysis nor increase above control hemolysis on prolonged storage, 3) a normal rate of loss of potassium from the red blood cells

Renal excretion of phosphate in the dog, influence of potassium, action of parathyroid extract C A M HOGGEN (by invitation) and J L BOLLMAN *Mayo Foundation, Rochester, Minn* The existence of a maximal tubular reabsorption of phosphate was confirmed utilizing plasma ultrafiltrates for the measurement of plasma inorganic phosphate During the course of a 6-8 hour experiment, the maximum will decline 25-50% Incorporation of potassium in the infusion fluid would, under certain conditions, restore the maximum The action of intravenous parathyroid extract was measured on the tubular reabsorption of endogenous phosphate in the intact dog for 2 hours after administration A slight rise in plasma phosphate and urinary excretion of phosphate resulted No evidence was obtained to support the hypothesis that parathyroid extract inhibited tubular reabsorption of phosphate Intravenous parathyroid extract to the intact dog was without effect upon the maximum tubular reabsorption of phosphate for 2 hours after administration Further studies will be reported on the action of parathyroid extract in the thyroparathyroidectomized dog

Pathology of an experimental flash-burn LEWIS HOGG, (by invitation), J THOMAS PAYNE (by invitation) and HERMAN E PEARSE *Univ of Rochester Atomic Energy Commission Project, Rochester, N Y* The importance of the flash burn as a clinical entity was pointed out by the Navy at Pearl Harbor, and by observers of the Hiroshima and Nagasaki casualties. An attempt was made to produce a flash burn, to study its morphology and the factors altering its healing. A series of about 75 such burns were produced on the pig, using magnesium explosions as the high intensity, short duration heat energy source. These lesions typically presented a zone of central pallor surrounded by abrupt erythema. The erythema and edema resolved over 4-7 days, leaving only a thin central crust which came free at about 10 days. Histologically, the lesions presented an abrupt, linear demarcation at their margins, as well as their depths, as manifest by hair follicle and dermal changes. Healing was by sequestration of the burned tissue, rather than by organization, and required 3-5 days. This is a unique picture and is intimately related to the transfer of heat through the cutaneous layers. The energy transfer measurements are still in progress.

Body content of radium in individuals with no known exposure J B HURSH and A A GATES (by invitation) *Dept of Radiation Biology, Univ of Rochester, Rochester, N Y* The commonly accepted maximum permissible level of radium in the body is 1×10^{-7} g. This level is about ten times higher than the lowest radium content known to have produced crippling bone lesions. It is, therefore, noteworthy that A Krebs, working in a German laboratory, measured the radium body content of eighteen individuals who died with no known radium exposure and found a range of body contents from less than 1×10^{-9} to 4×10^{-8} g radium, with an average of 1.4×10^{-8} g. If these findings have a general application, the margin between the 'safe' radium level and the level existing in 'unexposed' individuals is uncomfortably narrow. We have, therefore, repeated Krebs's measurements using crematory material available from local hospitals. Total body cremation was carried out at a temperature of 1600°F for two hours. The total ashes were weighed and ground. After mixing, an aliquot was taken for analysis. The sample was digested and put into clear solution. The radium disintegration product, radon, was allowed to accumulate for a known period of time. Radon gas was then boiled off the solution and the activity measured in an alpha counter. The results gave values from 1.3 to 3.6×10^{-10} g radium per total body with an average of 2.7×10^{-10} g. This is about two per cent of Krebs's average figure and introduces a more generous margin between the 'safe' and 'unexposed' value.

Localization of vital dyes in areas of inflammation HOWARD C HOPPS and BARBARA C REITER (by invitation) *Dept of Pathology, School of Medicine, Univ of Oklahoma, Oklahoma City, Okla* To elucidate further the mechanisms by which increased capillary permeability occurs in areas of inflammation, the manner of localization of trypan blue in dermal inflammatory lesions has been studied. In all, 58 inflammants have been investigated including such diverse agents as heat (conduction), cold, infra red and ultra violet rays, N-mustard, various cationic and anionic detergents, hyaluronidase, numerous essential oils (cantharidin, methyl salicylate, etc.), pyridine, chloroform, acetone, histamine, leukotaxine, and products of streptococcus pyogenes including intra-dermal infections as such. Quantitative data have also been secured as to the degree of localization of dye in relation to time and intensity of inflammatory reaction. These observations suggest that localization of such dyes as trypan blue differs qualitatively as well as quantitatively in response to different types of inflammatory stimuli.

Production of 'sickling' in normal red blood cells RAPHAEL ISAACS *Michael Reese Hospital, Chicago, Ill* If a drop of blood is mixed with a drop of concentrated 'fish gelatin' (e.g., Le Page's glue) the red blood cells will take on the sickle shapes at once. This phenomenon is noted in normal human blood, in the blood of animals, even those with nucleated erythrocytes, in sickle cell anemia and in spherocytosis. In an extensive list of diseases tested, the sickling response was the same. The cells are first aligned along the lines of stress in the thick jelly, but later retain the sickle form when thoroughly mixed. If the glue is allowed to dry, the sickling is preserved. In wet preparation, under a cover glass, the cells eventually become spherical, and later hemolyze. Sickling, then, is a property of all red blood cells. In sickle cell anemia and in sickle cell anemia some factor is present which makes the cells sickle under conditions which the normal cell resists.

Methionine and choline in the arrest of dietary cirrhosis of the liver in the rat ERNEST R JAFFE (by invitation), ROBERT W WISSLER (by invitation) and EARL P BENDITT *Dept of Pathology, Univ of Chicago, Chicago, Ill* An experiment with young albino rats is described in which it was possible to evaluate quantitatively the influence of methionine and choline on the progression of dietary cirrhosis. After a 70-day preparatory period on a low protein, high fat ration, the animals were offered diets containing crystalline amino acid mixtures in place of casein with various quantities of methionine, cystine, and choline for 32 days. When the rats ate 55 to 69 mg of methionine or 27 to 33 mg of choline chloride per day, cirrhosis was absent or much less marked than when they con-

sumed about 11 mg of methionine and no choline. No accentuation of hepatic fibrosis was noted that could be attributed to high levels of cystine, and the combination of cystine plus choline was not as effective in arresting the scarring as methionine and choline. The influence of methionine and choline on the cirrhosis was closely correlated with the ability of these dietary essentials to alter the large droplet fatty change present in rats after 70 days on the basal ration. These substances, perhaps through their lipotropic properties, caused a disappearance of the large globules, and this improvement in the structure of the parenchymal cells may, in part, have delayed the progression of the hepatic fibrosis.

Tocopherol requirements during the rat's menopause HANS KAUNITZ and C. A. SLANETZ (by invitation) *Depts. of Pathology and Animal Care, College of Physicians and Surgeons, Columbia Univ., New York City*. An inbred colony of albino rats was maintained for successive generations on a simplified diet supplemented by 3 mgs synthetic dl alpha tocopherol (Hoffmann-La Roche) per 100-gm diet. When the females were 3-6 months, mating resulted in 90% pregnancies, at 10-24 months, only 50% gestations (both normal or resorptions) were observed. When the tocopherol supplement was omitted, reducing the daily intake from about 300 to 30 micrograms, 50% pregnancies (nearly always resorptions) occurred when the females were 3-6 months. Those old rats on the 'complete' (3 mgs tocopherol) diets, which had become sterile, were given 30-60 mg tocopherol weekly, resulting in 16 pregnancies among 37 rats. More than half of the females on the 'complete' diet gradually developed a condition which could be termed menopause because it was associated with irregularities and finally with absence of the estrus cycle as well as with sterility. This is not necessarily a consequence of 'natural aging' because tocopherol supplements restored the estrus cycle and the fertility in a high percentage of the animals. The tocopherol requirements of the rat essential for the development of either a normal or a resorptive gestation increase steeply with age. In rats of over 1 year, they are roughly 10 times higher than at 3-6 months. This is one of the causes for the occurrence of menopause on a 'normal' diet.

Level of serum mucoprotein as indicator of disease activity in rheumatic fever V. C. KELLEY and R. A. GOOD (introduced by IRVINE MCQUARRIE) *Dept. of Pediatrics, Univ. of Minnesota, Minneapolis, Minn.* Winzler and co-workers, who determined the mucoprotein content of the serum in adult human subjects in health and in various disease states, demonstrated elevations of the levels of these proteins in malignancy, in certain acute infections, and in tuberculosis. Since they found a constant ratio to exist between total nitro-

gen, carbohydrate and tyrosine in the mucoprotein fraction, they used the relatively simple determination of mucoprotein tyrosine as an indicator of mucoprotein level. Using their methods modified to permit employment of the Evelyn photoelectric colorimeter, we determined the serum mucoprotein levels in 50 normal children and in a much larger number of children suffering from a variety of disease conditions, including rheumatic fever. Our values for normal children of different ages were in close agreement with those found in normal adults by these authors and by ourselves as well. The most notable feature of our data on sick children was the abnormally high mucoprotein levels found in a series of patients with rheumatic fever in its active phase. In comparison a similar series of rheumatic patients observed during the inactive or quiescent phase and a few patients with apparently active chorea minor showed entirely normal values. Serial determinations of the serum mucoprotein levels in a smaller group of rheumatic fever patients, followed throughout the cycle of active and inactive periods, confirmed the relationship. The degree of elevation of serum mucoprotein was found to be correlated with the state of rheumatic activity as determined by clinical manifestations and by erythrocyte sedimentation rate.

Sustained elevation of blood cholesterol and phospholipid levels in rabbits given detergents intravenously AARON KELLNER, JAMES W. CORRELL, and ANTHONY T. LADD (introduced by JOHN G. KIDD) *Dept. of Pathology, New York Hospital, Cornell Medical Center, New York City*. Sustained elevations of blood cholesterol and phospholipid were produced in rabbits maintained on a normal diet by the intravenous injection of the detergents Tween 80 and Triton A-20. A single injection of Tween 80 caused an increase in blood cholesterol and phospholipid to two to three times the baseline level, the values reaching a peak in 6 to 12 hours and returning to normal in 24 to 48 hours. Repeated injections of Tween 80 resulted in a sustained hypercholesterolemia with blood levels 5 to 15 times normal, and a parallel and somewhat greater increase in blood phospholipid levels. A single injection of Triton A-20 caused an increase in blood cholesterol and phospholipid levels to more than 5 times normal, with a peak at 2 days after injection and a return to normal after 5 to 12 days. A sustained elevation of blood cholesterol and phospholipid was maintained in 12 rabbits for 10 weeks by twice-weekly injections of Triton A-20. In most animals the blood serum became opalescent and then milky within a few hours after injection and cleared when the injections were stopped. Whether the increase in blood lipid depends on the actual presence of the detergent in the circulation, or on other factors, remains to be determined.

Fate of cancer cells implanted in susceptible and resistant hosts JOHN G KIDD and HELENE W TOOLAN (by invitation) *Dept of Pathology, The New York Hospital, Cornell Medical Center, New York City* The cells of two cancers—a mammary carcinoma and a lymphosarcoma—always grew progressively when implanted in the subcutaneous tissues of C3H mice of the sort in which they had originated, causing huge tumors that brought about death of the hosts within a few weeks after implantation. The cancer cells grew equally well for a time upon implantation in mice of the 'A' strain, but after developing into visible and palpable growths that reached 1-3 cm in diameter within 8 to 14 days, they regressed abruptly in the alien 'A' hosts, disappearing completely by the 10th to 16th days. The 'A' mice in which such tumors had regressed proved solidly 'immune' to re-implantation, no visible or palpable nodules forming after the subcutaneous implantation of large numbers of tumor cells. To learn more about the phenomena of resistance and immunity just described, a study was made of the cytological and histological changes in and around the cancer cells of regressing tumors, and those implanted in immune hosts. These findings were correlated with the viability of the cells as determined by transplantation tests. In many experiments, transplantable tumor cells were procured from regressing growths that had all but disappeared and at a time when only a few malignant cells remained, imbedded in a great mass of reactive host elements. The tumor cells likewise proved transplantable after a sojourn of 3 to 5 days or longer in the subcutaneous tissue of immune hosts.

Cardiac hypertrophy, an immediate response to Starling's Law of increased energy output of the heart CECIL A KRAKOWER and HELEN E HEINO (by invitation) *Univ of Illinois, College of Medicine, Chicago, Ill* The accepted mechanism for increasing the energy output of the heart per stroke by lengthening of the muscle fibers of the heart and that which underlies cardiac hypertrophy is the same. It has not been established experimentally, how soon cardiac hypertrophy sets in, once the energy output of the heart per stroke is increased. The speed of cardiac hypertrophy was studied in chicks standardized as to breed, age, sex, and body weight. They were fed a 6% NaCl food mixture over a period of nine hours. Food and water were then withdrawn. Observations on changes in cardiac mass were made at the end of 24 hours. It is demonstrated that in repeated trials, an increase of 7% or more in the heart weight-body weight ratio is obtained within a 24 hour period, representing an absolute increase in intracellular water, in fat-free dry substance and total nitrogen of the heart. The ratios of fat-free dry substance and nitrogen of heart to carcass were

likewise increased. It is concluded that cardiac hypertrophy is an immediate response to an increased energy output of the heart per stroke. It follows that cardiac mass in the normal individual is not stable but fluctuates with shifts in the work of the heart per beat beyond that which is customary. It will be demonstrated that a sound myocardium, responds to a given load with maximum hypertrophy within a few days, in the present experiments 20 plus % in 9 days.

Intravenous detergents in experimental atherosclerosis, with special reference to the possible role of phospholipids ANTHONY T LADD, AARON KELLNER, and JAMES W CORRELL (introduced by JOHN G KIDD) *Dept of Pathology, New York Hospital, Cornell Medical Center, New York* Rabbits were maintained on a normal diet without added cholesterol and were given twice daily intravenous injections of Tween 80 for 8 to 14 weeks. Controls were fed cholesterol but given no Tween 80. Despite the fact that the animals given detergent had considerably higher mean cholesterol levels than the controls, they had significantly less atherosclerosis. In the animals receiving detergent, the blood phospholipid levels were as high or higher than the corresponding cholesterol levels, whereas in the controls, the phospholipid levels were about half the cholesterol levels. Experimental atherosclerosis produced in rabbits by cholesterol feeding is usually accompanied by a great increase in blood cholesterol and only a slight increase in phospholipid. Other experiments in which rabbits were fed cholesterol and also given intravenous detergents indicate that despite a sustained hypercholesterolemia, the incidence and severity of atherosclerosis is decreased if the blood phospholipid is elevated concomitantly with the cholesterol. Intravenous detergents were ineffective in the resorption of atherosclerosis previously produced by cholesterol feeding. It seems probable that the cholesterol-phospholipid ratio is an important factor in experimental atherosclerosis, and that increased blood phospholipids may modify or prevent the development of atherosclerosis.

Observations on lung pathology following the inhalation of radioactive cerium HERMANN LISCO and M P FINKEL (by invitation) *Argonne National Lab, Chicago, Ill* Radioactive cerium (Ce^{144}) is one of the rare earths which is formed in the fission of uranium and of plutonium. It is a beta emitter (maximum energy 0.348 Mev) and it has a half-life of 275 days. Ce^{144} was administered to rats as an aerosol (CeO_2) in doses ranging from 3.2 to 200 μ c per animal. Although the material was deposited generally in all lobes of the lungs, its distribution was not uniform. Radioautographs showed small and large, irregularly distributed foci. A considerable portion of the inhaled cerium

remained in the lungs. However, in addition to excretion, some translocation occurred primarily to the liver, kidneys and skeleton. Histologic examinations were made of the lungs of most of the animals, especially those surviving beyond 200 days. The lesions observed will be discussed and illustrated. Particular emphasis will be placed on the occurrence of metaplastic changes in the bronchial epithelium and on malignant tumors arising therefrom.

Varying genetic resistance of rabbits to quantitative inhalation of human tubercle bacilli MAX B. LURIE, SAMUEL ABRAMSON and A. G. HEPPLESTON. *Henry Phipps Institute, Univ. of Pennsylvania, Philadelphia, Penna.* The fundamental variant tuberculosis caused by virulent bovine tubercle bacilli in highly inbred rabbits of varying genetic resistance to tuberculosis is the degree of localization of disease at the portal of entry and the chronicity of ensuing disease. Rarely, is the bovine infection completely overcome even by the most resistant family. Bovine disease in rabbits of different genetic resistance to infection differs only in degree, not in kind. It has been demonstrated that if genetically susceptible and resistant rabbits simultaneously inhale from 100 to 2000 virulent tubercle bacilli, human type, resistant rabbits overcome the infection completely so that at the end of 2 to 5 months after exposure the lungs are entirely free of any gross tuberculosis in 80% of the individuals, while in 90% of the susceptible animals there is a disease of varying extent often involving the greater part of the lung parenchyma. Thus, not only can the time necessary for determining resistance be greatly shortened, but the distinction between genetically resistant and susceptible rabbits becomes an all or none difference in the great majority of individuals. There is evidence that genetically resistant animals have an innate capacity to destroy or inhibit growth of inhaled human type tubercle bacilli more effectively than susceptible rabbits. Furthermore, and perhaps as a consequence of this differing capacity, genetically resistant rabbits tend to develop allergic sensitivity to tuberculin more rapidly than susceptible rabbits under these conditions.

Studies on the pathogenesis of experimental necrotizing arteritis J. H. McCORMICK, JR. (by invitation) and RUSSELL L. HOLMAN. *Dept. of Pathology, Louisiana State Univ. School of Medicine, New Orleans, La.* Previous studies from this laboratory have shown that acute necrotizing arteritis can be produced with regularity in dogs by feeding them a specified high fat diet for a period of 8 weeks or longer then sacrificing them through renal damage. To determine whether damage to tissues and organs other than the kidney would precipitate the lesions in properly fed dogs, 4 such animals were subjected to light chloroform

anesthesia for periods totalling 9 to 28 hours in 6 to 20 days. None of these 4 dogs showed either gross or microscopic lesions in the arterial system. Another group of 3 properly fed dogs were subjected to severe inflammatory reactions, one by means of sterile abscesses induced with turpentine and 2 by bacterial infections. None of these dogs showed arterial lesions. These experiments tend to emphasize the importance of the kidney in the pathogenesis of necrotizing arteritis, and suggest the possibility that the proximal convoluted tubules elaborate a substance that is necessary for the proper utilization of certain lipid substances.

Influence of alloxan diabetes of cholesterol atherosclerosis in the rabbit HENRY C. MCGILL (by invitation), LOUIS E. PARRISH (by invitation) and RUSSELL L. HOLMAN. *Dept. of Pathology, Louisiana State Univ. School of Medicine, New Orleans, La.* Nine control rabbits fed Rockland rabbit diet with 0.3–5 gm. of cholesterol in olive oil per day were sacrificed after intervals of 38 to 72 days on this diet. There was an average gain in body weight of 38% and an approximate seven-fold increase in total blood cholesterol. All 6 of these animals sacrificed beyond the 48th day showed gross or microscopic atheroma in the aorta and elsewhere, and the degree of these lesions increased with time. Eight rabbits of similar age and original body weight were injected two or more times with alloxan (180 to 385 mg/kg. total) and after a satisfactory diabetic state was established they were fed the same diet and cholesterol in oil as the control animals. These diabetic rabbits were sacrificed after intervals of 18 to 98 days. Six of the 8 rabbits lost weight but 2 of them showed weight gains comparable to the control rabbits. Despite a ten-fold increase in the blood cholesterol level in this group only the rabbit fed for 98 days (which lost 10% in body weight) showed microscopic arterial lesions. Three rabbits injected with alloxan failed to develop sustained diabetes and had lesions comparable to the controls when sacrificed at 40, 52, and 69 days respectively. Contrary to anticipation these results indicated that the diabetic state induced by alloxan retards in both time and degree the development of cholesterol atherosclerosis in the rabbit. These experiments confirm the results of Duff and his co-workers.

Experimental papillary necrosis of the kidney EMANUEL E. MANDEL (by invitation) and HANS POPPER. *Dept. of Pathology, Northwestern Univ. Medical School, the Hektoen Institute for Medical Research of the Cook County Hospital and the U. S. Public Health Service, Chicago, Ill.* Necrosis of the renal medulla has been reported in old European papers to be produced in experimental animals by the administration of vinylamine. In order to study pathogenesis and functional changes incident to such a lesion, vinylamine (ethyleneimine) was

given to 20 rabbits in single or divided doses of 0.005 to 0.03 gm/kg body weight. On the first day following injection of a lethal dose, excessive hyperemia of all medullary vessels occurred, associated with edema and hemorrhage particularly around the bundles of vasa recta at the cortico-medullary junction. The cortex showed striking ischemia except for distended veins. Necrosis of epithelium of the proximal convoluted tubules was often present. In later stages (3-14 days after intoxication), when the cortical epithelium appeared either almost or completely normal or showed evidence of regeneration, conglutination thrombi of erythrocytes were seen in engorged medullary vessels. There were well circumscribed foci of ischemic necrosis of varying size in the papillae. These animals did not develop anuria but showed albuminuria, hematuria and progressive azotemia with almost complete abolishment of renal concentration power as indicated by a urine/plasma quotient of close to unity. In animals surviving the intoxication more than 2 weeks, fibrous scars were found in the papillae and also replacing the vasa recta bundles. These findings appear to throw light both upon a vascular pathogenesis of human papillary necrosis and upon the renal functional impairment occurring in predominantly medullary injury. They, furthermore, illustrate the duality of renal circulation (Trueta).

Hypoprothrombinemia due to loss of intestinal lymph J. D. MANN (by invitation), F. D. MANN (by invitation) and J. L. BOLLMAN *Mayo Foundation, Rochester, Minn.* When all of the intestinal lymph of the rat was drained externally, marked hypoprothrombinemia, as determined by the two-stage method, developed rapidly, usually within 24 hours. If adequate amounts of vitamin K were administered parenterally a normal level of prothrombin was maintained, despite loss of lymph and even of considerable amounts of blood as well. Transfusion of twice the animal's normal plasma volume did not maintain normal prothrombin while lymph was lost. Under the conditions of these experiments it appeared that vitamin K was absorbed practically exclusively through the lymph and very little of it was stored, while the turnover of prothrombin was extremely rapid.

An additional thermostable component in the mechanism of leukocytosis with inflammation VALY MENKIN *Agnes Barr Chase Foundation for Cancer Research, Temple Univ. School of Medicine, Philadelphia, Penna.* The leukocytosis-promoting factor (LPF), described previously, offers in part a reasonable explanation for the mechanism of leukocytosis with inflammation. Nevertheless, an acute experimental inflammation is frequently associated with a leukocytosis of such a high level that it cannot always be explained by liberation of LPF. This is especially seen in the later stages of

an inflammatory state when the exudate tends to be at an acid pH, and from which the LPF is not always readily recovered. It has been observed that the non-injurious pyrogenic factor, pyrexin, frequently recovered from acid exudates, induces not only an elevation in temperature, but also an initial leukopenia due to the presence of the leukopenic factor, described previously. The initial leukopenia is subsequently followed by a marked leukocytosis. Boiling pyrexin fails to inactivate this leukocytosis-promoting component. This latter is evidently thermostable. Its effect is very rapid. Even one hour following administration of boiled pyrexin, the effect of the thermostable component of the leukocytosis-promoting factor is manifested by the discharge of immature polymorphonuclear leukocytes. This thermostable component is found in boiled whole acid exudate. In alkaline exudates it is less pronounced, and in such boiled exudates only the discharge of immature leukocytes may be encountered. This thermostable component has been observed in the inflamed pleural cavity of dogs, inflammation being induced with either turpentine or croton oil in olive oil and in the inflamed pleura of guinea pigs with the reaction caused with croton oil in olive oil. The mechanism of leukocytosis with inflammation seems referable to two components: a thermolabile component distributed between the α 1 and α 2 globulins of exudate and a thermostable component associated with pyrexin in the euglobulin fraction found particularly in acid exudates.

Effects of thyroid feeding on histologically normal islets of rats receiving small doses of alloxan DAVID W. MOLANDER (by invitation) and ARTHUR KIRSCHBAUM *Dept. of Anatomy, Univ. of Minnesota Medical School, Minneapolis, Minn.* Impairment in glucose tolerance appeared in rats receiving 4 intravenous injections of 20 mg/kg of alloxan on alternate days. Fasting blood sugar levels were not elevated. Microscopically the pancreatic islets were normal. Rats receiving 10 injections of the same dose of alloxan showed glucose tolerances similar to those found in animals receiving four doses. Beta cell degeneration was present, however, in the islets. Neither glucosuria, polyuria, nor polydipsia occurred in either the 4 or 10 dose animals. Extensive beta cell degeneration was observed in rats receiving one dose of 30 mg/kg. These rats exhibited persistent hyperglycemia and glucosuria, and decidedly altered glucose tolerance. Growth was stunted, whereas it was normal in animals receiving the 4 or 10 injections. If thyroid (0.1 gm/kg daily by stomach tube for one month) was fed to rats following the 4 doses of alloxan, glucosuria and fasting hyperglycemia occurred. This condition persisted until the rats were autopsied one month after the last feeding. If rats were adrenalectomized preceding the administra-

tion of thyroid, clinical diabetes did not appear. The islets of thyroid-fed alloxanized rats revealed a reduction in the number of beta cells. In the rats that were adrenalectomized preceding thyroid treatment, and in which clinical diabetes did not appear, no changes in the islets were observed. Thus, by functionally altering the beta cells with small repeated doses of alloxan, the added metabolic strain imposed on islet tissue by thyroid feeding was detectable. Lower blood sugar (reduced gluconeogenesis) following adrenalectomy was probably important in preventing beta cell damage.

Induction of hepatic carcinoma in rats by 4-dimethylaminobenzene-1-azo-1-naphthalene. A S MURRAY and H I FIRMINGER (introduced by HAROLD L STEWART) *National Cancer Institute, Bethesda, Md.* Azonaphthalene (4-dimethylaminobenzene-1-azo-1-naphthalene) was fed to 80 Osborn-Mendel rats, in the dose of 0.075 gm/100 gm of diet for 8 months. The dye was suspended in corn oil, and this in turn was incorporated in a basic diet consisting of casein, sugar, vitamin mix, and salt mix. Following the azonaphthalene diet period, the animals were returned to their normal laboratory diet of Purina Chow pellets. They were examined at regular intervals, and the date of the first palpable tumor was noted. The clinical diagnosis of the tumor was confirmed at autopsy by microscopic examination in all cases. Fifty-six animals have been autopsied to date and 11 tumors have been found, an incidence of 19.5%. In this series, the first tumor appeared 8 months after the start of the experiment. If this interval is taken as the latent period for tumor induction by this compound, and the percentage of tumor incidence is then recalculated on the basis of those animals surviving the incubation period, the incidence of the tumor increases to 31%. The changes in the liver were similar to those induced by paradi-methylaminoazobenzene. Microscopic examination showed fatty change, fibrosis, bile duct proliferation, cirrhosis, cyst formation, and carcinoma. Most of the tumors were hepatic cell carcinomas. They infiltrated the adjacent tissues, and metastasized to the peritoneum, mesentery, and the lungs.

Renal cytoplasmic inclusions following the administration of large doses of streptomycin. CHARLES W MUSHETT and ROBERT B STEBBINS (by invitation) *Merck Institute for Therapeutic Research, Rahway, N. J.* Repeated subcutaneous injection of streptomycin either as the sulfate or the calcium chloride complex has resulted in the appearance of acidophilic 'colloid-like' droplets in the cytoplasm of the renal tubular epithelium of rats and dogs. The droplets, varying in size from small granules to relatively large globular bodies, have been seen within one week when daily injections

of drug (400 mg base/kg body weight) were given. After discontinuance of treatment in rats dosed daily for 21 days, it was observed that the droplets became smaller in size and had disappeared within 4 weeks. With hematoxylin-eosin stain the inclusions have a deep pink color, some give a positive reaction for hemoglobin by the Dunn-Thompson technique, they are not visualized by the usual fat staining methods. Salts of dihydrostreptomycin, the reduced form of streptomycin, also produced these inclusions. The feeding of natural stock diets, highly purified diets or diets containing 5% liver powder had no influence on the appearance of the inclusions. No abnormalities in renal function were observed in dogs whose kidneys were found to contain the 'colloid-like' cytoplasmic inclusions. The nature of the renal inclusions observed after treatment with streptomycin or dihydrostreptomycin is as yet undetermined.

Comparison of different lots of bacitracin for nephrotoxicity to rats and mice. A A NELSON and E C HAGAN (by invitation) *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.* Five commercial lots of bacitracin, suspected of varying in their degree of nephrotoxicity when given parenterally in man, were studied. Microscopic sections were made from 140 animals. All 5 lots caused severe kidney damage when 5000 U/kg in 2% solution were injected intraperitoneally twice a day in mice for 3 days. Variation in kidney damage between lots was no greater than that between different batches of mice when the same lot was used. The basic lesion was necrosis and sloughing of the convoluted tubular epithelium, evident as early as 1 hour after a single injection. Morphological effect paralleled dosage, and our impression was that when a certain concentration of bacitracin occurred in the tubule there was a direct toxic effect on the epithelium. Saline solutions were slightly less toxic than water solutions. In a general way the histological appearance was similar to that described by Scudt et al (*Proc Soc Exper Biol & Med* 64: 503 and 66: 558, 1947). In our rats, however, lesions were not 'insignificant', with equal dosage, rats showed even greater kidney lesions than did mice. Nephrotoxicity in rats from intramuscular administration was as great as from intraperitoneal, in mice it was much less. Intramuscularly, local inflammatory reaction was severe in rats and less so in mice, intraperitoneally, local reaction was slight. Organs other than kidney showed infrequent or little effect. Limited study of repair showed that in mouse kidneys the bacitracin picture persisted for about 15 days, changing after 30 days into one of a nonspecific nephritis.

Bone deposition of uranium. W F NEUMAN, M W NEUMAN, E R MAIN and B J MULRYAN

(introduced by F S ROBSCHT-ROBBINS) *Dept of Radiation Biology, School of Medicine & Dentistry, Univ of Rochester, Rochester, N Y* Previous studies have indicated that the major site of long-term storage of uranium injected into animals was the skeleton. Once deposited, the uranium was mobilized very slowly and underwent very little redistribution within the skeleton itself. A series of studies *in vitro* was conducted on the mechanism of this skeletal deposition. The results of these experiments indicate that uranyl ions undergo exchange with calcium ions in the surfaces of the bone, mineral crystals. In this exchange, uranium apparently combines with two adjacent phosphate groups in the mineral surface to form a but slightly dissociated complex.

Reticulocyte ripening and nutrition in anemic dogs A NIZET (introduced by F S ROBSCHT-ROBBINS) *Univ of Rochester School of Medicine and Dentistry, Dept of Pathology, Rochester, N Y* The ripening time of the reticulocytes has been measured by a technique previously described in anemic dogs and doubly depleted dogs (anemia and hypoproteinemia). Anemia is produced by blood withdrawal, double depletion by blood withdrawal plus a non-protein containing diet. The progressive disappearance of the various reticulocyte groups is studied in heparinized blood samples at body temperature. In the anemic dogs receiving a basal diet containing proteins the ripening time is the same as in non-anemic dogs, regardless of the supplementary diet factors. In the anemic hypoproteinemic dogs (double depletion) in contrast receiving a non-protein basal diet the ripening time depends upon the supplementary diet factors. With the non-protein diet alone in these doubly depleted animals the ripening is almost completely blocked. Following administration of poor supplementary diet factors (low hemoglobin regeneration) the ripening time is greatly prolonged. With an optimum supplementary diet (maximum hemoglobin regeneration) the ripening time is normal. The rate of hemoglobin regeneration *in vivo* and the ripening time of the young erythrocytes *in vitro* coincide very well.

Histopathology of the liver following histamine infusion W G NOTHACKER and RALPH W BRAUER (introduced by RUSSELL L HOLMAN) *Depts of Pathology and Pharmacology, Louisiana State Univ School of Medicine, New Orleans, La* When histamine diphosphate was infused continuously into the portal vein of a dog under sodium pentobarbital anesthesia, at such a rate as to depress the mean arterial blood pressure to approximately 50% of the initial level, there was a fall in the portal venous pressure, an increase in lymph flow from the liver, and a reduction of the rate of bromsulphthalein removal from the blood plasma. In animals sacrificed after 4 hours or more of such

infusion, the liver was enlarged, and mottled red and red brown with prominent portal areas. Upon histological examination these livers showed neutrophils and lymphocytes about the portal veins, dilatation of portal lymphatics, leukostasis of neutrophils in the sinusoids, constriction of central veins and occasional hemorrhages into the portal areas. When histamine infusion was stopped, at any time during the experiments, the mean arterial blood pressure regularly rose to the initial level within ten minutes or less and the animal survived. Control animals subjected to the same experimental procedures but infused with normal saline showed none of the above physiological and anatomical changes. These studies indicate that histamine in sublethal doses is capable of producing definite liver injury.

Replacement of a portion of common bile duct by a segment of uterine horn CHARLES M O'LEARY (by invitation), WILLIAM T SNODDY (by invitation), and BÉLA HALPERT *Depts of Surgery and Pathology, Univ of Oklahoma School of Medicine, Oklahoma City* The right uterine horn of the dog was resected with its blood supply from the ovarian artery left intact. The common bile duct was severed near its junction with the duodenum and the duodenal end ligated. The proximal end of the common bile duct was then telescoped into the proximal end of the uterine horn while the distal end of the horn was pulled through a longitudinal slit made in the duodenal wall about 2 cm anterior and medial to the choledochoduodenal junction. Both ends of the uterine horn were secured in place by cotton sutures. Of 20 dogs surviving the operation 12 lived for 3 to 21 days, the rest were sacrificed between 4 and 27 weeks following the operation. Detailed morphologic studies are in progress to determine the mechanism by which the continuity of the flow of bile into the duodenum was maintained and the effects of the replacement of the terminal segment of the common bile duct on the biliary system.

Restoration of estrous cycle by alpha tocopherol in old rats S Y P'AN, HANS KAUNITZ, and C A SLANETZ (by invitation) *Depts of Pharmacology, Pathology, and Animal Care, College of Physicians and Surgeons, Columbia Univ, New York City* An inbred colony of albino rats was maintained for successive generations on a simplified diet containing 3 mg (synthetic alpha tocopherol (Hoffmann-La Roche) per 100 gm, this permitted a daily intake of approximately 300 micrograms of tocopherol as usually provided for laboratory rats. Daily vaginal smears were made on 64 females of 10-20 months for 4 weeks or more. In 16, the estrous cycle was of 4-5 days duration, in 34, it lasted more than 8 days, and in 15 of this latter group, it lasted longer than 17 days in 80% and more than 12 days in 20%. The latter 15 females received 30-

60 mgs alpha tocopherol weekly by mouth. In 3 rats, cycles of 4-5 days and in 5, cycles of 6-8 days were observed within 2 weeks after treatment was begun. There was no change in the remaining 7 animals. It is improbable that the shortening of the cycle was due to spontaneous changes because 4 untreated controls with cycles of 9-12.5 days were smeared during the period of observation with lengthening of the cycles. It seems evident that, in a high percentage of old rats maintained on a diet with 'normal' tocopherol supplement, lengthened estrous cycles may occur which can frequently be shortened by additional alpha tocopherol.

Blood cholesterol value of normal men in relation to their basal metabolic rate. ANNIE LAURIE PEELER (by invitation), OPAL E HEPLER, VELMA MILLER KINNEY (by invitation), LILLIAN E CISLER (by invitation), and FREDERIC T JUNG (by invitation) *Depts of Pathology and Physiology, Northwestern Univ Medical School and Passavant Memorial Hospital, Chicago, Ill.* Because of the large range of normal cholesterol values the correlation was determined between the blood cholesterol of normal men and their basal metabolic rates. Bloor's method adapted to the Evelyn photoelectric colorimeter was used to determine cholesterol in serum. Values for 73 male medical students ranged from 131-303 mg/100 ml of serum with a mean of 189 mg and a standard deviation of ± 33 . Four fell below 150 and 6 above 250, leaving 86% with a range from 150 to 250 mg. The metabolism tests were performed under basal conditions using a Benedict-Roth machine. The results presented are the percentages above and below normal using the Boothby and Sandiford modification of Dubois standards. Values for 73 men ranged from -24.9 to +10% with a mean of -9.5% and a standard deviation of ± 7.2 . No correlation was found between these basal metabolic rates and cholesterol values as shown by the correlation coefficient of -0.10. Both cholesterol and basal metabolic rates were repeated on 40 of the men after a 38-day period which included 2 weeks of vacation. The coefficient of correlation between the first and second cholesterol values was +0.73 which indicates that the blood cholesterol is maintained at a fairly stable level. This was also true, to a lesser extent, of the basal metabolic rates which gave a correlation coefficient of +0.65 between the first and second periods.

Quantitative correlation between results of hepatic tests and morphologic liver cell damage. HANS POPPER, FREDERICK STEIGMANN and PAUL B SZANTO (by invitation) *Hektoen Inst for Medical Research of the Cook County Hospital, the Dept of Pathology of Northwestern Univ Medical School and the Dept of Internal Medicine of Univ of Illinois College of Medicine, Chicago, Ill.* The degree of liver cell damage was graded 0 to 3 plus

in 257 liver biopsy specimens obtained from patients with various diseases, mostly hepatobiliary in nature. The results were compared with those of a series of hepatic tests performed simultaneously with the biopsy. In confirmation of previous studies concerning correlation of presence or absence of pathologic findings with results of hepatic tests, it was noted that the higher the grade of liver cell damage in a group, the higher the average of the cephalin flocculation, thymol turbidity, serum globulin, urinary urobilinogen, alkaline phosphatase, prompt reacting and total bilirubin, and the lower the albumin, albumin/globulin ratio and prothrombin time. No parallelism was found with total serum protein, total cholesterol, non-protein nitrogen, sedimentation rate and fecal urobilinogen. The described parallelism was not necessarily present in individual cases. Moreover, in extrahepatic biliary obstruction without secondary infection, cephalin flocculation and thymol turbidity did not parallel the degree of liver cell damage, the cephalin flocculation being negative and the thymol turbidity only slightly elevated even with severe damage. Contrarily, the results of these two tests paralleled the degree of liver cell damage if bacterial infection complicated extrahepatic obstruction. To exclude liver cell damage, cephalin flocculation and urinary urobilinogen appeared the most valuable tests. The number of tests with abnormal results paralleled the degree of liver cell damage in the different groups. In general, the frequent exception in individual cases do not contradict a general tendency to a quantitative relation between functional and structural hepatic alterations.

Electron microscopy of clinical marrow aspirates. J W REBUCK and H L WOODS (introduced by F W HARTMAN) *Dept of Labys, Henry Ford Hospital, Detroit, Mich.* Direct electron microscopy of sternal aspirates was accomplished as follows. After aspiration of the specimen the customary air-dried smears were prepared from a portion for light microscope studies. The remaining material was utilized, with a modification of Schleicher's technic, for electron microscope studies. One or more gross marrow units were selected and affixed to the blunt end of a wooden applicator. Imprints were then made by touching the marrow unit lightly against formvar-covered glass slides. The marrow cells adherent to the surface of the formvar were fixed over osmic acid vapor and washed in distilled water. The specimens were mounted directly by the modified stripping technic which we have previously reported. Electron micrographs of hematocytologic features were thus obtained. Developing neutrophil granules were observed to be more vacuolar than granular in structure. The bizarre nuclear structure of leukemic reticulum cells from a case of reticular myelosis was depicted.

Superimposition of small osmophilic spherules was noted. Myeloma cells from a case of multiple myeloma were micrographed. A small, extremely dense particulate substance presented in the cytoplasm of such a cell. In one such case complicated by an acquired hemolytic anemia, interesting structural changes were present in erythrocytes individually and in their agglutinated relationships. Upon erythrocytic desiccation in rouleaux-like aggregates, hemoglobin shrinkage resulted in delineation of the isolated corpuscular 'membrane'. The thickness of this human surface ultrastructure was measured as being between 330 and 1000 Å with a mean of about 550 Å.

Effect of antihistamine on the localization of trypan blue in xylene treated areas of skin. R. H. RIGDON *Laby of Exptl Pathology, Medical Branch, Univ of Texas, Galveston, Texas*. Localization of dye in areas of skin injected with histamine (*J Lab and Clin Med* 27: 1554, 1942) and in areas of inflammation produced by xylene (*Arch Surg* 41: 101, 1940) has been studied. In the latter it was shown that localization and concentration of this dye was determined by the interval between the application of xylene and the injection of the dye. Furthermore, the localization of the dye was not related to the presence of hyperemia. The influence of antihistamine on the hyperemia produced by xylene and the localization and concentration of dye in these areas were studied in the rabbit. Pyrrolidineethylphenothiazine hydrochloride (Pyrrolazate) and N,N-Dimethyl-N' (alpha-pyridyl)-N'-(alpha-thenyl)-ethylenediamine hydrochloride (Thenylene) were dissolved in saline (10 mg/ml). The former was given intravenously and the latter intravenously and intraperitoneally in large amounts. The antihistamine was given frequently before the intravenous injection of trypan blue (10 ml of a 0.5% solution). Xylene was applied to the skin with a cotton applicator at intervals preceding the injection of the dye. Reaction to the xylene was the same in antihistamine treated animals as in the controls. Furthermore, the localization and concentration of trypan blue was the same as in the controls. Trypan blue localized and concentrated at the site of intradermal injection (0.2 ml) of antihistamine. It is suggested that histamine is not the primary factor responsible for the localization and concentration of trypan blue in areas of inflammation produced by xylene and, furthermore, antihistamine, when injected intradermally in the rabbit, causes a localization and concentration of dye in a manner similar to the intradermal injection of histamine.

Multiple injections of potassium as a test for adrenocortical function, with modifications induced by desoxycorticosterone. THEODORE ROBERTSON (introduced by JOHN G. KIDD) *Dept of*

Pathology, New York Hospital—Cornell Medical Center, New York City. As a means of studying the function of the adrenal cortex a modification has been made of the potassium toxicity test previously employed by others. Four intraperitoneal injections of potassium chloride solution were given at 2-hour intervals to mature rats and mice. Dosage was calculated in milligrams of KCl/10 gm of body weight. Significant differences were found when the test was applied to normal and adrenalectomized animals. For the L D 50 in normal rats was about 4.5 mg/10 gm, and in normal mice was slightly less than 5 mg/10 gm, whereas in adrenalectomized animals given salt and tested on the sixth postoperative day, the L D 50 values were approximately 2.0 and 2.6 respectively in the two species. A consistently increased resistance to potassium was obtained by the administration of small amounts of desoxycorticosterone acetate in various ways to normal and adrenalectomized animals. The only variation attributable to sex was found in adrenalectomized rats about 11 weeks old. When tested with 2 mg KCl/10 gm there was a 90% mortality in males (18 of 20) and a 15% mortality in females (2 of 13). The results obtained with the potassium toxicity test suggest an ovarian influence on potassium metabolism as an addition to those influences previously recognized. The test has also been applied with interesting results to animals in which a depletion of the zona glomerulosa of the adrenal cortex had been effected by means of pellets containing desoxycorticosterone.

Intradermal reaction to streptococcal thermal antibody and antigen in persons having epidemic respiratory infections. EDWARD C. ROSENOW *Bacteriological Research, Longview Hospital, Cincinnati, Ohio*. The solutions of thermal antibody and of antigen injected intradermally consisted of the bacteria-free supernatants of saline suspensions of streptococci isolated in studies of respiratory infections. The former after autoclaving suspensions containing twenty billion streptococci/ml for 96 hours and adding an equal volume of saline containing 0.4% phenol, the latter after heating suspensions containing ten billion streptococci/ml at 70°C for one hour and adding 0.2% phenol. Three hundredths ml of each and of control solutions were injected into the skin of the anterior aspect of the forearm of 272 persons having respiratory infection and of 483 well persons. Reactions to the 'respiratory' thermal antibody indicating specific antigen in skin or blood and of a streptococcal infection occurred in nearly all persons having respiratory infection. The reactions were largest during the acute stage of the disease and gradually diminished as recovery ensued. In sharp contrast reactions to 'respiratory' antigen indicating antibody were minimal at the onset, in-

creased as recovery occurred and then diminished as antigen disappeared. The incidence of significant reactions to the 'respiratory' antibody indicating antigen in well persons during summer was minimal. It increased in autumn and remained relatively high during winter and spring but was uniformly less in persons having symptoms of respiratory infection. Reaction to the 'respiratory' antigen was absent or least pronounced in well persons remote from respiratory infections and increased with the seasonal incidence. Reactions in well persons to 'poliomyelitic' streptococcal antibody and antigen were in reverse of those to 'respiratory' antibody and antigen.

Contagiousness of coccidioidomycosis. SOL ROY ROSENTHAL *Univ of Illinois, College of Medicine, Chicago, Ill.* Experiments conducted in 3 phases show that coccidioidomycosis is a contagious disease. 1) Spherule containing exudates from humans or guinea pigs when instilled into the trachea of guinea pigs produced a primary form of the disease very similar to that found in humans. 2) Spherule containing exudates of humans kept in the refrigerator for periods up to 110 days failed to produce mycelial threads and chlamydozoospores but retained their spherule forms that were infective to guinea pigs. Spherule containing sputum exposed out of doors to the shade or the sun with or without earth for periods up to 230 days retained their spherule forms, albeit in a certain percentage of cases mycelial forms were found. 3) In a limited number of cases placing normal guinea pigs in the same cages with animals infected intratracheally with the spherules of *coccidioides immitis* resulted in infection of the control animals.

Electrocardiograms of normal and malarial infected monkeys. ARTHUR RUSKIN (by invitation) and R. H. RIGDON *Heart Station and Lab of Exptl Pathology, Univ of Texas, Medical Branch, Galveston, Texas.* Electrocardiograms in 14 normal *M. rhesus* monkeys weighing from 2 to 6 kg simulated those of normal humans with the following differences. The QRS wave was usually of very low voltage in Lead I. The T wave in Lead I was likewise usually very low. It was flat in 7 monkeys. However, in 1 it was slightly negative on another occasion. The T wave in Lead II was flat in 2 cases, in both slightly negative on other occasions. T4 was flat in 2 animals and slightly negative in one. These findings controvert those of deWart and Storm. Heart rate varied from 190 to 280, P-R interval from 0.06 to 0.10 sec, QRS from 0.02 to 0.04 sec, Q-T from 0.14 to 0.18 sec, the axis from 50° to 115° . Marked S-T segment depression occurred particularly in Lead II and III in 6 cases. An auricular ectopic beat was the only arrhythmia noted. Electrocardiograms in 12 monkeys infected with *P. knowlesi* did not vary significantly from normal. This is consistent with

the absence of specific pathologic lesions in the heart of malarial infected monkeys. T waves in Lead I were flat in 6 of 12 infected monkeys. In 2 of the same group on different days T waves in Lead I were negative, however, both monkeys showed similar findings before infection. In 2 monkeys with severe parasitemia, the Q-T intervals were 0.20 at a ventricular rate of 188, and 0.23 at a rate of 159. One also showed left axis deviation of -48° , another very ill animal had an axis deviation of -40° . With these three exceptions, electrocardiograms in malarial infected monkeys were similar to normals.

X-ray induced developmental abnormalities in the mouse. LIANE BRAUCH RUSSELL (introduced by F. S. ROBSCHT-ROBBINS) *Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.* In an attempt to determine the effect of penetrating radiation on developing mouse embryos, pregnant females were irradiated at various stages of gestation. Each female was given a single exposure to 250 krp x-ray total body irradiation, doses ranging from 200-500 r at an intensity level of around 80 r/min. Gestation was timed from the observation of a vaginal plug and the stages examined cover almost the entire range of embryonic life. Highly inbred C57 Black strain females, made pregnant by NB strain males, yielded the genetically uniform F₁ fetal population. Except for a few cases in which the litters were raised, the irradiated fetuses were allowed to develop only to birth (or the expected date of birth), at which time they were carefully examined externally and then processed to give cleared and stained skeletal preparations. Our results indicate that certain structures are sensitive to x-ray effect only during definite periods in development. Dosage-effect relations at particular stages have proved to be interesting. It is hoped that the determination of sensitive stages for each structure, the entire syndrome produced at a particular stage and the order in which certain changes are produced—all considered in conjunction with the known facts of normal development and of hereditary abnormalities—may shed some light both on the nature of the radiation effect and the nature of development.

Prompt and indirect reacting serum bilirubin in jaundice. FENTON SCHAFFNER (by invitation), HANS POPPER and FREDERICK STEIGMANN *From the Hektoen Inst for Medical Research of the Cook County Hospital, the Dept of Pathology of Northwestern Univ Medical School, and the Dept of Internal Medicine of Univ of Illinois College of Medicine, Chicago, Ill.* Serum bilirubin determinations (1 minute and total) were obtained on 195 jaundiced patients with cirrhosis (152), acute hepatitis (171) and extrahepatic biliary obstruction (99). Results were correlated in 132 instances with liver biopsies. The ratio of prompt reacting to total bili-

rubin followed similar distribution curves with almost half the values lying between 40 and 50%. Too few cases of hemolysis were observed to evaluate statistically but in each instance the ratio of prompt to total bilirubin was below 15%. When the ratio was plotted against total bilirubin, ratios ranged in random fashion between 12 and 73% when the total was below 10 mg/100 cc. When above this, 82.5% of the ratios were between 30 and 50%. As the level rose, the ratio fell. It was below 40% in 65% of the determinations when the total was above 40 mg/100 cc. No relation between ratio and degree of liver damage was noted. The only significance of an elevated prompt bilirubin in the presence of jaundice is its exclusion of hemolysis. This does not support the view that bilirubinemia arises in a different manner in parenchymal and obstructive jaundice. The evidence suggests that indirect bilirubin is changed to prompt reacting in the Kupffer cells from where it is transmitted to the liver cells. Inability of Kupffer cells to accept indirect bilirubin explains the elevated indirect level in non-hemolytic jaundice, while leakage from the Kupffer cells or regurgitation explains the elevated prompt levels.

Preparation and properties of mammalian striated myofibrils ARMIN F. SCHICK, (by invitation) and GEORGE M. HASS, *Rush Dept. of Pathology, Presbyterian Hospital, and the Dept. of Pathology, Univ. of Illinois College of Medicine, Chicago, Ill.* A physico-chemical method for isolation and purification of myofibrils from mammalian skeletal and cardiac muscle has been devised. Blocks of living muscle were frozen and cut into sections with a microtome. Sections were transferred to a proteolytic enzyme buffer solution (0°C, pH, 7, ionic strength, 0.25). After 30 to 45 minutes, the myofibrils were separated by mechanical agitation. Then, the segregated myofibrils were separated from other components of muscle by centrifugation. The isolated myofibrils obtained in large quantities had characteristic properties. They varied in length and breadth but retained microscopic structural detail, plasticity and birefringence, characteristic of myofibrils in their intracellular location. They were soluble in a slightly alkaline reagent (0.5 N KCl - 0.03 N sodium bicarbonate) giving viscous solutions which displayed birefringence of flow. In buffer solutions with an ionic strength of 0.15, myofibrils dissolved acid to pH 4.0 and alkaline to pH 10.0. In phosphate buffer solutions with an ionic strength of 0.5, myofibrils dissolved when solutions were alkaline to pH 6.3. In phosphate buffer solutions with ionic strength increased from 0.15 to 0.5 by addition of potassium chloride, myofibrils were soluble when solutions were alkaline to pH 6.0. Isolated myofibrils, although probably modified by tryptic action and mechanical agitation during isolation at 0°C, ex-

hibited property of contractility when placed in solutions of adenosine triphosphate. Under these conditions, myofibrils isolated from skeletal muscle contracted rapidly so that long fibrillar structures were converted irreversibly into spherical masses.

Electrophoretic findings in normal and abnormal pregnancy NEVIN S. SCRIMSHAW (by invitation) and ERIC L. ALLING, *School of Medicine and Dentistry, Univ. of Rochester, Rochester, N. Y.* Electrophoretic patterns of nearly 200 normal and abnormal pregnant women reveal a tendency for albumin values to be lowered in preeclampsia. In 46 of 52 mild and severe pre-eclamptics, the albumin value was below 2.80. It was below this figure in only 10 of 77 apparently normal pregnancies. The pattern in pregnancy with essential hypertension does not differ from that of normal pregnancy. However, when pre-eclampsia is superimposed on previously existing hypertension, the pattern is that of pre-eclampsia. This is strong evidence that pre-eclampsia and essential hypertension are separate conditions which frequently co-exist during pregnancy. It is usually possible to determine by means of the electrophoretic pattern whether a blood pressure elevation late in pregnancy actually represents pre-eclampsia. In occasional cases, the lowering of albumin has been noted to precede the appearance of clinical signs and symptoms of preeclampsia. The changes in the globulin fractions are less consistent, but the shapes of the peaks as well as the absolute values may be of significance. If a small amount of normal hemoglobin is added to normal plasma before electrophoresis, it migrates with, or just behind, α_2 globulin. In most cases of severe pre-eclampsia, normal hemoglobin so added, migrates more slowly, with β globulin. After delivery the migration soon becomes normal. This phenomena has also been observed in all cord blood examined. It has never been observed in plasma from pregnant women without pre-eclampsia.

Chorionic gonadotrophin as luteotrophin in women ALBERT SEGALOFF, CORNELIA J. GASKILL (by invitation), WILLIAM STERNBERG (by invitation), and RICHARD L. COPPEDGE (by invitation), *Depts. of Medicine, Physiology and Pathology of Tulane Univ., the Alton Ochsner Medical Foundation, and the Charity Hospital of Louisiana, New Orleans, La.* Five patients were given chorionic gonadotrophin (A.P.L.—Ayerst, McKenna and Harrison) in a dosage of 10,000 i.u./day starting after the ovulatory rise in the cycle. Preliminary studies and studies during treatment were made on the urinary excretion of gonadotrophic hormones, 17-ketosteroids, glycogen depositing steroids (cortin) and prolactin. Endometrial biopsies were obtained in the previous cycle and during therapy. Three of the patients required gynecologic surgery and were given the chorionic gona-

drotrophin up to the day of surgery, and the surgical specimen studied. There were no significant changes in the urinary excretion of 17-ketosteroids or cortin. There was a small but consistent rise in the urinary excretion of prolactin, the significance of which at present is obscure, and there was a great increase in the excretion of gonadotrophic hormone due to the urinary spillage of the chorionic gonadotrophin. In all instances, the next menstrual period was delayed significantly, and in only one instance did spontaneous menstruation occur while the medication was being given. The endometrial biopsies and surgical specimens obtained during the administration of chorionic gonadotrophin all showed a definite decidual type of reaction. All of the endometria obtained in the cycle prior to the administration of chorionic gonadotrophin showed typical progestational phase.

Vitamin B₆ deficiency in the Syrian hamster
GREGORY SHWARTZMAN and LOTTE STRAUSS *Division of Bacteriology, Labys of The Mount Sinai Hospital, New York City*. Male weanling Syrian hamsters were maintained on a purified diet containing the essential vitamins and biotin but deficient in vitamin B₆. This led to arrest of growth after a depletion period of 2 to 3 weeks, diminished food and water intake, progressive malnutrition, muscular weakness and changes of the fur. Increased quantities of xanthurenic acid were found in the urine. The deficient animals did not survive beyond 12 to 13 weeks showing loss of fat tissue and marked atrophy of the lymphoid tissues, notably the thymus, even when malnutrition was mild. There was arrest of sexual maturation. Paired controls receiving 50 γ pyridoxine HCl daily showed arrest of growth and atrophy of lymphoid tissues, but failed to develop fur changes, muscular weakness, or cachexia. Controls fed ad libitum and receiving daily 50 γ of pyridoxine HCl showed good growth and nutrition and normal activity. Their fur was normal and there was no atrophy of the lymphoid tissues. Upon treatment of the deficient animals with daily injections of 50 γ of pyridoxine HCl after a depletion period of 9½ weeks, there was in most animals a resumption of appetite and growth, deposition of fat and general return to normal behavior and appearance. Fur changes were repaired within one week of treatment. A single injection of pyridoxine HCl to a deficient animal seemed to bring about similar results, the effect lasting for about two weeks. Although the presence of unsaturated fatty acids in the diet seemed to delay the onset of deficiency symptoms, the addition of corn oil to the diet of animals in an advanced state of depletion did not have any beneficial effect.

Retardation of the reaction time in parathyroidectomized rats CARL C SMITH (by invitation) and HERBERT C STOERK *Merck Inst for Therapeutic*

Research, Rahway, N J. 'Reaction time', the time necessary to elicit a response to a painful stimulus, appears to be a complex sum of events involving perception, conduction along sensory and motor neurons and synaptic transmission. Since a state of hyperexcitability of the motor nerve endings is known to exist in parathyroid insufficiency, it was thought that in parathyroidectomized rats reaction times may be measurably shortened. Contrary to expectation it was found that reaction times were significantly prolonged in parathyroidectomized rats. The method of estimating reaction times was that of D'Amour and Smith in which light from a small bulb is focused by a reflector on the blackened tip of a rat's tail. The reaction times of parathyroidectomized rats, ten days after operation, were 10-50% greater than those of controls. Simultaneous measurements as expected demonstrated increased excitability of motor nerves. The prolonged reaction times could be obtained as soon as two days after parathyroidectomy when Na₂HPO₄ (230 mg/kg) was administered orally. The analgesic effect of methadone and the retardation of reaction time due to parathyroid insufficiency appear to be additive.

Effect of malnutrition on the infection of mice with influenza virus DOUGLAS H SPRUNT *Division of Pathology and Bacteriology, Univ of Tennessee College of Medicine, Memphis, Tenn*. We have previously reported that adult mice, which were fed a diet low in protein but adequate in all other respects, were more resistant to infection with the swine influenza virus than were mice given an adequate diet. The mice were fed the low protein diet for 2 weeks before inoculation with the virus and until the end of the experiment 2 weeks after inoculation. Since this report we have found that this increased resistance to viral infections disappears if the mice are kept on the low protein diet for a longer period of time. It has also been found that the resistance of immature mice cannot be increased by a low protein diet. The immature mice are more susceptible to infection even though they are on the low protein diet. In all these experiments an equal number of mice have been kept without virus in order to see if the malnourished mice are dying of malnutrition. In every instance the diet has proven adequate to support life. At present we do not have sufficient data to explain the above phenomenon but believe it is due to the fact that animals on a low protein diet call upon their reserves of protein and hence are not on a low protein diet until these are depleted. The immature mice we believe are more susceptible because no appreciable reserve exists.

Determination of minimal daily essential amino acid requirements in protein-depleted male albino rats C H STEFFEE (by invitation), R W WISSLER (by invitation), E M HUMPH-

REYS (by invitation), E P BENDITT, R L WOOLRIDGE (by invitation), and P R CANNON *Dept of Pathology, Univ of Chicago, Chicago, Ill* The response of a group of 24 protein-depleted rats fed a relatively complete amino acid ration was used as the standard for determining the quantitative requirements for each of the nine essential amino acids for adequate regain of weight in ten days For this purpose 247 protein-depleted rats were used With different amino acid intakes, weight gains were directly parallel to changes in carcass protein By varying the intake of each amino acid successively, without altering other dietary components, the requirements for each were ascertained These requirements represent the minimal quantity of each essential amino acid which, in conjunction with the remainder of the standard ration, produced weight gains equal to those induced by feeding the complete standard ration Moreover, excellent weight gains followed the feeding of an identical ration, containing only these minimal quantities used have no absolute value, except under these experimental conditions, the proportions of amino acids required in relation to one another may have wide significance For example, the repleting rat utilizes from one to four times the quantities of essential amino acids needed for maintenance The requirements for leucine and lysine are greatest, and these amino acids are found in high concentrations in mammalian muscles It is suggested that the variant results obtained by other investigators with amino acid rations may be due to their use of inadequately proportioned mixtures of amino acids

Influence of nutrition on plasma vitamin A alcohol FREDERICK STEIGMANN, HANS POPPER, HATTIE A DYNIEWICZ (by invitation) and IRENE A MAXWELL (by invitation) *Heiktoen Inst for Medical Research of the Cook County Hospital, Dept. of Pathology, Northwestern Univ Medical School, and Dept of Internal Medicine, Univ of Illinois College of Medicine, Chicago, Ill* The plasma vitamin-A level depends upon endogenous factors like diseases (influencing release of vitamin A from the liver and its intestinal absorption) as well as upon nutritional factors This explains the equivocal correlation between plasma vitamin-A level and nutritional state Recent experiences indicated that the vitamin-A alcohol level responds more readily than that of vitamin-A ester to changing vitamin-A nutrition Therefore, vitamin-A alcohol and ester plasma levels were studied in 19 patients with either conditions without known influence upon vitamin-A metabolism (controls) or with liver diseases They were kept for 1 to 4 weeks on different levels of vitamin-A nutrition (from zero to 20,000 μ) These first periods were alternated with others with increased or reduced vitamin-A intake In controls kept for more than

one week on diets not exceeding 1500 μ vitamin A, the alcohol level dropped, whereas, the esters revealed irregular variations After prolonged periods on low intake, rise of alcohol and ester vitamin A might ensue, possibly as result of release from the liver After complete depletion of over one week, intake of 10,000 μ raised the alcohol level consistently without influence upon esters After a pre-period of 1500 μ intake, 3000 μ caused a rise in half the cases Secondary depletion and repletion periods of 1 week duration revealed less consistent results The curves in patients with liver diseases were erratic Serial follow up of the plasma vitamin-A alcohol level may, therefore, in normals with certain reservations mirror the status of vitamin A nutrition and possibly permit estimation of the human vitamin A requirement

Studies of leukocyte production and distribution factors in plasma and in leukocytes BERNHARD STEINBERG and RUTH A MARTIN (by invitation) *Toledo Hospital Institute of Medical Research, Toledo, Ohio* One of the significant questions in hematology concerns the mechanism which maintains the solid constituents of the blood at a constant definite level Our investigations (*Proc Soc Exp Biol & Med* 56 50-52, 1944, *J Immun* 51 421-426, 1945, 52 71-75, 1946, 53 137-141, 1946) of the immunochemical characteristics of various types of leukocytes of variable ages and physical states suggested the possibility of the presence of regulating substances within the cells Normal and leukemic plasma with and without contact with normal or leukemic cells as well as leukocytes only were injected into rabbits The animals were either normal or prepared by injections of benzene and extracts acting on the marrow Studies were made of marrow, other organs and of the peripheral cell content The tissues as well as the blood gave indications that intact leukocytes contain a substance which is secreted into plasma and which acts on the marrow to inhibit the expulsion of cells Degenerating leukocytes, on the other hand, secrete a substance which accelerates cell expulsion from the marrow Effect of various temperatures on the active substances were determined There is suggestive evidence that these substances are of the nature of enzymes Correlations were made with other materials such as milk, which apparently induce a change in the peripheral leukocyte picture by an increase in cell expulsion

Influence of chorionic gonadotrophin on the hilus cells of the human ovary WILLIAM H STERNBERG (by invitation), ALBERT SEGALOFF, and CORNELIA J GASKILL (by invitation) *Depts of Pathology and Medicine, Tulane Univ, the Alton Ochsner Medical Foundation and the Charity Hospital, New Orleans, La* The hilus and mesovarium of the human ovary normally contain nests of cells morphologically identical with Leydig cells They

contain lipids, acidophilic granules, lipochrome pigment and crystalloids of Reinke as do the interstitial cells of the testis. They are intimately associated with non-myelinated nerves and vascular spaces. During pregnancy and at the menopause they are especially conspicuous. We have recently studied 3 instances of tumor and 3 of hyperplasia of these cells all associated with masculinizing syndromes. Five regularly menstruating patients were treated with chorionic gonadotrophin and their ovaries examined histologically. The dosage was 10,000 u of chorionic gonadotrophin daily to each for periods of 12 to 19 days following ovulation. This dosage usually delays menstruation and produces a decidual reaction. Morphologic changes were noted in the ovarian hilus cells. The cells were present in larger than average numbers, and the cytoplasm more granular and acidophilic. In one case mitoses were present, although mitoses are not ordinarily seen either in hilus cells or in testicular Leydig cells. In 3 cases focal areas of degeneration were noted, including pyknosis, clumping of nuclei, and loss of cell outlines. Similar changes were noted in a case of chorionepithelioma of the uterus, and a teratoma of the ovary in which urinary gonadotrophin was elevated. The findings suggest that ovarian hilus cells are responsive to chorionic gonadotrophin. It is likely that they are one of the sources of androgen in the human female.

Mechanical and osmotic fragility in relation to age of dog erythrocytes tagged with radio-iron. W B STEWART, J M STEWART, M J Izzo and L E YOUNG (introduced by C L YULE) *Depts of Pathology and Medicine, Univ of Rochester School of Medicine and Dentistry, Rochester, N Y*. The mechanical and osmotic fragilities of dog erythrocytes of known age tagged with radio active iron were measured and compared with the fragilities of the total cell population. Under the stimulation of severe bleeding prior to intravenous administration of a small dose of radio-iron, the radio activity could be confined to a group of cells whose age was known within reasonable limits. As the cells matured and aged, measurements were made of their abilities to resist destruction by rotating glass beads and to resist hemolysis in hypotonic solutions of sodium chloride. Hemolysis of the total cell population was determined by conventional methods and hemolysis of the tagged cells by measurements of radioactivity. Comparison of the figures obtained revealed that cells up to 3-6 weeks of age were more resistant to mechanical trauma than the total cell population. The 2 fragility measurements then became almost identical until 12-15 weeks of age when the tagged cells were found to become increasingly fragile. The ratios then reversed after the tagged cells reached the end of their life span. A transient drop was noted in the total circulating radio-iron at this

point corresponding to a life span of about 17 weeks for the tagged cells. The osmotic fragility of the tagged cells was considerably greater than that of the total cell population for the first week, confirming results obtained earlier by Cruz, Hahn, Bale, and Balfour (*Am J Med Sci* 202, 157, 1941). Data for the older cells were inconclusive.

Parathormone and renal reabsorption of phosphate. HERBERT C STOERK and ROBERT H SILBER *Merck Inst for Therapeutic Research, Rahway, N J*. Although it has been established that parathormone (PTH) activity is not dependent upon renal function, evidence has been presented both for and against an effect of PTH upon the reabsorption of phosphate by the renal tubules. In the present experiments, the renal excretion of phosphate during a 5-hour period was determined in parathyroidectomized and in intact rats after administration of graded amounts of phosphate (0, 4 and 8 mg of P as Na_2HPO_4) and parathyroid extract (5-160 u , Lilly). In intact rats it was found that urinary phosphate excretion was not altered by the injection of 5 to 160 u of parathyroid extract. In parathyroidectomized rats the renal excretion of phosphate, but not of creatinine, was greatly suppressed. This defect was corrected by the administration of 20 u and no additional effect upon phosphate excretion was obtained with larger amounts of parathyroid extract (up to 160 u). In parathyroidectomized rats, injected with 20 or more u of extract, as well as in intact rats, an increased intake of phosphate led to an increase in urinary phosphate. It therefore appears that excessive amounts of parathyroid extract (more than 20 u per rat) exert no additional effect on the urinary excretion of phosphate. Thus the influence of PTH upon tubular reabsorption of phosphate is essentially an 'all or none' effect and can not be demonstrated in the presence of adequately functioning parathyroid tissue.

Eclampsia-like condition in pregnant rats injected with progesterone. A SYMEONIDIS (introduced by H L STEWART) *National Cancer Institute, Bethesda, Md*. Pregnant rats (24, strain M-520) injected with 20 mg of progesterone, intramuscularly (5 mg every day) in late pregnancy became ill. Some of them aborted dead embryos following which they recovered. In other rats the embryos were absorbed. Approximately 40% of the treated animals died. The liver and the kidney showed striking gross and microscopic lesions resembling those of eclampsia. Elevation of the non-protein nitrogen of the blood and albuminuria were found. The blood pressure was significantly elevated in 5 animals examined. Pregnant females receiving the same treatment prior to the 15th day of pregnancy and virgin tested rats failed to show any changes.

Relations between volumes of closed hypo-

thermal cerebral lesions and symptoms in rabbits
C BRUCE TAYLOR (by invitation), GEORGE M HASS and JOHN E MALONEY (by invitation) *Rush Dept of Pathology, Presbyterian Hospital, and Dept of Pathology, Univ of Illinois College of Medicine, Chicago, Ill* Acute closed cerebral lesions characterized by hemorrhage, necrosis and progressive edema were produced hypothermally in rabbits without interrupting continuity of the calvarium or introducing variables incidental to mechanical trauma The dimensions and locations of lesions were controlled so that they could be reproduced in successive animals Although hemorrhage, edema and necrosis varied slightly in lesions otherwise identical, the variations were restricted to discrete volumes of injury When unilateral or bilateral lesions occupied less than 9.4 volumes % of the brain, symptoms were negligible When lesions occupied 9.4 to 18.5 volumes % of the brain, severe symptoms developed in many animals The minimum lethal volume of cerebral damage in 50 % of more than one hundred animals was 14.3% of the volume of the brain Severe clinical courses with an average postoperative duration of 7 hours and fatal termination always occurred when lesions occupied more than 18.5 volumes % of the brain Most animals that died had a normal postoperative period of behavior Secondary lapse into stupor was a dependable indication of coma and eventual death within 24 hours after operation These data indicate that a quantitative experimental approach to treatment of acute expanding closed intracerebral lesions characterized by local necrosis, hemorrhage and edema can now be made The postoperative duration of life can be predicted from the magnitude of lesions produced and this duration is sufficient to permit evaluation of most therapeutic methods, now used empirically in treatment of similar lesions in humans

Proteinuria in dogs ROGER TERRY (introduced by G H WHIPPLE) *Dept of Pathology, School of Medicine and Dentistry, Univ of Rochester, Rochester, N Y* If homologous plasma is given parenterally to normal dogs for a sufficient period, proteinuria develops Additional experiments to those previously reported have been designed to help elucidate the mechanism of this proteinuria Circulating plasma protein levels must be elevated above a critical 'threshold', and there appears to be a lag period between the development of hyperproteinemia and the onset of conspicuous proteinuria This suggests that the 'threshold' for plasma protein molecules may be related to a large accumulation of these protein molecules within the renal tubule cells

Actions of 4-amino-pteroylglutamic acid in dogs with special reference to hematopoiesis J B THIERSCH and F S PHILIPS (introduced by C P RHOADS) *Division of Experimental Chemotherapy,*

Sloan-Kettering Inst for Cancer Research, New York City Accepting 4-amino-pteroylglutamic acid as a folic acid antagonist from studies in rats (Philips and Thiersch, *J Pharm Exper Therap*, *in press*) its actions in dogs were examined Acutely toxic doses proved fatal within 3 to 4 days while daily doses (0.05 to 0.10 mg/kg) were fatal to approximately 50% of animals within 10 days Signs of intoxication included occasional emesis, anorexia, losses in weight and electrolyte, hemorrhagic diarrhea, leucopenia, hemoconcentration, and terminal collapse In acutely intoxicated animals serial aspirations revealed rapid degeneration of bone marrow Within 24 hours abnormal erythroid elements could be found including nuclear remnants, pathologic mitoses, and megaloblasts After 96 hours erythroid elements were almost absent Simultaneously, myeloid elements and megakaryocytes became decreased in number Pathologic metamyelocytes and myelocytes appeared In chronic intoxication, similar but less rapid degeneration of bone marrow occurred although megaloblasts were not found in all animals In poisoned dogs lymphopoiesis was less affected, by comparison, than was either erythro- or myelopoiesis Edema of intestinal mucosa and desquamating colitis with hemorrhage were present in all dogs

Thermostable inhibition of bacterial hyaluronidases by serum of normal human beings ROBERT T THOMPSON and FRANCES E MOSES (introduced by M A BLANKENHORN) *Dept of Internal Medicine, College of Medicine, Univ of Cincinnati, Cincinnati, Ohio* This study investigated the inhibition of similar test strengths of hyaluronidases in culture filtrates of Type 3 Pneumococcus, hemolytic *Staphylococcus aureus*, *Clostridium perfringens*, beta hemolytic Streptococcus (group A), and purified bovine testicular hyaluronidase by the serum of 50 normal human beings who were between 20 and 40 years of age Hyaluronidase inhibition was measured by a mucoprotein clot prevention test using egg albumin as the protein component of the substrate Each serum was tested immediately as it was removed from the clot, and again after it had been heated at 56°C for 30 minutes Results tabulated below reveal thermostable inhibition of Pneumococcus and Staphylococcus hyaluronidases by normal serum

Hyaluronidase tested	No sera tested	Inhibiting sera	Thermostable inhibiting sera
Pneumococcus	50	47	45
Staphylococcus	49	23	19
Cl Perfringens	50	16	10
Streptococcus	50	12	7
Testicular	48	46	2

Tests upon consecutive daily sera of 1 person revealed consistent inhibition of the pneumococ-

cus, staphylococcus, and testicular hyaluronidases. However, there was fluctuation in the inhibition of the *Streptococcus* and *Cl. perfringens* hyaluronidases, so that results of the inhibition of these 2 enzymes tabulated above are of undetermined significance. The inhibition of testicular hyaluronidase which disappeared after heating could be restored by the addition of complement to the heated serum. The complement used was a 1:30 dilution of normal guinea pig serum, which itself did not inhibit the test strength of hyaluronidase.

Association of lymphoid elements with cancer cells undergoing distinctive necrobiosis in resistant and immune hosts. HELENE W. TOOLAN (by invitation) and JOHN G. KIDD, *Dept. of Pathology, The New York Hospital—Cornell Medical Center, New York City*. Regression of C3H cancers in 'A' mice proceeds inwards from the periphery, the tumor cells in groups in the centermost parts of the regressing nodules, and often those surrounding blood vessels, continuing to undergo mitosis and showing no signs of injury until their more peripheral fellows are overcome. Scrutiny of the cells about the edges of the regressing tumors made it plain that they do not die 'en masse', but instead succumb one by one, manifesting as they do so a distinctive type of necrobiosis. This is characterized by a gradual but progressive shrinkage of the cell with intensified basophilism of its nuclear and cytoplasmic constituents, and often with the accumulation of fluid around it, eventually with further shrinkage the nuclear membrane breaks down and the condensed and darkly-staining cellular remains dwindle and disappear without the intervention of phagocytes. The tumor cells never exhibit the necrobiotic changes just described until lymphoid elements provided by the host accumulate in force about them and attach themselves to first one and then another of the outlying cancer cells, adhering closely to them and often curving like crescents about their rims as they shrink, while adjacent tumor cells without attached lymphocytes remain unaltered. Further studies showed that cancer cells of both types proliferate for a short time when implanted in 'A' mice in which they had previously grown and regressed, but with the accelerated arrival of lymphoid elements they are promptly overcome as described above.

Passive transfer of age resistance to an avian malaria parasite. WILLIAM TRAGER and R. BARCLAY MCGHEE (by invitation), *Dept. of Animal and Plant Pathology, Rockefeller Institute for Medical Research, Princeton, N. J.* If young chicks are inoculated intravenously with the erythrocytic stages of *Plasmodium lophurae* they undergo an acute, severe infection. If adult chickens are similarly inoculated, few of them exhibit any appreciable parasitemia. It has now been found that the

plasma of adult chickens contains a material which when injected into young chicks infected with *P. lophurae* is capable of reducing the extent of multiplication of the parasites. One-week old chicks were given a dosage of *P. lophurae* sufficient to produce an initial parasite count of 1 to 2 parasites per 100 red cells. They were then given an intravenous injection once daily for 2 to 4 days of 3 ml. of either heparinized adult chicken plasma, buffered saline (pH 7.4) or 6% bovine albumen in buffered saline. The chicks receiving adult chicken plasma consistently developed less severe infections than those receiving saline or bovine albumen. For example, in one experiment the averages of the peak parasite numbers were, as parasites per 100 red cells, 102 for the group receiving albumen, 47 for the group receiving hen plasma and 102 for the group receiving saline. The activity of the hen plasma was not destroyed by heating it for one half hour at 56°C.

Radioresistance and radiosensitivity of swine exposed to atomic bomb radiation. JOHN L. TULLIS (introduced by F. S. ROBSCHT-ROBBINS), *Commander, MC, U. S. Navy*. Irradiation of the total body with an atomic bomb source affords a fairly accurate method of studying the differences in radiosensitivity of tissues. The variables introduced by the body thickness of swine and the differences in specific density of the tissues are minimized by the use of atomic bomb radiation, but they are by no means eliminated. The most radiosensitive tissues in swine are the lymphoid elements, the bone marrow, the gonads and the intestinal epithelium. It is significant and interesting that the reticular cells (the stem cells) of the lymph nodules and bone marrow, the indifferent cells (the stem cells) of the immature testis, the primordial ova, macrophages and plasma cells are relatively radioresistant. That is to say, doses of total body irradiation from an atomic bomb, or other source of penetrating ionizing radiations, sufficient to kill swine often cause no morphologic alteration in the cells listed. If treatment during the acute phase of the injury is successful in maintaining life, there is histologic evidence that regeneration of radiosensitive cells can be accomplished by certain radioresistant apparently-uninjured stem cells.

Intradermal vaccination with infectious psittacosis virus: pathological changes. JOSEPH VICTOR and JOHN C. WAGNER (by invitation), *Biological Dept., Chemical Corps, Camp Detrick, Frederick, Md.* Injection of psittacosis virus intramuscularly in monkeys (Rivers and Schwenker, *J. Exp. Med.* 60:211, 1934) and intradermally in guinea pigs has produced resistance to respiratory challenge. Pathological effects of intradermal injection of guinea pigs of strain 6BC grown in chick embryo yolk sac (6BCYS) were studied at intervals during

5 weeks after injection. Distribution of virus in tissues of these animals is reported elsewhere. The infectious material produced acute inflammation at the injected site. The skin lesion later became granulomatous with fibroblast and giant cell formation after 3 weeks. Ceroid pigment occurred within histocytes and giant cells 3 weeks after injection of 6BCYS. Ceroid was identified from its fluorescence in U-V light, acid fastness with carbol-fuchsin and its affinity for basic and lipophil dyes. Regional lymph nodes became hyperplastic and hypertrophied reaching maximum size at about 2 weeks. Ceroid appeared in these nodes at 5 weeks and seemed to have migrated from the skin lesion. Liver contained midzonal areas of focal necrosis for 2 weeks. After 3 weeks, collections of histocytes replaced the midzonal necrosis. Control animals injected intradermally with normal chick embryo yolk sac or formalinized yolk sac infected with 6BC revealed no changes comparable to those treated with infectious virus. Lesions in skin, regional lymph node and liver persisted longer than detectable virus. No virus was found in livers with focal necrosis.

Comparison of two types of experimental ascites in the dog. WADE VOLWILER (by invitation), JESSE L. BOLLMAN and JOHN H. GRINDLAY *Mayo Foundation, Rochester, Minn.* Ascites in the dog has been regularly produced by 1) chronic passive congestion of the liver from the application of a cellophane band to the thoracic inferior vena cava and 2) plasmapheresis following the application of a cellophane band to the abdominal inferior vena cava and portal vein. Although portal and inferior caval venous pressures are elevated to the same moderate degree in both types of preparations, the character of the ascites is markedly different. The ascites accompanying Preparation 1 is rapidly progressive, high in protein content, and regularly associated with marked engorgement of the extrahepatic liver lymphatics, whereas the abdominal fluid appearing in Preparation 2 is less extensive and difficult to maintain, low in protein content and without associated lymphatic enlargement. Evans blue dye injected intravenously into dogs of Preparation 1 is found in the ascites in one hour, the dye does not appear in the abdominal fluid of Preparation 2 in detectable amounts in 48 hours. Assays for antidiuretic substances in the urine concentrates of dogs of Preparation 1 have been made using male rats having suprapubic cystotomies for individual urine collections. Since previous studies have shown that the protein content of hepatic lymph is approximately $\frac{1}{3}$ that of plasma and that the rate of flow of liver lymph is markedly increased from hepatic congestion, it is suggested that increased transudation of fluid through the dilated extra-hepatic liver lymphatics

contributes importantly to the formation of the ascites associated with hepatovenous congestion.

Intradermal vaccination with infectious psittacosis virus. **virus distribution** JOHN C. WAGNER (by invitation) and JOSEPH VICTOR *Biological Dept., Chemical Corps, Camp Detrick, Frederick, Md.* Production of immunity to psittacosis virus introduced into respiratory tract has been reported in monkeys by Rivers and Schwentker, following intramuscular inoculation of infectious virus. Similar observations were made following intradermal administration of psittacosis virus, in guinea pigs. This study attempts to elucidate factors concerning this resistance. Tissues of guinea pigs injected with psittacosis virus were studied for distribution of virus and pathological changes. Psittacosis virus, 6BC strain, cultivated in chick embryo yolk sac, was inoculated intradermally in a concentration of 300,000 egg LD₅₀ in 0.5 ml broth suspension. Autopsies were performed 1, 2, 3, 6, 9, 14, 21 and 35 days thereafter. Tissues were tested as 10% suspensions in nutrient broth by intracerebral inoculation of mice. Presence of virus in guinea pigs was established by demonstration of elementary bodies in smear preparations of brains from injected mice that died. Evidence obtained indicated that treated guinea pigs harbored virus for 6 days at site of inoculation, for 14 days in regional lymph nodes and sporadically for 9 days in the liver. Spleen suspensions yielded virus on the first day in only 1 of 3 animals. No virus was detected in suspensions of contralateral lymph nodes, whole blood, heart, lung or kidney. Virus distribution data indicated that psittacosis virus inoculated intradermally migrated from site of injection to regional lymphatics and thence to internal organs. Pathologic studies confirmed this. Resistance to respiratory infection occurred in the absence of any demonstrable virus or lung lesions.

Effect of renal ischemia on the production of nephrosis in jaundiced rabbits. W. B. WARTMAN, J. M. TUCKER (by invitation), A. P. RUSTERHOLZ (by invitation) and R. JENNINGS (by invitation) *Dept. of Pathology, Northwestern Univ. Medical School, Chicago, Ill.* The purpose of these experiments was to test the assumption that the presence of previous kidney damage might be a factor in the development of bile nephrosis. Using rabbits, one kidney was damaged by temporarily occluding the right renal artery and vein for 15 to 20 minutes, and the common bile duct was divided. Lesions developed in the kidneys of approximately 45% of animals after 3 days. They were chiefly in the cortex of the kidneys and consisted of dilatation of both proximal and distal segments of the tubules, flattening, degeneration and necrosis of the lining epithelium and numerous reddish brown, granular, heme casts. There were no clinical evidences of kidney disease. No changes were dis-

covered in rabbits in which only the common bile duct was cut, nor in animals in which only the right renal artery and vein were obstructed, nor did rabbits in which the common bile duct and right renal pedicle were dissected out but not otherwise manipulated show changes in the kidney. Prolonged and severe restriction of water intake had no demonstrable influence on the development of the nephrosis. It is concluded that under the conditions of these experiments, a peculiar type of bilateral nephrosis may be produced in rabbits by a combination of jaundice and temporary, unilateral, renal ischemia, and that it is produced neither by jaundice alone nor renal ischemia alone. Restriction of fluid had no effect upon the development of the lesion.

Bactericidal action of dog blood against *Br suis*
E. BUIST WELLS (by invitation), JEAN CLEMENTS (by invitation), ABRAHAM D. POLLACK (by invitation) and JOSEPH VICTOR, *Biological Dept., Chemical Corps, Camp Detrick, Frederick, Md.* Irwin et al (*J. Inf. Dis.* 58: 15-22, 1936) have reported that bovine plasma had greater bactericidal activity than whole blood against *Br suis* and *Br abortus*. The present study indicated that in the dog whole blood possessed great bactericidal activity against *Br suis* whereas plasma had none. A standardized number of organisms of a virulent strain was mixed with either normal dog blood, red blood cells, buffy coat or plasma. Mixtures were agitated in rotating tubes. Bacterial numbers were counted by dilution and plating. The influence on bactericidal action of blood of citrate, heparin, temperature, time of mixing, volume of blood and interval between addition of fresh blood was studied. Citrate suppressed bactericidal activity of blood whereas heparin did not. Bactericidal activity of blood was the same both at room temperatures and at 37°C after 2 hours. In contrast, after 4 hours bacterial destruction was greater at 37°C than at room temperatures. Repeated additions up to 4 hours of fresh blood to the mixtures did not influence bactericidal action. Activity of buffy coat was similar to that of whole blood. Plasma was inactive. Blood from which almost all leukocytes had been removed by centrifugation possessed much less activity than whole blood. It appeared that bactericidal power of dog blood for *Br suis* depended on the presence of leukocytes.

Clearance of rose bengal by livers of normal and carbon tetrachloride-treated mice
W. LANE WILLIAMS, *Dept. of Anatomy, Univ. of Minnesota, Minneapolis, Minn.* Livers of untreated mice were cleared of rose bengal (intraperitoneal, 0.4 cc, as a 1% saline solution 25 gm body weight) within 4 to 6 hours post injection. Twenty-four hours after treatment with 0.4 cc of CCl₄ (subcutaneously in mineral oil) total clearance time was 9 to 11 hours, at 48 hours, 11 to 14 hours. These livers showed

typical centrilobular damage. Nine days subsequent to a single injection of 0.01 cc of CCl₄, livers showed no histological evidence of parenchymal damage but dye clearance time was 8 to 9 hours. One day after injection of the larger amount of hydrocarbon all parenchymal cells (including peripheral ones) were more deeply stained (cytoplasm) than equivalent cells in livers of untreated animals. Greatest amount of dye was in centrilobular cells of injured livers. Although these central zones eventually showed necrosis the deeply stained centrilobular cells were still able to excrete dye 48 to 60 hours after injection of CCl₄. Intracytoplasmic rose bengal is difficult to determine in cytological studies because of its light pink color. However, the presence of a greater intracytoplasmic concentration of dye transiently 'labels' and differentiates damaged parenchymal cells. All such cells were free of dye within 8 to 14 hours after injection. In ultraviolet light 10μ paraffin sections of normal livers showed a faint yellow fluorescence during the period of greatest dye content. In CCl₄-damaged livers a definite orange fluorescence was observed during the interval of maximum dye content.

Vitamin deficiency and protein synthesis with special reference to riboflavin-protein interrelationships
ROBERT W. WISSLER (by invitation), EARL P. BENDITT, C. HAROLD STEFFEE (by invitation) and ROBERT L. WOOLRIDGE (by invitation), *Dept. of Pathology, Univ. of Chicago, Chicago, Ill.* The omission of all vitamins from the diet of normal young adult albino rats produced little change in weight gain or diet consumption for about 30 days. Under similar conditions protein-depleted rats showed poor diet consumption and weight loss in 2 or 3 days after being shifted to a low vitamin ration adequate in protein. When each vitamin was removed singly from protein-repletion rations, groups of protein-deficient rats displayed marked reduction in diet consumption and weight gain only when the diets were devoid of riboflavin or thiamin. Riboflavin deficiency usually began to become apparent in 3 or 4 days, thiamin in 7 to 10 days. Antibody production was markedly reduced in the riboflavin-deficient rats. Pair-feeding experiments revealed that the poor weight gains shown by the protein-depleted rats repleting on riboflavin-deficient diets were not the result of inanition alone. Analyses of the various tissue compartments revealed that the smaller weight gains of these riboflavin-deficient rats were the result of interference with protein synthesis as well as fat storage. Liver and carcass analyses indicated that riboflavin storage is decreased in the protein-depleted rats despite adequate riboflavin intake. Succinic dehydrogenase and other enzymic activity was reduced in the livers of protein-depleted animals and remained low in those repleting without

dietary riboflavin Protein-depletion interferes with enzyme activity and riboflavin storage possibly by limiting available protein prosthetic groups

Response of the rachitic metaphysis in the rat to excessive administration of vitamin A S B WOLBACH and CHARLOTTE L MADDOCK (by invitation) *Division of Nutritional Research, Children's Hospital, Boston, Mass* It has been shown (*Proc Inst of Medicine of Chicago* 16 1946, and *J Bone and Joint Surg* 29 171, 1947) that in the growing young animal the administration of excessive amounts of vitamin A accelerates growth and maturation of epiphyseal cartilage cells and the remodelling sequences of the bones of the skeleton This has been demonstrated for the rat, mouse, guinea pig and dog It causes rapid consumption of the epiphyseal cartilage and, in appropriate species, premature closure of epiphyses, also excessive rapidity of remodelling processes in regions which undergo remodelling in normal growth The present communication is for the purpose of describing effects upon the accumulated cartilage in rachitic metaphysis which results from failure of cartilage cells to mature The rachitogenic diet employed was composed with the advice of Dr Otto A Bessey and contains all essential materials with the exception of adequate phosphorus content Under the influence of excessive vitamin A administration there is rapid repair of rachitic lesions, including resumption of normal epiphyseal cartilage cell cytomorphosis and calcification of cartilage matrix and osteoid Partial to complete closure of the distal, femoral and proximal tibial epiphyses takes place in spite of the fact that in these locations epiphyses remain open for most of the lifetime of the rat Acceleration of remodelling

sequences occurs as in rats upon normal diets and in conformity with normal growth patterns Our results support the idea that epiphyseal cartilage cell sequences are influenced by a factor other than those concerned in calcification of matrices

Effects of protein deficiency upon natural and acquired immunity to Friedlander's bacillus ROBERT L WOOLRIDGE (introduced by PAUL R CANNON) *Dept of Pathology, Univ of Chicago, Chicago, Ill* The effects of protein depletion on natural and acquired immunity against infection with Friedlander's bacillus were studied in adult male albino rats following intranasal or intratracheal introduction of a standardized inoculum Forty-one markedly protein-depleted rats showed a significantly lower agglutinin response on the 6th and 14th days after antigenic stimulation, with a concomitantly lower survival rate, than did 40 well-nourished controls When the animals were sacrificed the controls had only a few focal pulmonary abscesses whereas the protein-depleted rats had many large chronic pulmonary abscesses which showed evidence of progressive enlargement with approximately 50% of each lung consolidated Marked protein depletion also lowered natural resistance in that an intratracheal inoculum which was sublethal for the control animals killed all of the protein-depleted rats within 54 hours, with an associated septicemia Histological studies revealed marked differences in the cellular response and degree of phagocytosis For example, in the protein-depleted rats the ability to circumscribe the infection and repair the damaged tissue was definitely impaired whereas the phagocytic ability of the control animals during the early stage of the infection was adequate to protect them until acquired immunity was developed and the infection localized

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(For possible corrections in any of the following abstracts see the June issue)

Leucine content and utilization of amino acid mixtures JOSEPH T ANDERSON (by invitation) and E S NASSET *Dept of Physiology and Vital Economics, Univ of Rochester School of Medicine and Dentistry, Rochester, N Y* The utilization of the N of amino acid mixtures in the adult rat was investigated by the method previously described (Anderson and Nasset, *J Nutrition* 36 703, 1948) in which a 'complete' amino acid mixture simulating egg protein is compared with a mixture in which a portion of one of the essential amino acids has been replaced by an isonitrogenous amount of of glutamic acid Reduction of L-leucine to 21.1 mg leucine N/gm total N, which is $\frac{1}{3}$ the value in the 'complete' amino acid mixture, caused a significant decrease in the utilization of the dietary N as indicated by a decrease in N balance index and an increase in N requirement for equilibrium Further reduction of L-leucine by one-half caused a further decrease in N utilization but not so great as would be predicted from the theory that N retention is limited by the supply of limiting essential amino acid Substitution of an equal quantity of DL-leucine for the L-leucine (21.1 mg leucine N/gm total N) resulted in no significant reduction in N utilization Apparently about $\frac{1}{3}$ the maintenance requirement of L-leucine in the rat can be filled by D-leucine

Ammonium persulfate as a flour maturing agent AARON ARNOLD and FRANS C GOBLE (by invitation) *Sterling-Winthrop Research Inst, Rensselaer, N Y* The need for chemical agents for rapidly maturing flour is well established and accepted Nitrogen trichloride, which has been used extensively for this purpose, is no longer acceptable in view of Mellanby's findings of deleterious effects in laboratory animals fed flour thus treated This fact has focussed attention on the suitability of alternative agents Ammonium persulfate has been known for some time to be an effective flour maturing agent, and, in fact, is used extensively abroad, this report presents data obtained in feeding trials of this compound with rats and dogs In chronic experiments, rats were fed diets high in flour or air-dried bread made from persulfate treated flour The levels of treatment were 0.02 and 0.1%, i.e., levels considerably in excess of commercial requirements The rats fed the treated flours or breads

showed no adverse effects The effects were judged on the basis of growth and reproduction performance and histological examination of representative tissues of first and second generation rats sacrificed after 6 months on test Dogs fed ammonium persulfate treated flour not only did not develop the running fits syndrome but were cured of running fits when transferred from a diet containing nitrogen trichloride treated flour to one containing persulfate treated flour As in the case of the rat feeding trials, no adverse effects could be detected in dogs fed flour treated with 0.15% persulfate as compared with dogs fed untreated flour The effects were judged from weight records, kidney and liver function tests, and histological examination of representative tissues after 5 months' feeding Ammonium persulfate appears to be suitable from all view points for use as a flour maturing agent

Effect of diet on treatment of parathyroidectomized dogs AARON ARNOLD *Sterling-Winthrop Research Inst, Rensselaer, N Y* It has been well demonstrated that vitamin D and dihydrotachysterol will substitute for parathyroid hormone in parathyroidectomized dogs However there is a disagreement as to the amounts of the calcemic steroids required For 16 months, 2 adult thyro-parathyroidectomized dogs were fed a mixture of 2 parts fresh ground meat and 1 part cereal diet (in percentage: corn 26, wheat 26, skim milk powder 24, linseed meal 8, casein 4, peanut oil 4, yeast 3, alfalfa meal 3, bone meal 1, CaCO_3 0.5, and NaCl 0.5) The composite diet supplied 0.58% Ca and 0.73% P The dogs were given, in addition, 0.3 gm thyroid powder daily and the usual vitamin supplements orally once weekly In agreement with the results of McChesney and Giacomino (*J Clin Invest* 24 680, 1945), the dogs were observed to require approximately 0.0875 mg of dihydrotachysterol or 0.2 mg of calciferol daily per 10 kg dog This amount of calciferol was about 200-fold greater than that found necessary by Kozelka *et al* (*J Biol Chem* 100 715, 1933) The requirement does not appear to be correlated with the dietary calcium and phosphorus but may be related to the general nature of the diet Specifically, dogs fed a diet with a substantial proportion of fresh meat require much greater quantities of vitamin D or dihydrotachysterol than do dogs fed diets com.

posed largely of cereal grains. This suggestion may apply also to the marked difference in the amount of vitamin D required to prevent the onset of rickets in dogs as reported by the Fleischmann Laboratories (unpublished data, 1936) and by Arnold and Elvehjem (*J Am Vet Med Assoc* 95:187, 1939).

Influence of heredity on the crude protein and carotene contents of corn L W AURAND (by invitation) and R C MILLER *Dept of Agricultural and Biological Chemistry, Pennsylvania State College, Penna*. Crude protein and carotene values have been determined on corn produced in 3 different series of experiments, each of which had all possible single cross combinations of 10 different inbred lines of corn. Each experiment was completely replicated on at least 3 experimental plots which had the same fertilizer treatment. Separate samples of corn from each single cross combination on each plot were analyzed for protein and carotene, and average values were computed from all of the individual data for each single cross on each experiment. Significant differences in the protein and carotene values for single crosses definitely reflected the influence of the breeding of the inbred lines on the resulting single crosses. The latter had crude protein contents ranging from 7.95 to 12.58% and carotene contents ranging from 0.53 to 1.95 mg/lb, all values being expressed on the basis of 15.5% moisture content of the corn. The definite influence of inbred lines was shown in characteristically high or low crude protein and carotene contents of the resulting single cross combinations. Although these relationships were true for both crude protein and for carotene there was no correlation between the crude protein and carotene contents of corn tested. The results show a definite influence of heredity on the carotene and crude protein content of corn and point the way for improvement of the nutritive value of corn by the plant breeder.

Nutritional requirements of men living under survival conditions in cold weather C G BLY (by invitation), R E JOHNSON and C F CONSO LAZIO (by invitation) *U S Army Medical Nutrition Lab, Chicago, Ill*. Balance studies were conducted when 32 volunteers, fully acclimatized to Florida winter conditions and unaccustomed to cold weather and its hardships were flown in January from Florida (mean temperature +57°F) without layover to Manitoba, Canada. On arrival they were placed in a field survival situation for 12 days (mean temperature -3°F), where they lived in one of four segregated areas, 8 men per group. The 4 group rations (per man per day) contained 1100 Cal (low calorie control), 1650 Cal, 1940 Cal, 4850 Cal (high calorie control). All urine and feces were collected daily. From analytical data on these, together with analytical data on all

foods and fluids consumed, nutrient balances were computed. For these young men, well outfitted against the cold, with adequate clothing, fuel, and tentage, doing no physical work other than necessary to keep themselves comfortable, nutrient balance could be maintained for 12 days by palatable rations supplying daily for an average 70 kg man 3400 Calories, 2.7 liters water, including beverage, food, and metabolic water, 50 gm protein, mostly animal, 135 gm fat, 400 gm carbohydrate, 1.1 gm calcium, 1 gm phosphorus, 100 mEq sodium, 85 mEq chloride, 20 mEq potassium. These estimates are not applicable for men required to perform any hard physical work.

Animal protein factor and growth of mice fed diets containing succinylsulfathiazole D K BOSSHARDT, J W HUFF (by invitation), WINIFRED J PAUL (by invitation) and R H BARNES *Dept of Biochemistry, Medical Research Division, Sharp and Dohme, Inc, Glenolden, Penna*. The addition of 2% succinylsulfathiazole to purified diets fed to mice caused a growth retardation that was not corrected by the feeding of biotin and pteroylglutamic acid nor by supplements of dehydrated, defatted whole liver. When, however, the biotin, pteroylglutamic acid, and liver were fed together growth rates were obtained comparable with those of mice fed a succinylsulfathiazole-free diet containing liver. Liver has been shown to contain a factor, or factors, referred to as the animal protein factor that is necessary for growth. The results of Ott *et al* (*J Biol Chem* 174:1047, 1948) suggest that vitamin B₁₂ would replace liver in the diet of succinylsulfathiazole-fed mice. The vitamin B₁₂ concentrate was prepared from liver and was found by microbiological assay, using *Lactobacillus leichmannii*, to contain approximately 1% vitamin B₁₂. A vitamin B₁₂ concentrate supplied by the Merck Laboratories was used as a standard. This preparation when administered to iodinated casein-fed mice (Bosshardt *et al* *J Nut*, in press) gave a maximum growth response with a daily intraperitoneal dosage of 0.5 µg. The vitamin B₁₂ concentrate administered by intraperitoneal injection at a level of 1 µg/day to mice fed a diet containing biotin, pteroylglutamic acid, and succinylsulfathiazole produced a marked growth response. However, the growth response did not appear to be as great as that obtained with crude liver.

Riboflavin and nitrogen metabolism of 6 women with active tuberculosis WILMA D BREWER (by invitation), HELEN L TOBEY (by invitation) and MARGARET A OHLSON *Dept of Foods and Nutrition, School of Home Economics, Michigan State College, East Lansing, Mich*. The riboflavin and nitrogen metabolism of 6 women with moderately advanced, active tuberculosis was studied. The subjects were non-ambulatory patients of the county sanatorium and the regular hospital diet

was served throughout the experiment. The riboflavin and nitrogen contents of the diets were varied by adjustment of the milk intake. During periods 1 and 2, the milk intake was restricted to 300 gm daily and for periods 3 and 4, the intake was increased to 1200 gm of milk per day. Daily supplements of 5 mg riboflavin were given during periods 2 and 4. Fourteen days of dietary adjustment preceded each 7-day balance period during which the intakes and excretions of nitrogen and riboflavin were determined. The urinary excretion of riboflavin following a test dose of 3 mg riboflavin was measured on the last day of each balance period. The average daily intakes of riboflavin for the four periods were 1.35, 6.41, 2.60 and 7.79 mg. The average daily excretions were 0.31, 3.53, 1.08 and 5.63 mg riboflavin, respectively. The retentions of nitrogen by the tuberculous women were similar to those of healthy women at comparable intakes. All of the subjects were in nitrogen equilibrium or retaining nitrogen at intakes above 10.00 gm daily. Analysis of covariance has been applied to the data and the possible interrelationships of riboflavin and nitrogen metabolism will be discussed.

Lysine requirement for growing pigs. M. J. BRINEGAR (by invitation), J. K. LOOSLI, HAROLD H. WILLIAMS, and L. A. MAYNARD, *Depts. of Animal Husbandry and Biochemistry and Nutrition, Cornell University, Ithaca, N. Y.* Various levels of L-lysine monohydrochloride were added to a diet containing linseed meal supplemented with histidine and methionine for pigs averaging 19 kg in weight. The basal diet consisting of 10.6% protein contained 0.34% lysine as determined microbiologically using *Leuconostoc mesenteroides*. The experimental diets were made up to contain 0.34%, 0.42%, 0.50%, 0.58%, and 0.74% pure L-lysine. The first 4 levels of lysine were fed to 3 pigs each and the 0.74% level was fed to 2 pigs. Protein efficiency ratios over a 4-week feeding period and biological values during a 5-day nitrogen balance were studied. The averages of the gm gain in weight per gm protein consumed were 2.6, 2.85, 3.12, 3.47, and 3.49 for each of the respective lysine levels of 0.34%, 0.42%, 0.50%, 0.58%, and 0.74%. The average biological values for the corresponding lysine levels were 52, 51, 61, 73, and 72. Each level of dietary lysine up to 0.58% increased the protein efficiency and the biological value, but the 0.74% level caused no further improvement. These data show that with a diet containing 10.6% protein, having a biological value of 73, growing pigs require approximately 0.6% of L-lysine in the ration.

Acetylation in diabetic rats. FRIXOS CHARALAMBOUS (by invitation) and D. MARK HEGSTED, *Dept. of Nutrition, Harvard School of Public Health, and Dept. of Biological Chemistry, Harvard Medical School, Boston, Mass.* The ability of alloxan-diabetic rats to acetylate p-aminobenzoic

acid (PAB) was compared to that of normal rats by injecting 1 mg of PAB intraperitoneally, collecting the following 24-hour urine, and analyzing the urine for total and free PAB. All animals received a purified diet containing casein, salts, glucose, corn oil, cod liver oil and riboflavin, thiamine, nicotinic acid, pantothenic acid, and pyridoxine. Acetylation gradually decreased after the onset of diabetes and eventually fell to 62% of the dose as compared to 76% in the non-diabetic controls. Induced diuresis in the normals did not decrease acetylation. Acetylation in the diabetic animals returned to normal after the injection of insulin, citrate, α -ketoglutarate, succinate, fumarate, malate, oxaloacetate, diacetyl, acetylphosphate, and ATP. Increasing the level of fat in the diet from 5 to 30% had a similar effect, as did starvation for 24 hours. Injections of pyruvate, lactate, glyceryl diacetate, acetoin, butylene glycol, methyl ester of acetoacetic acid, phosphate salts, adenylic acid, and pantothenic acid failed to restore acetylation to normal in the diabetic rats. None of the compounds tested affected the acetylation in normal animals. These findings are in agreement with the *in vitro* work of Lipmann *et al.* (*J. Biol. Chem.*, 160, 173, 1945) that acetylation requires high energy phosphate compounds. It appears that in the diabetic animal the regeneration of such high energy compounds is below normal.

Excretion of ascorbic acid on supplements of cantaloupe and crystalline ascorbic acid. SHIH DZUNG CHEN (by invitation) and CECILIA SCHUCK, *Nutrition Lab., School of Home Economics, Purdue Univ., Lafayette, Ind.* Four senior college women majoring in nutrition served as subjects. The study was divided into 3 periods: a saturation period of 5 days during which time the subjects, while consuming their regular diets, were given 200 mg of ascorbic acid daily in tablet form; a test period of 6 days on a constant basal diet supplemented daily with 40 mg of pure crystalline ascorbic acid; a second test period with approximately the same ascorbic acid intake, but with cantaloupe as the supplement to the basal diet. The 2,4-dinitrophenylhydrazine method for reduced and dehydroascorbic acid (*J. Biol. Chem.* 174:201, 1948) was used for the analysis of the diet and the cantaloupe supplement and for determining the urinary excretion. Since the excretions for the first 2 days of the 6-day test periods reflected the large intake of ascorbic acid during the saturation period these were not considered in the evaluation of the data. With an intake of 74 mg of ascorbic acid (reduced and dehydro) the average daily amounts excreted during the crystalline ascorbic acid period by the 4 subjects were 49.24, 47.65, 47.43, and 38.30 mg respectively. Corresponding amounts excreted during the cantaloupe period with a daily intake of 72 mg ascorbic acid

were 57.54, 58.63, 41.88, and 34.81 mg. The figures show an increased excretion during the cantaloupe period for 2 of the subjects and a slightly decreased excretion for the other two.

Determination of SLR factor in the liver and urine of rats fed on various diets. BACON F CHOW, LOIS HALL (by invitation), SHIRLEY DE BIASE (by invitation) and LOIS BARROWS (by invitation) *Division of Pharmacology, The Squibb Inst for Medical Research, New Brunswick, N. J.* Protein depletion causes a marked decrease in protein and enzyme contents of the liver. This report deals with the effect of inanition on the concentration of the SLR factor (Elvehjem, J. Biol. Chem. 167: 86, 1947) in the liver of rats. It was found that if the liver were homogenized with an ice cold saline solution immediately after its removal from the animal, the activity of such a suspension increased some 10-fold upon incubation at 37°C. A maximum was, however, reached after 8-24 hours at this temperature. The results of assay of this factor in the homogenized livers of normal and seven-days-fasted rats, after appropriate length of incubation, demonstrated that inanition brought about a marked loss in the total amount of the potential activity, when expressed in units of activity per hundred grams body weight of the animals before fasting. The excretion of the SLR factor in urine was also studied. The renal excretion of this factor decreases on feeding a diet low in protein but adequate in calories and vitamins, but increases, if liver is given as a supplement either orally or intraperitoneally.

Weight of liver and liver glycogen of rats after dietary restriction and rehabilitation. RUTH M. CLAYTON and ESTHER DA COSTA (introduced by ROBERT E. JOHNSON) *U. S. Army Medical Nutrition Lab., Chicago, Ill.* Liver weights of 72 male rats fed during 13 weeks either a low calorie, modified or synthetic vegetable diet and of 120 rats changed from these diets to either high protein, high fat or high carbohydrate were determined. The glycogen of the liver of half of these rats was determined. The average weight of 42 control livers was 12.16 gm. with a total glycogen of 0.24 gm. The average weights of livers following the low calorie, modified carrot or synthetic vegetable diet were 7.13, 7.5 and 5.47 gm., respectively. The average total liver glycogens following the low calorie, modified carrot or synthetic vegetable diet were 0.12, 0.24 and 0.06 gm., respectively. After 72 hours of rehabilitation with a high protein, high fat or high carbohydrate diet following the low calorie diet were 13.2, 10.19 and 10.98 gm., respectively. Average liver weights from rats refed following the modified carrot or synthetic vegetable diet followed a similar pattern of increased weight. After 72 hours of rehabilitation liver glycogen increased in all diets but decreased after 96 hours.

After 13 weeks of rehabilitation on a high protein diet, liver glycogens were at the control level, on a high fat diet below and on a high carbohydrate diet above the control level.

Function of the ceca in the domestic fowl. J. R. COUCH (by invitation), D. R. KNIGHT (by invitation), HARRY GERMAN (by invitation) and P. B. PEARSON *Agricultural and Mechanical College of Texas, College Station, Texas.* Broad-breasted Bronze mature turkey toms and White Leghorn pullets were each selected at random from flocks on range and killed. The gastrointestinal tracts were removed and separated into two parts for sampling. The first section was made up of the ceca while the second consisted of the tract below the duodenum less the ceca. The contents were removed from each of these sections and analyzed for riboflavin, niacin, pantothenic acid, folic acid and biotin. The cecal contents contained approximately 1.5 times as much niacin, twice as much riboflavin, 2.5 times as much pantothenic acid, 6 to 8 times as much folic acid, and 10 times as much biotin as the intestinal contents. Identification studies of the microflora of the two sections of the GI tract showed that essentially the same flora existed in the ceca as in the rest of the tract. The ceca was removed from pullets which were placed in individual cages with raised screen bottoms. After 2 months a 24-hour collection of feces was made from the cecectomized birds and from normal pullets. There was no difference in the riboflavin, niacin, pantothenic acid, folic acid and biotin content of feces of cecectomized and normal birds fed a practical all-mash diet. These birds were then placed on a low biotin diet with starch as the carbohydrate. Biotin content and hatchability of the eggs will be reported as measures of intestinal synthesis in birds with and without the ceca.

Desoxypyridoxine and development of the chick embryo. W. W. CRAVENS and E. E. SNELL *Depts. of Poultry Husbandry and Biochemistry, Univ. of Wisconsin, Madison, Wis.* Injecting 1000 μ g of desoxypyridoxine into the hen's egg at 0 hours of incubation results in nearly 100% mortality of the embryos by the end of the fourth day of incubation and all embryos died prior to hatching. 250 and 500 μ g injected at 0 hours caused a reduction in hatchability, but some live chicks were obtained. These inhibitory effects were prevented by simultaneous injection of sufficient amounts of pyridoxal HCl, pyridoxamine 2HCl or pyridoxine HCl. The ratio of vitamin to inhibitor which permitted approximately 50% of the embryos to produce live chicks were pyridoxal HCl 1/20, pyridoxamine 2HCl 1/50, and pyridoxine HCl 1/100. In contrast to the results at 0 days, injection of 100 μ g of desoxypyridoxine at 6 days of incubation was innocuous. At this time the toxic level was found to be 2.5-5.0 mg/egg. However,

this toxicity was not due to a competitive anti-vitamin action of the inhibitor since it could not be prevented by any of the three forms of vitamin B₆. At similarly high levels (more than 2.5 mg/egg) pyridoxal HCl proved toxic. Pyridoxine HCl was somewhat less toxic while pyridoxamine 2HCl was non-toxic at the highest level tested (10 mg/egg).

Effect of dietary restriction and rehabilitation on serum protein of male rats ESTHER DA COSTA and RUTH CLAYTON (introduced by ROBERT E. JOHNSON) *U S Army Medical Nutrition Lab, Chicago, 9, Ill*. The mean total serum protein of 42 control rats was 6.4 gm % and the A/G ratio was 1.34. Seventy-two rats kept 3 weeks on a low calorie, modified carrot or synthetic vegetable diet had total serum protein values of 6.14, 5.41 and 6.21 gm % and A/G ratios of 1.43, 1.16 and 1.05 respectively. After 13 weeks of rehabilitation following the 3 types of restriction 120 rats were rehabilitated with a high protein, high fat or high carbohydrate diet. After 48 and 96 hours of refeeding, the high protein and high fat rats had slightly higher serum proteins than the controls but the high carbohydrate rats had significantly lower serum proteins. After 13 weeks of rehabilitation all types of re-fed rats showed normal protein values. During the first 48 hours of rehabilitation the A/G ratio of the rats on the high protein and high fat diets fell below 1.0, i.e. due to a lowering of the albumen. By 96 hours of refeeding all A/G ratios had reached 1.0 and by 6 weeks of refeeding they returned to normal. The rats kept on a low calorie, modified carrot or synthetic vegetable diet after 13 weeks lost an average of 39.6, 42.2 and 44.5% in weight but only the rats on the modified carrot diet had serum protein concentrations below normal.

Tryptophan-nicotinic acid relationship in the developing chick embryo CHARLES A. DENTON (by invitation) W. L. KELLOGG (by invitation), W. E. ROWLAND (by invitation) and H. R. BIRD *Bureau of Animal Industry, Agr Res Adm U S D A, Beltsville, Md*. The developing chick embryo synthesizes nicotinic acid and at the same time decreases in tryptophan content. The eggs contained on the average 36 micrograms of nicotinic acid, whereas the average content of the chicks was 1080 micrograms. The tryptophan decreased from 116 mgs (average content of the eggs) to 72 mg (average content of the chicks). Injection of 25 mg of tryptophan into the eggs on the 10th day of incubation resulted in a significant increase in the nicotinic acid content of the chicks. Injection of 10 mg of nicotinic acid into the eggs caused a slight increase in the nicotinic acid content of the chicks, but did not change the tryptophan content.

Effects of pteroylglutamic acid, injectable liver extract, and ascorbic acid on dietary glycine toxicity JAMES S. DINTING (by invitation), CECILIA K. KEITH (by invitation), JOHN R. TOTTER

and PAUL L. DAY *Dept of Biochemistry, School of Medicine, Univ of Arkansas, Little Rock, Ark*. A study was made of the effect of pteroylglutamic acid (PGA), injectable liver extract (LE), and ascorbic acid on growth and fecal porphyrin excretion of rats receiving purified diet containing 10% glycine. The purified diet without glycine was fed to a control group. The average weekly gains over a 5-week period for the groups were: control 25.3 gm, high glycine 5.6 gm, high glycine + PGA 19.8 gm, high glycine + LE 12.9 gm, high glycine + PGA + LE 21.1 gm, and high glycine + ascorbic acid 9.3 gm. Both PGA and LE significantly improved growth when added to the high glycine diet ($p < 0.01$). PGA was more effective than LE in improving growth under these conditions and a combination of PGA and LE did not improve growth above the level obtained by PGA alone. Although addition of ascorbic acid to the high glycine diet improved growth slightly, the increase was not statistically significant. The fecal porphyrin excretion of rats receiving the high glycine diet was considerably lower than that of the control rats. Additions of PGA, LE, or ascorbic acid to the high glycine diet increased the fecal porphyrin excretion. The greatest increase in porphyrin excretion was obtained by the addition of a combination of PGA and LE to the high glycine diet; fecal porphyrin excretion under these conditions was elevated above that of the control rats.

Vitamin B₁₂—a growth factor for young rats GLADYS A. EMERSON, ELIZABETH WURTZ (by invitation) and MARY E. ZANETTI (by invitation) *Merck Inst for Therapeutic Research, Rahway, N J*. The rat requires a factor which is present in liver and other animal proteins for normal growth and successful propagation. The influence of vitamin B₁₂ on the progeny of rats maintained during gestation and lactation on a diet containing 60% soybean meal has been investigated. One group of animals received 5 γ of vitamin B₁₂ daily from impregnation while a like number of untreated females served as controls. The size and birth weights of the litters cast by the rats in each group were the same. However, the weaning weights (28 days) of the young from mothers receiving vitamin B₁₂ averaged 50% more than did those from the untreated females. The offspring from each group were segregated at weaning into 2 subdivisions, in one the animals were given 0.5 γ of vitamin B₁₂ daily for 90 days and the others were unsupplemented. The weight increments of the males from the several groups were as follows: control mothers, young undosed, 151 gm; control mothers, young dosed, 257 gm; treated mothers, young undosed, 229 gm; treated mothers, young dosed, 283 gm. The feeding of vitamin B₁₂ to the young of control mothers resulted in body weights that were 100 gm in excess of those of the unsupplemented animals.

Since the undosed young of the treated mothers grew for 2 months at a rate approximating that of the dosed young from the control mothers, it would appear that vitamin B₁₂ is stored during the suckling period. The red and white cell counts of the individuals of all groups fell within the normal range.

Relation of choline intake to survival in rats fed p-dimethylaminoazobenzene R W ENGEL *Lab of Animal Nutrition, Alabama Polytechnic Inst, Auburn, Ala*. When immature male and female rats of approximately 200 gm body weight were subsisted for 2 months on a low-protein diet deficient in choline, they gained an average of 1.2 gm/rat/week. Inclusion in the diet of p-dimethylaminoazobenzene at the 0.6% level for a subsequent 7-week feeding period resulted in the death of 7 of 8 rats with an average survival period of 28 days. When the diet was fortified with choline (2 gm/kg), the average body weight gain during the initial 2-month feeding period was 3.0 gm/rat/week and 8 of 8 rats survived the subsequent 7-week feeding period when p-dimethylaminoazobenzene was included in the diet at the 0.6% level. All the animals receiving the choline deficient diet exhibited gross evidence of liver cirrhosis and a marked accumulation of a clear serous fluid in the body cavities at autopsy. Only mild kidney damage was evident. The livers of the control animals receiving choline were normal in appearance, and no gross pathologic changes were evident in any tissues at autopsy after 7 weeks of p-dimethylaminoazobenzene feeding. The basal diet employed consisted of alcohol-extracted casein 2, alcohol-extracted peanut meal 10, sucrose 63.5, salts 4.4, L-cystine 0.1, lard 19, and cod liver oil 1, and was fortified with known accessory factors.

Chronic vitamin A deficiency in 3 inbred strains of mice PAUL F FENTON (by invitation), RUTH E SHRADER (by invitation) and GEORGE R COWGILL *Yale Nutrition Lab, Dept of Physiological Chemistry, and Dept of Anatomy, Yale Univ, New Haven, Conn*. Weanling mice of the C₅₇, A and I strains were fed an artificial diet containing no added vitamin A. Growth rates of the C₅₇ mice were less than those of the A strain although substantial growth occurred in both strains. Growth was more seriously retarded when the corn oil of the diet was replaced by hydrogenated cottonseed oil and vitamin E was omitted. A striking early feature of vitamin A deficiency in C₅₇ mice was the graying or bronzing of the normally black fur. Distribution of the fur changes differed markedly from that seen in pantothenate deficiency. The vitamin A content of the livers was reduced to a very low level. Most mice survived many months without outward symptoms of deficiency. A substantial group was sacrificed when about 9 months old and organ weights determined. The most consistent findings

regarding the blood picture were reductions in the red cell counts, hemoglobin levels and hematocrit values. Infection was frequently present. Many animals retained extensive fat depots despite 8 months on the deficient diet. Histological and histochemical studies were carried out on liver, kidney, spleen, thymus, testes and adrenals, and, when necessary, on other tissues. Several vitamin A-deficient male mice were injected with three doses of estradiol benzoate. Two months later the accessory reproductive structures were found to be greatly distended. They presented a histologic picture similar to that seen in mice treated for a long time with large doses of the estrogen.

Unidentified vitamins of the B complex required by certain insects G FRAENKEL (introduced by H E CARTER) *Dept of Entomology, Univ of Illinois, Urbana, Ill*. Many insects which normally live on grain or flour can be easily grown on 'synthetic' diets and are excellent material for nutritional studies. It is now an established fact that insects in general require all the known vitamins of the B-complex, including biotin and pteroylglutamic acid, with the exception of paraaminobenzoic acid and possibly inositol. Some insects seem to develop optimally on 'synthetic' diets in the absence of any sources of unidentified factors. With the mealworm, *Tenebrio molitor*, there is clear-cut evidence for the need of one additional factor present in yeast or liver, tentatively called B_T, which is not adsorbed on charcoal and has proved very elusive in all attempts to be precipitated from the aqueous phase. In this respect, and certain chemical characteristics as well, it bears a close resemblance to streptogenin, though the absence of B_T activity in casein excludes identity of the two factors. The need for at least 2 other unidentified members of the B-complex has been demonstrated for the flour beetle, *Tribolium confusum*. Growth on a 'synthetic' diet is slow by comparison with an optimal natural diet—wholemeal flour. A certain improvement is achieved by adding 2½% of the water-insoluble fraction of yeast, but a still greater improvement results from adding 1% dry yeast. For reasons still unexplained these deficiencies are more marked if starch is used as the carbohydrate, as compared with glucose. B_T concentrates failed to have any effect on *Tribolium* in diets deficient in one or two of the missing factors.

Effect of gossypol on the body weight of rats ALFRED H FREE and HELEN M FREE (by invitation) *Miles Research Labs, Elkhart, Ind*. Gossypol is an organic compound present in the pigment gland of the cottonseed. It has long been recognized that the inclusion of large amounts of unheated cottonseed meal in the diets of animals has a detrimental and toxic effect which has been ascribed to gossypol. Zucker and Zucker (Abstracts of Papers, 111th meeting, American Chemical So-

ciety, page 47B, 1947) have suggested that gossypol has a specific effect in reducing food intake. Further experiments have been carried out in this laboratory to determine the effect of gossypol on the body weight of rats. Gossypol was prepared from whole cottonseeds by the method of Boatner et al. (Jour Am Chem Soc 69 1268, 1947). Growth studies with young rats were made in which the animals were maintained on a standard diet and were given daily supplements of gossypol suspensions by stomach tube. In 7 experiments of this type employing 3 different preparations of gossypol, uniform results were obtained in which animals on higher intakes of gossypol died, whereas those on intakes that were $\frac{1}{2}$ or $\frac{1}{3}$ of the toxic amount grew at rates comparable to the control group which did not receive gossypol. Adult rats showed no significant weight change when given a daily supplement of gossypol that was one-half the toxic amount. Starvation experiments with rats indicated that the weight loss of animals receiving minimal lethal quantities of gossypol was much less than the weight loss of animals dying of starvation.

Rat-repletion studies with amino acid solutions

Rôle of non-essential amino acids. DOUGLAS V. FROST and HARRY R. SANDY (by invitation) *Nutritional Research Division, Abbott Labs., North Chicago, Ill.* Weight response of adult protein-depleted rats to dry proteins, liquid protein hydrolysates or solutions of amino acids fed separately from protein-free diet was studied at controlled critical levels of nitrogen. A 5% solution was fed to supply twice Cannon's essential amino acid minima of tryptophan 13, phenylalanine 37, leucine 77, isoleucine 66, methionine 35, threonine 45, lysine 51, histidine 22 and valine 53 mg/rat/day. Discounting the D-isomers of isoleucine, threonine and valine, this provided about 100 mg N/day. Only half of the liquid allotment representing this mixture was consumed and the weight response was low. Intake and response were nearly doubled by isonitrogenous replacement of 10 parts of essentials by arginine or glycine. Nitrogen efficiency ratios were high in each instance and compare favorably with the highest values calculated for standard proteins. Glutamic acid, in presence of arginine, glycine and alanine, led to a further improvement in acceptability and weight response. Complete hydrolysates of fibrin fortified with tryptophan were taken with greater avidity than amino acid mixtures, even though nitrogen efficiency was somewhat lower for the former. These effects were checked by repeated experiments in paired groups. The data suggest that absence of nitrogen other than essential amino acid N imposes a physiologic burden which is reflected in decreased appetite, but not in decreased nitrogen efficiency. Possible inhibitory effects of the D-amino acids may account

in part for the lesser avidity for amino acid mixtures than for complete protein hydrolysates.

Decrease in the storage of thiamine in tissues from supplementing the diet with live bakers' yeast. FUNG-HAAN FUNG (by invitation), RUTH J. AANESS (by invitation) and HELEN T. PARSONS *Dept. of Home Economics, Univ. of Wisconsin, Madison, Wis.* It has been established that when live bakers' yeast is ingested by human subjects, not only the yeast thiamine but also food thiamine consumed simultaneously fails to be returned in the urine in normal amounts, presumably because it is taken up in the digestive tract by yeast cells. However, about 80% of the nitrogen of the yeast cells was returned in the urine of the subjects. The hypothesis of an anti-enzyme analogous to folic acid anti-conjugase was untenable inasmuch as free thiamine was itself influenced by live yeast. Hence it became important to establish by direct proof whether or not absorption and storage of thiamine were actually interfered with by live yeast. Weanling pigs were found to be satisfactory test animals. Those fed live bakers' yeast in addition to the basal ration stored only about $\frac{1}{2}$ as high a percentage of the total thiamine intake as pigs without this supplement. In all animals tested, about $\frac{1}{10}$ of the total storage occurred in the skeletal muscles. Supplementing with live yeast depressed the urinary thiamine and increased the fecal values quite comparably with previous results in this department on college women. This fact, considered with the decrease in thiamine storage in the tissues of pigs fed the yeast dose, substantiates the earlier conclusion that the ingestion of live bakers' yeast interferes with the absorption of thiamine by human subjects.

Biotin deficiency in ducklings and the histopathology of the heart. JAMES W. GODDARD and ROBERT E. OLSON (introduced by FREDERICK J. STARE) *Dept. of Nutrition, Harvard School of Public Health, Boston, Mass.* Studies in biotin-deficient ducks (J Biol Chem 175 503, 1948) have shown that the respiration, pyruvate and succinate utilization of cardiac muscle slices *in vitro* is markedly depressed below normal. In view of this the histopathology of the heart in biotin deficiency was investigated. Biotin deficiency was induced in one-day old ducklings by feeding a purified diet low in biotin and supplemented with 20% egg white. Deficient ducks were killed by decapitation at intervals after their growth had ceased and perosis developed, the hearts removed, weighed, fixed *in toto* in Romeis' fixing fluid and paraffin sections stained with Masson's trichrome, Mallory's phosphotungstic acid hematoxylin, and Heidenhain's iron hematoxylin. Grossly, the hearts from the deficient ducks were larger, heavier (50%) and paler than those from pair-fed controls. Microscopically, the hearts from the deficient ducks showed disruption

and lysis of myocardial fibrils, disintegration of anisotropic hands, and granularity and vacuolation of the muscle fibers. The Purkinje cells showed marked disorientation and disappearance of their myofibrils. In severe cases, these myofibrils and even whole Purkinje cells were replaced by connective tissue. The endocardial cells showed occasional hyperplasia, chiefly in areas over involved Purkinje cells, and desquamation. The pericardium and vessels were essentially negative.

Resurvey of nutritional status in Norris Point, Newfoundland. GRACE A. GOLDSMITH, WILLIAM J. DARBY, RUTH STEINKAMP (by invitation), ANNE STOCKELL (by invitation) and ELLEN McDEVITT (by invitation). *Dept of Medicine, Tulane Univ School of Medicine, New Orleans, La, Depts of Biochemistry and Medicine, Vanderbilt Univ School of Medicine, Nashville, Tenn.* Evaluation of the nutriture of 126 persons from 4 population segments—pre-school, pre-adolescent, mothers of children under 2 years, and other adults—was made in Norris Point, Newfoundland, in July, 1948. Following a comparable study in 1944 (*J Lab Clin Med* 30:475) compulsory enrichment of flour was instituted. Dietary patterns were similar in the 2 studies, $\frac{1}{4}$ or more of the calories of half the subjects being derived from flour and cereals. Low intakes of ascorbic acid, calcium, vitamin A and riboflavin occurred in 25 to 50% of the examinees. The incidence of subjective complaints attributable to malnutrition was essentially the same in both surveys. Physical findings in 1948, as compared to 1944, showed somewhat less nasolabial seborrhea, thickening and pigmentation of pressure points, circumcorneal injection, neurologic changes, and a definite decrease in the incidence and severity of lingual lesions. The same or increased incidence was noted for cheilosis, conjunctival injection, dental caries, gingival changes, follicular keratosis and angular fissuring. Less conjunctival thickening was observed in pre-school children, an equal amount in other groups. Serum protein, hemoglobin, and vitamin A levels were generally satisfactory. Distribution of serum ascorbic acid was 0.13, 0.39, and 0.65 mg/100 cc for the first, second, and third quartiles, respectively. Four-hour urinary excretion, after an oral test dose of vitamins, was frequently low for riboflavin, but usually comparable to that in a group of normal laboratory subjects for thiamine and N'-methylnicotinamide. Correlations between dietary intake, physical signs, and laboratory findings will be presented and the significance of the data discussed.

Interrelation of age, serum cholesterol, and basal metabolism of women. MARY R. GRAM (by invitation) and RUTH M. LEVERTON. *Human Nutrition Research Lab, Univ of Nebraska, Lincoln, Neb.* Serum cholesterol and basal metabolic rate were determined on 130 women between

the ages of 18 and 70 years and living on self-chosen diets. Total serum cholesterol was determined fasting blood samples by the Liebermann-Burch reaction modified by Hoffman. Basal metabolic rate was determined with a Benedict-Roth respiration calorimeter and the results compared with normal values calculated on the basis of ideal weight by the method of DuBois as modified by Boothby and Sandiford. The women studied were in the following age groups: 18-25 yr, 52 subjects; 30-40 yr, 28 subjects; 40-50 yr, 28 subjects; 50-60 yr, 32 subjects; and 60-70 yr, 20 subjects. The mean serum cholesterol for each age group was 177.4, 200.7, 250.6, and 257.4 mg % respectively. Analysis of variance indicates this increase of cholesterol with increasing age is significant. The mean basal metabolic rate for all women was 7% below the standard. The means of the basal metabolic rates for each age group were not significantly different. The relationship of basal metabolic rate to serum cholesterol and other blood values is being investigated for each age group.

Goiter in West Virginia. ISIDOR GREENWALL. *Dept of Chemistry, New York Univ College of Medicine, New York City.* In 1928, Kimball, relying upon statements by 'older physicians' in Charlestown, claimed that goiter had been 'exceptionally rare' in the Kanawha valley before 1900 and that the high incidence in 1922 was due to replacement of local salt by a refined product, "the only change in food or water" that had occurred in the interval. The Journal of the American Medical Association has recently reiterated that claim (135:434, 1947). The only information available regarding the iodine content of the local salt is given by Grimsley (West Virginia Geological Survey, Reports, vol. 4) the brine of the one well open in 1909 contained only one part of NaI per 61,400 parts of NaCl. Moreover, Drake, writing from 'among the upper waters of the Kanawha' in 1850, stated "Goitre. Cases are scattered here and there. I think they are not more numerous in proportion to the population than in the valleys of the Scioto and Cuyahoga" (Western Lancet 11:558). Earlier reports of goiter in West Virginia are mentioned by Barton (Memoir Concerning Goiter, 1800). In 1893, goiter had disappeared from many parts of the country in which it had been endemic (Osler, *Am J Med Sci* 106:503). Later, the disease reappeared. This sequence was well illustrated at Edmonton. There, too, an 'older physician' denied that the disease was present before 1900. However, cretins and large goiters were common in 1820 and many young girls had goiters as late as 1863 (Greenwald, *Bull His Med* 17:229, 1945).

Effect of various amino acids on rats receiving niacin-tryptophan deficient rations. L. V. HANKES, L. M. HENDERSON (by invitation) and C. A. ELVEHJEM. *Dept of Biochemistry, College of Agri-*

culture, *Univ of Wisconsin, Madison, Wis* Previous work has shown that 0.078% DL-threonine or 0.204% DL-phenylalanine will cause a growth inhibition in rats on a 9% casein sucrose ration deficient in niacin. The active isomers were found to be L-threonine and D-phenylalanine. When the 0.2% L-cystine, which is normally included in the ration, was omitted, almost normal growth was obtained in the presence of 0.078% DL-threonine, 0.208% DL-phenylalanine, 2% acid hydrolyzed casein, or 2% glycine. However, the addition of 0.2% L-cystine with any of the above materials caused growth inhibition. When 0.2% DL-methionine replaced 0.2% L-cystine a growth inhibition occurred only with 0.078% DL-threonine or 2% acid hydrolyzed casein. Urine excretion studies indicated that acid hydrolyzed casein did not affect the amounts of N-methylnicotinamide or nicotinic acid excreted. DL-threonine (0.078%) in the presence of either L-cystine or DL-methionine did not affect the nicotinic acid levels in the urine. Since rats on a 9% casein dextrin ration showed a growth inhibition only with 6% gelatin, and not with 0.078% DL-threonine or 2% acid hydrolyzed casein, the effect of coprophagy on growth inhibition was studied. Six % gelatin caused a growth inhibition when the rats were in a square cage, but not when in a tube cage. Feces were analyzed for niacin.

Use of enriched cereals in certain diets consumed in the south. HARRIET A. HARLIN and BEULAH D. WESTERMAN (introduced by MARTHA M. KRAMER) *Dept of Foods and Nutrition, Kansas Agr Exp Sta, Kansas State College, Manhattan, Kan* Growth studies were made on young albino rats fed diets based upon the food consumed by certain low income groups in the South, to determine the significance of the use of enriched flour as a source of the B vitamins. The Negro tenants of the Mississippi Delta consume diets made up primarily of molasses, fat, cereals and black-eyed-peas with small quantities of eggs, vegetables, fruits and milk. When 58% of the calories in such a diet was derived from cereals, the growth of the rats consuming the diets containing enriched flour as the cereal component was greater than those consuming an equal quantity of non-enriched flour. When 45% of the total calories were derived from cereal, there was no difference in the growth gains made by the rats on the diets containing enriched and non-enriched flour. Certain people in South Carolina consume diets consisting of limited quantities of fat, meat, milk, eggs, vegetables, fruits and large quantities of cereals. When such diets were fed to rats and 50% of the total calories of the diet was furnished by enriched flour the animals with enriched flour in the diet made on the average 10 gm greater growth gains than did those with non-enriched flour in the diet. When the enriched and non-enriched flour were fed at a level of 40 and 33%

of the total calories in the diet no difference was noted in the average weight gains of the rats.

Concurrent feeding of amino acids. RICHARD HENDERSON (by invitation) and ROBERT S. HARRIS *Nutritional Biochemistry Labs, Massachusetts Inst of Technology, Cambridge, Mass* Groups of weanling rats were fed a basal diet containing casein (30), corn starch (55.4), hydrogenated fat (10), tryptophan (0.17), histidine (0.13), salt mixture (4) and vitamin mixture (0.36). Control groups received the basal diet + 1% lysine. Test groups received the basal diet and the basal diet + 2% lysine at alternate feedings. The lysine replaced equal weights of corn starch in the diet. Groups were fed (1) one hour and fasted 7 hours, (2) one hour and fasted 5 hours and (3) one hour and fasted 3 hours throughout successive experiments lasting 27 days. A time-interval feeder was devised for the automatic opening and closing of the feeding jars. Significant differences were noted in nitrogen retention and in body weight gain, weight gain/gram of diet eaten, weight gain/gram of lysine eaten. These differences were greater as the feeding intervals lengthened. Thus a delay of 3 hours or longer, in the feeding of lysine as a supplement to a lysine-low diet interfered with metabolism. Lysine must be fed concurrently with other amino acids in the diet for most efficient utilization by the rat.

Vitamin B₁₂ in swine nutrition. A. G. HOGAN and G. C. ANDERSON (by invitation) *Depts of Animal Husbandry and Agricultural Chemistry, Univ of Missouri, Columbia, Mo* Six pigs were taken from their mothers at 2 days of age and transferred to cages with wide-mesh wire floors. They were reared on synthetic milk which included 'vitamin-free' casein, sucrose, corn starch, lard, mineral salts, and generous supplies of vitamins A, D, E, K, thiamine, riboflavin, pyridoxine, pantothenic acid, niacin, choline, biotin, folic acid and inositol. Three of the pigs were given intramuscular injections of crystalline vitamin B₁₂ (Merck) at 3-day intervals, until they were 38 days old. One pig received a total of 50, a second received 100, and the third received 200 mcg. All 6 pigs grew at about the same rate for the first 6 weeks. In the following 4-week period the pigs that received the injections made an average gain of 26.8 lb, the others gained 15.9 lb. In the following 6-week period one pig that did not receive vitamin B₁₂ died, unexpectedly, and one has not gained consistently. The third began to decline and when injected with a total of 15 mcg of vitamin B₁₂ it began to gain at a moderate rate. The 3 that had received the vitamin injected grew at a uniform rate with an average gain of 58.8 lb, which is exceptional for that age. Three sows in all have been reared on synthetic diets. Two farrowed but were unable to rear their litters. The third never became

pregnant, presumably because of a constriction in each Fallopian tube

Role of methionine in the protein metabolism of the rat CECILE HOOVER (by invitation), PEARL SWANSON, and HELEN MERRIAM STEWART (by invitation) *Nutrition Laby, Foods and Nutrition Section, Iowa Agr Exp Sta, Iowa State College, Ames, Iowa* Methionine, when added as a supplement to a nitrogen-poor diet offered to rats for a period of 21 days, exerts a marked body-sparing action. It has been postulated that in its absence, the body raids its own tissues to secure this compound for the fabrication of an essential metabolite(s). This hypothesis is being tested by studies of the partition of urine excreted by rats under various experimental conditions. The reduced excretion of urinary nitrogen resulting from the dietary addition of methionine is due to a marked decrease in both the total and relative amounts of urea nitrogen. Effects induced by the supplementary feeding of certain analogs of methionine also have been investigated. An accelerated catabolism, for example, accompanies the administration of ethionine. Although the quantity of nitrogen excreted is increased by 148 mg, the excretion of urea remains constant. Ethionine apparently acts like an antagonistic substance, thereby indicating that methionine occupies a key position in some metabolic reaction, and is important in this connection as a whole molecule rather than by virtue of specific constituent groupings. With caloric restriction, the elimination of fat from the test ration of rats results in a striking increment in urinary nitrogen due to increased quantities of urea. The addition of methionine averts the catabolic action of the low fat diet under these conditions. The ameliorative influence of dietary fat appears to rest on its ability to reduce the catabolism of compounds, the end products of which show up in the non-urea nitrogen fraction of urine.

Fatal lung hemorrhage and liver damage in rats prevented by vitamin E E L HOVE, D H COPELAND (by invitation) and W D SALMON *Laby of Animal Nutrition, Alabama Polytechnic Inst, Auburn, Ala* Young rats died suddenly after an average of 53 days on a Vitamin E-free, low-casein, high-fat diet. A sex difference in incidence was apparent: death came to 80% of the males (30/37) but only 30% of the females (3/10). No spontaneous deaths occurred among 28 litter mate controls receiving 7 mg DL alpha tocopherol weekly. The diet contained water-washed casein 10, lard 19, cod liver oil 1, salt mixture 4, sucrose 66, choline 0.2, and an abundance of B vitamins. Necropsy revealed 3 major types of lesions: massive hemorrhage in the lungs, distension of small and large blood vessels of the body, and grossly evident liver damage which histologic section showed to be zonal necrosis. Evidence of Vitamin-E deficiency was shown by

blanching of the teeth, testicular atrophy, creatin-urea, and depressed growth (0.86 gm/day as compared with 1.26 gm/day for controls). Methionine, cystine or theophylline (0.1%) did not protect, although a higher casein level did. With 18% casein in the E-low diet, growth was optimum, and the rats remained in apparent good health for a year. The protective factor in casein could be destroyed by hydrogen peroxide. Death and liver damage occurred in 5 of 6 rats on E-low diets containing 18% oxidized casein fortified with tryptophane and methionine. The DL-methionine requirement for maximum growth was over 1% although this level did not protect against the acute Vitamin-E deficiency.

Influence of vitamin E on the creatine and creatinine excretion of rats poisoned with CCl₄ E L HOVE *Laby of Animal Nutrition, Alabama Polytechnic Inst, Auburn, Ala* A sharp increase in creatine excretion and a reduction in creatinine excretion has been observed in rats poisoned with CCl₄. The same condition occurs as the result of a chronic vitamin-E deficiency. Therefore a study was made of the influence of vitamin E on the creatine-creatinine excretion pattern following CCl₄. Groups of rats on a 10% casein, E-low diet were fed 0, 0.5, 2.5 and 12.5 mg of DL, alpha tocopherol daily for 10 days prior to injections of single doses of 1 cc of CCl₄/kg body weight. The maximum creatine excretion level, expressed as mg/rat/day, was 25 for the E-free group, but only 7 for the group fed the highest tocopherol level. The fall in the creatinine excretion was delayed in the tocopherol fed group and the return to normal values was more rapid. The lower tocopherol levels gave intermediate values. Half of the E-free control group died as the result of CCl₄. None of the tocopherol-fed rats died. The values given for the creatine-creatinine excretion of the E-free group are based on survivors.

Role of the gastrointestinal tract in the conversion of tryptophan to nicotinic acid JAMES M HUNDLEY (introduced by F S DAFT) *Experimental Biology and Medicine Inst, National Inst of Health, Bethesda, Md* Several investigators have advanced evidence indicating that the intestinal bacteria can supply a part or perhaps all of the requirement for nicotinic acid in certain species. Since tryptophan is known to be a dietary precursor of nicotinic acid, the idea has been advanced that intestinal bacteria may convert tryptophan to nicotinic acid. To test this possibility, we have studied the efficiency of conversion of tryptophan to nicotinic acid in rats before and after surgical removal of the gastrointestinal tract. The amount of urinary N'-methylnicotinamide (NMN), expressed as γ per 100 gm of rat in 24 hours, was used as an index of the amount of nicotinic acid synthesized. 100 mg of L(-)-tryptophan given subcutane-

ously in 10 cc of saline was the test dose in all instances. 26 male rats weighing about 250 gm each were maintained on a basal purified 'nicotinic acid free' diet. The rats excreted 120 γ of NMN on this diet alone. Tryptophan increased the excretion to 1274 γ . After surgical removal of the entire intestine, excepting the stomach, the basal excretion of NMN was 246 γ . Tryptophan increased the excretion to 2156 γ . After gastrectomy only, the basal excretion was 182 γ which tryptophan increased to 1864 γ . These data have been interpreted to mean that neither the stomach nor the bacteria of the intestinal tract are involved in the synthesis of nicotinic acid from tryptophan.

Comparison of sugars in the purified diet of baby pigs S. R. JOHNSON, *Dept. of Animal Industry, Univ. of Arkansas, Fayetteville, Ark.* Five litters of baby pigs were fed 'synthetic' milk in which all nutrients were in purified form. When the carbohydrate was sucrose, acute diarrhoea quickly developed, resulting in rapid weight loss and death within 48 hours unless a ration change supervened. Hemorrhagic kidney syndrome, probably like that observed in young rats on low choline sucrose-base diets, resulted from the use of sucrose. When the ration was quickly changed to cows' milk, recovery occurred. If the pigs were started on cows' milk and changed very gradually to the sucrose-base diet, they tolerated the sucrose. Addition of 16% yeast, 0.4% d-l methionine, or a double allowance of pure vitamins did not prevent the sucrose syndrome. Glucose ('Cerelease') instead of sucrose in the purified diet resulted in uneventful growth with little or no diarrhoea or kidney damage. Lactose was satisfactory in the starting ration but pigs on the lactose-base diet eventually developed chronic diarrhoea, grew more slowly than those receiving glucose, and one pig continued on the lactose diet developed probable biotin deficiency. Levulose was unsatisfactory as the carbohydrate (2 cases), and addition of sucrase enzyme to the sucrose-base diet did not improve performance (2 cases). Baby pigs could be started very successfully with only one small nursing of colostrum, cows' milk while learning to drink the first day, then with the purified diet with either glucose or lactose as the carbohydrate.

Perspiration as a factor influencing the iron requirement FRANCES A. JOHNSTON and GLADYS C. HAGEN (by invitation), *N. Y. State College of Home Economics, Cornell Univ., Ithaca, N. Y.* In studies of the iron requirement the loss of iron in perspiration is not usually taken into consideration. Unless the loss is extremely small it will raise the requirement appreciably because of the low percentage of iron absorbed from the diet. The problem of collecting perspiration free of contamination is so difficult that all we could do was obtain a value which would set a top limit to the amount of iron

that might be present. The iron content of filtered water collected from washing 2 subjects after perspiring for 2 hr at 100°F and 59-67% relative humidity was 0.02, 0.02 mg respectively. This would amount to 0.24 mg for 24 hr of profuse perspiration. Since perspiration seldom continues at a profuse rate for 24 hr, a loss as large as this would be unusual. Perspiration induced by high temperatures and humidity and collected without dilution was found to contain a mean value of 0.06, 0.05, 0.03, 0.02 and 0.04 mg/l for each of 5 subjects. Except under extreme conditions of temperature, humidity or activity, the excretion of perspiration probably does not exceed 1 l/day. Thus, under normal living conditions in the north temperate zone, the loss of iron in perspiration is negligible and probably does not exceed 0.02 to 0.06 mg/day. The possibility that perspiration contains no iron can not be ignored. In 3 of the 19 samples collected from the 5 subjects no iron was present.

Color measurement in products of rice milling MARINUS C. KIK, *Univ. of Arkansas, Fayetteville, Ark.* Whole kernel samples of mill products (brown, first break, second break, and head or white rice) of Blue Rose, Zenith, California Pearl and Nira, tested for color with the Hardy self-recording photoelectric spectrophotometer, gave average percentage reflectances at 460 m μ and at 660 m μ for brown rice, 25 and 51, for first break, 33.6 and 59.2, for second break, 36.2 and 61.3 and for head rice, 36.1 and 60.6. In the whole visible region (especially in the yellow), reflectance values between brown and white rice differed considerably, were small between first break and nonappreciable between second break and white. The photovolt photoelectric reflectometer, with blue, green and red filters, gave similar results. Blue and green filters were more effective than the red filter. In these latter studies rice samples with different bran removal (1 to 10%) were tested for color. The widest spread of readings between samples of one variety is obtained when measurements are made with 'suppressed zero'. Using the blue filter and for brown rice a setting at 0, gave readings for first break, second break and head rice of 50, 58 and 69 respectively. The results of these reflectometer studies might find application in government grading, in description of standards for rice and in controlling rice milling to a specific degree of whiteness.

Pteroylglutamic acid studies on patients with tropical sprue Factors affecting urinary excretion levels. ELIZABETH M. KNOTT and RAMÓN M. SÁENZ (introduced by R. R. WILLIAMS), *School of Tropical Medicine, San Juan, Puerto Rico*. The urinary excretion of pteroylglutamic acid has been assayed with Lactobacillus casei, for 313 24-hour specimens and 237 2-8-hour collections, from 65 individuals. Patients with tropical sprue were diagnosed in accordance with the criteria defined by

Suárez, et al (*Ann Int Med* 26 643, 1947) Results are reported as micrograms The average total daily excretion for sprue patients was 1 109 (range for 6 patients 0 166–0 778, and 4 patients 1 180–3 068) Eight staff members averaged 1 693 (range 0 920–4 100) Two non-sprue, anemic patients excreted 0 418 and 0 243 on a low protein diet, increased to 0 650 and 0 433 when niacin was added, and increased to 1 544 and 1 273 when given the regular hospital diet Three anemic patients on a diet high in protein and vitamins, plus injections of liver extract, excreted an average of 4 340 (range 3 909–4 715) The night rate of excretion, in $\mu\text{g/hr}$, was 0 022 for sprue patients, 0 057 for other hospital patients, and 0 076 for staff members During fasting in the morning, the rate of excretion decreased for sprue patients to 0 016, but increased to 0 094 and 0 119 for the other hospital patients and staff members One staff member, although receiving an excellent diet, excreted amounts of pteroylglutamic acid comparable to the lower figures for sprue patients, and had a tendency for repeated episodes of a spruelike syndrome Women during the last trimester of pregnancy had night excretion rates of 0 068 and fasting rates of 0 101, but decreased to 0 049 and 0 091 during the first week postpartum

Paradoxical relationship between serum level and liver content of vitamin A R F KRAUSE (introduced by H B PIERCE) *Dept of Biochemistry, College of Medicine, Univ of Vermont, Burlington, Vt* An examination has been made of the relationship between serum level and liver content of vitamin A in normal rats and those under the influence of varying degrees of vitamin-A depletion An inverse relationship has been found between the blood and liver levels of vitamin A in normal rats and in depleted animals whose liver content ranged as low as 600 IU /total liver However, under conditions of more severe depletion when the total liver content fell below 600 IU , there was a parallelism between blood and liver levels This evidence supports the hypothesis that there is a regulatory mechanism controlling blood vitamin-A levels of the rat which, under the conditions described above, operates to give an inverse relationship between blood level and liver content of vitamin A This mechanism, however, fails to function under conditions of extreme depletion

Some inter-relationships of thiamine and fat in the nutrition of the rat M M KRIDER (by invitation) and N B GUERRANT *Dept of Agricultural and Biological Chemistry, Pennsylvania State College, State College, Penna* Studies, with regulated intakes of isocaloric diets, designed to clarify the probable inter-relationship of the thiamine and fat intake of the rat and the amounts of thiamine and fat stored in the rat's body have yielded data that permit the following conclusions 1) Increasing the thiamine intake from 2 to 1000 μg daily had less

effect on increasing the body-weights of rats than did increasing the fat content of the diet from 0 to 20% This increase in body-weight was due primarily to greater fat deposition attained by the rats receiving the fat-containing diets 2) The cardiac thiamine of rats was found to depend primarily on the thiamine intake and was not markedly affected by the fat content of the diet 3) Under the conditions of the experiments, the fat content of the rat's body was found to increase with the fat content of the diet and was not markedly influenced by the thiamine intake where the intake of this vitamin was 50 μg or more/day 4) The index of refraction of the body fat of rats was found to vary with the fat (Crisco) content of the rat's diet and to decrease with an increase in the amount of fat present in the rat's body

Effect of dietary restriction and rehabilitation upon the spontaneous activity of rats HARRY KRZYWICKI and ESTHER DA COSTA (introduced by ROBERT E JOHNSON) *U S Army Medical Nutrition Laboratory, Chicago 9, Ill* During an average of 14 weeks, a control group of 12 male rats fed ad libitum gained 121% in weight with an average food intake of 14 9 gm/rat/day and showed an average activity of 1101 rotary cage revolutions/ rat/day Eighteen rats fed a quantitatively restricted but otherwise complete diet lost 42% in weight with a food intake of 8 gm/rat/day and an average activity of 7854 cage revolutions/ rat/day The peaks in activity of the restricted rats occurred during the 6th, 9th and 10th weeks Six rats fed 8 gm/rat/day of a low protein, high salt diet lost 32% in weight and showed an activity of 3351 cage revolutions/ rat/day During dietary rehabilitation, spontaneous activity decreased promptly and showed an average of 1143 revolutions/ rat/day with a high fat diet, 1176 revolutions/ rat/day with a high carbohydrate diet and only 640 revolutions/ rat/day for a high protein diet Thus both types of food restriction caused increased activity but different types of refeeding led to significantly different reductions of spontaneous activity

Nitrogen excretion of women related to the distribution of animal protein in the daily meals RUTH M LEVERTON and MARY R GRAM (by invitation) *Human Nutrition Research Laboratory, Univ of Nebraska, Lincoln, Neb* This study is a preliminary investigation of whether the time element in the feeding of complete proteins affects the utilization of the nitrogen by human subjects Fourteen college-age girls were kept on a constant diet which furnished 62 gm of protein daily for 36 days During the first 18-day period a fruit-bread products-coffee breakfast, which furnished no protein from animal sources, was served and all of the animal protein was given in the noon and evening meals During the second 18-day period a glass of milk was taken from the evening meal and added

to the breakfast. Metabolism data were secured on all subjects for the last 9 days of each of the 2 periods. The average daily excretion of nitrogen for all subjects for the first and second periods was 9.07 and 8.57 gm respectively for urinary nitrogen, and 0.57 and 0.54 gm respectively for fecal nitrogen. Differences between daily nitrogen excretion of the 2 periods were not always statistically significant. On the same level of protein intake there was, however, a persistent trend toward a lower nitrogen excretion when the animal protein from a glass of milk was in the breakfast than when the animal protein was present only in the noon and evening meals. All subjects were in nitrogen equilibrium during the first period and retained an average of 1.03 gm nitrogen daily during the second period.

Studies involving protein stores in man. STANLEY LEVEY, JOHN E. HARROLD and CHARLES J. SMITH (introduced by ARTHUR H. SMITH). *Wayne County General Hospital, Eloise, Michigan, and the Depts. of Physiological Chemistry and Medicine, Wayne Univ. College of Medicine, Detroit, Mich.* The plasma volume (dye method), total circulating proteins and albumin were determined before and 30 minutes after the infusion of 1000 ml of physiological saline in 4 normal, healthy males and in 6 male patients exhibiting various degrees of undernutrition. The undernourished subjects were then placed on a high caloric (4000-5000), high protein (2.0 to 4.0 gm/kg/day) diet for a period of at least 3 weeks after which the above procedure was repeated. The subjects with normal nutrition demonstrated a constant response to the saline infusion characterized by an increase in plasma volume, approximately 350 ml, a decrease in serum protein concentration, but an increase in total circulating proteins. The albumin changes did not follow a consistent pattern. The response to the saline infusion exhibited by the malnourished patients was characterized by decrease in both the protein concentration and in the total circulating proteins, in spite of an increase in plasma volume. The malnourished subjects who were followed through the controlled dietary period demonstrated an increase in circulating proteins after the saline infusion similar to the normal controls. The major interest of this study centers on the changes in the circulating proteins following the saline infusion. The increase in circulating proteins demonstrated by the normal patients is explained by a washing into the circulation of proteins from a labile protein store. The decrease in circulating proteins exhibited by the undernourished patients is explained by a lack of labile protein stores. The change from the malnourished to the normal pattern after the period of dietary treatment demonstrates the repletion of the protein stores.

Human urinary and fecal elimination of vitamin B₆ and 4-pyridoxic acid on a controlled diet

HELEN LANKSWILER and MAY S. REYNOLDS (introduced by HELEN T. PARSONS). *Dept. of Home Economics, Univ. of Wisconsin, Madison, Wis.* The pyridoxine intake and the pyridoxine and 4-pyridoxic acid elimination by 12 normal human subjects on a controlled dietary intake have been determined. The diet furnished from 0.5 to 1.0 mg of B₆ and from 19 to 32 gm vegetable protein per day by analysis. Supplements of thiamine, riboflavin, niacin and calcium lactate were given. Calories were adequate to maintain weight. A period of 9 days with no pyridoxine supplement was followed by a 9-day period during which 15 mg pyridoxine per day were given. In the first period, the pyridoxine and 4-pyridoxic acid eliminated ranged from 0.85 to 3.7 mg in excess of the pyridoxine intake. 4-pyridoxic acid accounted for approximately 80% of this total excretion. Intestinal synthesis of B₆ in man has been assumed by other investigators but has not been previously demonstrated. Studies of the effect of sulfasuxidine on synthesis are in progress. When the diet was supplemented with 15 mg of pyridoxine HCl daily only 50 to 60% of the total intake was eliminated. 4-pyridoxic acid excretion during this period ranged from 75 to 80% of the total metabolites determined.

Age and calcium utilization. C. M. MCCAY, F. E. LOVRIER (by invitation), C. H. LEE (by invitation) and G. KALL (by invitation). *Laboratory of Animal Nutrition, Cornell Univ., Ithaca, N. Y.* Rats, starting at 38 days of age stored 90% of the calcium from a diet containing 0.3% Ca, until the animals were 110 days old. At 160 days of age these rats stored 55-65% of the Ca. Littermate rats fed a similar diet containing enough oxalate to tie up $\frac{1}{3}$ of the dietary Ca stored only the free Ca until 70 days of age. After this age the utilization improved until 80% of all dietary Ca was stored at 100 days of age. After this Ca storage was only 10% below the controls in spite of the presence of oxalates. If oxalates were fed with meat in the morning and calcium fed as milk 5 hours later, the utilization of Ca was improved during the months shortly after weaning, when assimilation of Ca was difficult. Analogous studies with young puppies gave similar results. In order to determine the effect of the lipids of the diet upon the wastage of dietary calcium in old rats, balances were run upon animals ranging from 450 to 900 days of age. On a weight basis the fat in the diet was varied from 2 to 30%. Under conditions of a constant but marginal daily intake of Ca, the higher fat levels created a greater negative balance. A similar effect was found when the percentage of Ca in the diet was kept constant but the Ca ingested was increased, due to the consumption of more of the low fat food.

Effect of carbohydrate on the nutritive value of heated protein. IVAN J. MADER (by invitation), LAWRENCE J. SCHROEDER (by invitation) and

ARTHUR H SMITH *Dept of Physiological Chemistry, Wayne Univ College of Medicine, Detroit, Mich* A nitrogen balance study was used to determine the possible role of carbohydrate in altering the nutritive value of heated protein. A synthetic diet containing all the known dietary essentials was fed to three adult female dogs. Commercial and 'carbohydrate-free' lactalbumin, heated under various conditions, served as the protein nitrogen sources. All diets fed were isocaloric and daily nitrogen intakes kept at a constant minimal level for each dog. Based on the partition of nitrogen excretion between the urine and feces, no changes in digestibility or nutritive index occurred when the protein was dry-heated at 120°. Using the original lactalbumin as a criterion, significant decreases in both digestibility and nutritive value did occur when the lactalbumin was autoclaved. This decrease in nutritive value was proportional to the severity of heat treatment. Commercial lactalbumin contains large amounts of reducing substances, chiefly lactose. Water extraction allowed almost complete removal of the carbohydrate. The extracted lactalbumin, heated in the same manner as the commercial lactalbumin, was fed in another series of experiments. After removal of the carbohydrate, an increase was observed in both the digestibility and nutritive index of the autoclaved protein. The extracted lactalbumin was reconstituted with lactose with respect to the carbohydrate content of the original protein. Upon heating, the carbohydrate content diminished and severe negative nitrogen balances resulted. Decreased digestibility of the autoclaved lactalbumin, as evidenced by increased fecal nitrogen, accounted for the decrease in its nutritive value. Utilization of absorbed nitrogen remained uniformly high in all cases.

Effect of various degrees of starvation on gonadotrophic and lactogenic content of rat pituitaries **JOSEPH MEITES** and **J O REED** (introduced by **C F HUFFMAN**) *Dept of Physiology and Pharmacology, Michigan State College, East Lansing, Mich* Five groups each of intact and ovariectomized mature rats were put on full, $\frac{1}{2}$, $\frac{1}{3}$, $\frac{1}{4}$ and no-feed regimes. The first 4 groups were killed at the end of 14 days and the 5th group at the end of 7 days. Pituitary gonadotrophic content was determined by injecting an aqueous aliquot of 5 pituitaries from each group into 5 immature female rats during a 5-day period, and weighing the ovaries and uteri on the 6th day. Lactogenic potency was measured by injecting an aqueous aliquot of 2 pituitaries from each group intradermally over the crop glands of 5 pigeons during a 4-day period, and rating the crop responses on the 5th day. Regardless of the degree of starvation in either intact or ovariectomized rats, there was no reduction in pituitary gonadotrophic content. This occurred

despite the marked atrophy of the ovaries and uteri of the intact rats, and the high initial pituitary gonadotrophic content of the ovariectomized rats. Pituitary lactogenic potency was reduced in both intact and ovariectomized rats on the $\frac{1}{2}$, $\frac{1}{3}$ and no-feed regimes, corroborating the report of Meites and Turner (*Mo Agr Exper Sta Res Bul* 416, 1948) that mother rats starved during lactation show a decrease in pituitary lactogenic content. It is concluded that during starvation, pituitary gonadotrophic content is not and pituitary lactogenic content is an accurate index of the amount of hormone released into the blood stream.

Folic acid deficiency and the respiration and pyruvate utilization of duckling erythrocytes and cardiac muscle **O NEAL MILLER** (by invitation), **ROBERT E OLSON** (by invitation) and **FREDRICK J STARE** *Dept of Nutrition, Harvard School of Public Health, and Dept of Biological Chemistry, Harvard Medical School, Boston, Mass* Folic acid deficiency was induced in 4-day-old white Pekin ducklings by feeding a purified diet supplemented with sulfaguanidine. After the onset of anemia, the deficient birds were taken for respiration studies by the direct method of Warburg. Controls fed the deficient diet plus folic acid were tested simultaneously. Blood was drawn from the jugular vein into heparinized syringes, centrifuged, and washed once with cold phosphate-saline at pH 7.4. Slices of heart ventricle from the same birds were prepared. The washed red cells and the cardiac muscle slices were then incubated at 37°C for 1 hr after equilibration in an atmosphere of oxygen. When pyruvate was added as substrate, it was initially present in the medium. The disappearance of pyruvate and formation of lactate was determined chemically and the net pyruvate utilization, i.e., the conversion of pyruvate to non-lactate products, calculated. Data obtained with heart muscle slices are expressed in terms of (Q) while that obtained with the red cell suspensions are expressed in terms of microliters of substrate or oxygen utilized per ml of standard blood (hematocrit = 38). Slices of ventricle from folic acid deficient ducks showed no depression in oxygen consumption below that of controls either with added pyruvate ($QO_2 = 12$) or without pyruvate ($QO_2 = 9$). The conversion of pyruvate to lactate, however, was increased, and the conversion of pyruvate to non-lactate products was decreased approximately 20% by the deficiency. The erythrocytes from deficient birds showed a decrease in both respiration and pyruvate utilization.

Fate of ascorbic acid, dehydroascorbic acid and diketogulonic acid in the animal body **MARY B MILLS** (by invitation), **CHARLOTTE M DAMRON** (by invitation) and **JOSEPH H ROE** *Dept of Biochemistry, School of Medicine, George Washington Univ, Washington, D C* Normal guinea

pigs maintained on a stock diet supplemented with cabbage were injected or fed by stomach tube with pure solutions of ascorbic acid, freshly prepared dehydroascorbic acid, or a calcium diketogulonate. After 1 or 2 hours, the animals were sacrificed and the various tissues analyzed for ascorbic acid, dehydroascorbic acid, and diketogulonic acid. Ascorbic acid was recovered as such to the extent of about 40-50% of the amount administered. Dehydroascorbic acid was recovered to about 30-40% of the amount given and some was reduced to ascorbic acid or converted to diketogulonic acid in the animal body. Diketogulonic acid was recovered to about 25-30% of the amount given and there was no evidence of transformation to the other two compounds being analyzed. Young guinea pigs about 50-100 gm in size were used as a check upon the total recoveries obtained with adult pigs. The animals were injected with the test compound and killed 1 or 2 hours after injection. The animal was skinned and the carcass was ground into a slurry in the Waring blender under 10% stannous chloride solution containing CO_2 . The slurry was made to a satisfactory volume with 5% H_2PO_4 and analyzed for total ascorbic, dehydroascorbic and diketogulonic acids. Recoveries were a little higher with ascorbic and dehydroascorbic acids, and a little lower with diketogulonic acid, than those obtained with adult animals. The data obtained with young guinea pigs confirmed the observations on the fate of the three compounds in adult animals.

Essentiality of vitamin B_{12} for the baby pig, with preliminary quantitative data. A. L. NEUFMANN (by invitation), MARIAN F. JAMES (by invitation), J. L. KRIDER (by invitation) and B. CONNOR JOHNSON, *Dept. of Animal Science and Division of Animal Nutrition, Univ. of Illinois, Urbana, Ill.* Isolated soybean protein 'synthetic milk' fed to baby pigs was improved by supplementation with a vitamin B_{12} concentrate, both from the standpoint of growth and of physiological well-being. Forty-two micrograms of vitamin B_{12} /kg dry matter in the diet produced the maximum growth response and improved hematopoiesis. Grossly, the vitamin B_{12} deficient pigs were extremely irritable, sensitive to touch, sluggish in their movements, and a number of the pigs were unable to stand upon their rear legs. All the vitamin B_{12} pigs on the two highest levels fed grew better and showed none of the above symptoms. One group fed a manure factor concentrate showed the same gross symptoms as the vitamin B_{12} deficient pigs but grew almost as rapidly as those fed the higher levels of vitamin B_{12} . Hematopoiesis was rather severely strained in all the pigs fed the B_{12} deficient diets as well as in those fed the manure factor, as indicated by bone marrow and blood studies, even though 0.4 gm pteroylglutamic acid

was included per kg dry matter in the diet. Histological information on the nerves, gastro intestinal tract and other organs will be presented, as well as data on the blood and bone marrow condition. The growth effect of the injection of crystalline vitamin B_{12} in baby pigs fed the same basal diet will also be reported as well as some preliminary data on a pteroylglutamic acid vitamin B_{12} relationship.

Biochemical studies on primigravidae on self-selected diets differing greatly in nutritive value. HILLY OGDEN, BLANCIE BLUM-SHIFT (by invitation) and THIRMA PORTER, *Dept. of Home Economics, Univ. of Chicago, Chicago, Ill.* Primigravidae, living at home on self-selected diets, were studied for 10 day periods during each of the last 5 months of pregnancy. All food eaten during the first 7 days of each period was weighed and samples were saved for thiamine, riboflavin, niacin, nitrogen and tryptophane analyses. Intakes of other nutrients were calculated. Complete collections of excreta were made during the 7 days and aliquots analyzed. On the 7th, 8th, 9th and 10th days, after identical breakfasts, 1 hour urine specimens were collected. That on the 7th day served as a control and those on the 8th, 9th and 10th days as a measure of riboflavin, niacin and thiamine test doses, respectively, which were given with breakfast. The average daily intakes of the different subjects ranged as follows: calories, 400 to 2300, protein, 10 to 100 gm, Ca , 0.1 to 2.7 gm, iron, 4 to 150 mg, vitamin A, 600 to 16,600 IU, vitamin D, 500 to 3000 IU, ascorbic acid, 6 to 160 mg, thiamine, 0.35 to 19 mg, riboflavin, 0.23 to 20 mg, niacin, 4 to 86 mg. High intakes of vitamins and minerals were due for the most part to voluntary supplementation, the low, to poor dietary habits. The proportion of the thiamine intake which was excreted was relatively constant whereas that of riboflavin varied directly with the intake. Test dose returns of both thiamine and riboflavin were very low on low intakes. Excretion of niacin metabolites on low intakes usually exceeded the amount of niacin ingested, sometimes by as much as 100%. Niacin test dose returns of these subjects also tended to be high. Consistently negative nitrogen balances were found only when the average daily protein intakes were less than 30 gm.

Metabolism of radioactive acetate and pyruvate by cardiac muscle from normal and pantothenic acid-deficient ducklings. ROBERT E. OLSON (by invitation) and FREDRICK J. STARE, *Dept. of Nutrition, Harvard School of Public Health, and Dept. of Biological Chemistry, Harvard Medical School, Boston, Mass.* Acetate labeled in the carboxyl with C^{14} and 2 isomers of pyruvate, labeled respectively in the carboxyl group and the alpha carbon atom were synthesized. Slices and dilute homogenates of heart ventricle from normal and

pantothenic acid-deficient ducklings were incubated with these substrates in Warburg flasks. The gas phase in the slice experiments was O_2 , in the homogenates, air. Total pyruvate disappearance and lactate appearance were determined chemically and the conversion of pyruvate to non-lactate products expressed as net $-Q_{\text{pyruvate}}$. The rate of oxidation of the labeled carbon atom in the respective substrates was determined by counting the $C^{14}O_2$ trapped in the center well. All substrates were tipped in from side arms after equilibration and gave a final concentration of 5 mM/l. The following representative data were obtained in a series of normal ducklings

	Net -Q Pyruvate	$QC^{14}O_2$ Pyruvate Car- boxyl	$QC^{14}O_2$ Pyruvate α -carbon	$QC^{14}O_2$ Acetate Car- boxyl
Slice	5.2	2.8	1.8	1.1
Homogenate	3.8	3.7	2.2	0.7

These data indicate that in the slice, but not in the homogenate, considerable amounts of added pyruvate are metabolized to non-lactate products without the loss of CO_2 . The oxidation of added pyruvate in slices of ventricle from pantothenic acid-deficient ducks was essentially normal. In homogenates of deficient ventricle, however, there was a marked depression in pyruvate oxidation. The oxidation of the alpha-carbon atom was reduced more than the rate of decarboxylation. The oxidation of acetate was reduced in both slices and homogenates.

Relationship of riboflavin deficiency to adrenicortical function as shown by carbohydrate metabolism in anoxia. BLUEBELL READE (by invitation) and AGNES FAY MORGAN *Laby of Home Economics, Univ of California, Berkeley, Calif*. Young rats were subjected fasting to anoxic anoxia after 1, 3, 5, 8 and 12 weeks on riboflavin-deficient diet. After the test period of 24 hours, blood sugar, liver glycogen and muscle glycogen were determined. Comparable animals kept at sea-level under the same conditions were similarly examined, as were rats given the same diet plus riboflavin under anoxia and at sea-level. The total carbohydrate content of the deficient animals was greater than that of those on normal diet through the third week but this decreased rapidly below the normal fasting level in the 5th to the 12th week. Injection of 0.1 mg riboflavin immediately before, or of 2 ml cortin in the 24 hours just preceding the test period, restored the carbohydrate-producing capacity of the deficient rats. Injection of desoxycorticosterone, 5 mg in oil, in the 48 hours preceding the test had little effect. Adrenalectomized rats on the control diet were not enabled to increase their carbohydrate content by injections of

0.1 mg riboflavin. Pair-fed normal controls exhibited increased gluconeogenetic capacity above that of the full-fed positive controls. The behavior of the deficient animals corresponded with that described by Selye as 'alarm reaction' during the first part of the deficiency and 'collapse' in the latter part. This may indicate that the state of riboflavin deficiency involves increased adrenicortical activity at first followed by adrenal cortex exhaustion. Either the complete cortical extract, cortin, or riboflavin substituted for, or restored adrenicortical function.

Relation of age to ascorbic acid metabolism in guinea pigs. MARY ELIZABETH REID *Expt'l Biol and Med Inst, N I H, Bethesda, Md*. Two groups of inbred male guinea pigs, one slightly over 2 months of age and the other ranging from 1 to over 3 years, were fed a commercial pelleted diet, supplemented with 1% of powdered yeast, vitamin E, and a small amount of fresh carrots. Daily intraperitoneal injections of ascorbic acid (5 mg/100 gm) were given for at least 3 weeks before the experiments were made. At periods varying from $\frac{1}{2}$ to 24 hours after the usual daily injection, blood samples were taken, following which the animal was killed. The liver, kidneys, spleen, adrenals, and the contents of the gastrointestinal tract were removed, assayed for ascorbic acid and the responses of the young and old groups to the experimental procedure were compared. The basal, pre-injection levels in the blood and liver were found to be lower in the young animals. Following administration of the vitamin, the levels in both groups rose rapidly in the blood, kidneys, and liver but showed no consistent variations in the adrenals and spleen. The rapidity of increase and the subsequent return to basal levels varied from organ to organ. The young animal showed a more rapid increase in the liver content but it also decreased earlier. The kidneys of the young animal maintained a high level much longer than the older group suggesting the possibility of greater tubular reabsorption. Assay of the contents of the digestive tract yielded no evidence of more rapid excretion and destruction in the younger group. It is known that the dietary requirements of vitamin C for young and old animals is approximately the same per unit of weight. However, these present results together with the results of earlier studies suggest that the younger animal actually stores and uses more vitamin C than the older animal. The evidence indicates that there is greater wastage in the older animals.

Germ-free chick nutrition development on synthetic-type diets. J. A. REYNIERS (by invitation), T. D. LUCKEY (by invitation), P. C. TREXLER (by invitation), R. F. ERVIN (by invitation), M. WAGNER (by invitation), H. A. GORDON (by invitation) *Germ-Free Life Division, LOBUND,*

Notre Dame, Indiana, R A BROWN, G J MAN-
NERING (by invitation) and C J CAMPBELL (by
invitation) *Research Labys, Parke, Davis & Co,*
Detroit, Mich The role of intestinal micro-
organisms in nutrition research is of considerable
theoretical and practical importance. Attention has
been largely directed toward the possible synthesis
of vitamins by the intestinal flora. This report
records a study to determine whether germ-free
chicks require a dietary source of unknown vita-
mins. Chicks were obtained and reared germ-free
according to the method of Reyniers *et al*
(LOBUND Reports, No 2). The animals were
fed autoclaved water and synthetic-type diets
(with and without liver and yeast) for 4 to 9 weeks.
Bacteriological tests (microscopic and cultural)
showed the chicks to be free from all demonstrable
living microorganisms. The diets used were ade-
quate as shown by chemical and biological methods.
The percentage of nutrients lost during auto-
claving at 122°C for 25 minutes is as follows:
thiamin, 90, riboflavin, 15, niacin, 3, pantothenic
acid, 45, pyridoxine, 31, biotin, 0, choline, 12,
folic acid, 64, vitamin A, 19, vitamin E, 46, vita-
min K, 38, lysin, 0, tryptophane, 8. Feather de-
velopment was excellent in the germ-free chicks.
When 4 weeks old, they were somewhat heavier
than the control chicks fed the same diet in 4 of
7 experiments. The presence of liver and yeast
powder or fresh liver extract did not significantly
increase growth rate or hemoglobin formation.
Hematopoiesis was normal except for some devia-
tions in the white blood cell picture. Perosis oc-
curred in some of the germ-free birds. The results
indicate that the nutritional requirements of germ-
free chicks are similar to those of the controls.
Therefore if the search for unknown vitamins is to
be carried further, it is evident that either the
diets or the animals used will have to be changed.

Amino acids and vitamin B₁₂ as supplements to
plant proteins for growing chicks. L R RICHARD-
SON, L G BLAYLOCK (by invitation), H L
GERMAN (by invitation) and R M SHERWOOD
(by invitation) *Texas Agricultural Experiment*
Station, College Station, Texas Amino acids and
vitamin B₁₂ were investigated as supplements to
commercial cottonseed meal low in gossypol and
to soybean meal for growing chicks. The protein
concentrates were added in quantities to supply a
total of 20% protein in the diet. One typical diet
contained corn meal, protein concentrate, steamed
bone meal, manganese sulfate and all vitamins re-
quired by growing chicks. Another contained cere-
lose instead of grain and mineral mixture instead
of bone meal. The experimental period was 4 weeks.
Chicks receiving the corn cottonseed meal diet
weighed an average of 150 gm while those receiving
the same diet plus 0.5% of L(+) lysine weighed
250 gm. When the basal diet contained 3% sardine

meal the chicks weighed an average of 206 gm
without and 252 gm with lysine. The further addi-
tion of 0.1% of DL tryptophan and 0.2% of DL-
methionine to the cottonseed meal sardine meal
diet increased the weight to 282 gm. A vitamin B₁₂
concentrate was investigated as a supplement to
cottonseed and soybean meals. Three chicks re-
ceiving a cerelese cottonseed meal diet supple-
mented with 0.8% of DL-lysine in addition to
tryptophan and methionine weighed an average of
228 gm while 3 receiving the same diet plus 40
Mcg/kg of vitamin B₁₂ weighed an average of
300 gm. Two groups of 17 chicks each receiving
the cerelese soybean meal diet weighed an aver-
age of 250 gm without and 285 gm with vitamin
B₁₂.

Metabolism of p-aminobenzoic acid and some
of its conjugates by the rat. THOMAS R RIGGS
(by invitation) and D MARK HEGSTED *Dept of*
Nutrition, Harvard School of Public Health, Dept
of Biological Chemistry, Harvard Medical School,
and Dept of Biochemistry and Nutrition, Tufts
College Medical School, Boston, Mass The acetyla-
tion of p-aminobenzoic acid (PAB) in rats has been
shown to be diminished in pantothenic acid de-
ficiency (*J Biol Chem* 172: 539, 1948) and also to
be influenced by deficiencies of thiamine and ribo-
flavin and by the amount of PAB administered
(*J Biol Chem* in press). Since PAB can be con-
jugated at the carboxyl group (with glycine) as
well as at the amino group, it appeared that the
extent of acetylation of the amino group would
probably be influenced by the extent of conjuga-
tion of the carboxyl group. Determinations of
PAB, p-aminohippuric acid (PAH), and their
respective acetyl derivatives were carried out on
the urines collected for 24 hours after intraperi-
toneal injection of various amounts of these
compounds. Results showed interconversion among
the 4 substances, so that all four appeared in vary-
ing proportions after PAB, PAH, or acetyl-PAH
were injected. No acetyl-PAH was found after
acetyl-PAB was given. The latter observation in-
dicated that conjugation at the carboxyl end was
prevented once conjugation had occurred with the
amino group. Whenever the extent of acetylation
of PAB was diminished by nutritional deficiency
or by increasing the dose, conjugation at the car-
boxyl group was reciprocally increased so that the
total proportion conjugated at one position or the
other remained constant. The extent of acetylation
as affected by pantothenic acid deficiency and by
glycine supply (modified by preadministration of
benzoic acid or of glycine) has been studied after
administering PAB, PAH, and sulfanilamide.

Availability of pantothenol and calcium panto-
thenate in relation to food intake. S H RUBIN,
L DREKTER (by invitation), M E MOORE (by
invitation) and R PANKOFF (by invitation)

Nutrition Laboratories, Hoffmann-La Roche Inc., Nutley, N J Previous work in this laboratory (*J Nutrition* 35 499, 1948) has indicated that the availability of calcium or sodium pantothenate to humans at elevated dosage levels is depressed by food intake to a considerably greater extent than is that of panthenol. Further study of this effect in rats by an excretion technique (*J Am Pharm Assn* 37 498, 1948), at dosage levels within the linear working range of 1 to 10 mg/kg permits the following conclusions to be drawn: 1) During *ad libitum* feeding, panthenol is approximately 200% (range 193-267%) as available as equivalent amounts of calcium pantothenate at all doses fed (A value of 100% denotes equal availability). 2) Starvation for 24 hours (postabsorptive state) enhances the absolute urinary excretion of pantothenic acid in response to the 'loading' test doses at all levels, especially after calcium pantothenate. 3) The relative availability of panthenol and calcium pantothenate in this postabsorptive state is of the order of 220, 140 and 116% at the respective dosage levels of 10, 5 and 2 mg/kg. 4) In contrast with Silber's results with calcium pantothenate in dogs (*Arch Biochem* 7 329, 1945), the post-absorptive urinary responses are not profoundly affected by the administration of food with the dose. However, a significant increase in fecal pantothenic acid does occur when 10 mg/kg of calcium pantothenate is given with food, but not with 5 or 2 mg/kg. These results confirm and extend the previous findings in humans that the availability of panthenol is better than that of calcium pantothenate under conditions of higher dosage and *ad libitum* feeding, and is more nearly equal in the postabsorptive state at lower doses.

Vitamin A requirement of the turkey poult fed a purified diet WALTER C RUSSELL, M WIGHT TAYLOR and ROBERT VAN REEN (by invitation) *Dept of Agricultural Biochemistry, New Jersey Agr Exp Sta, Rutgers Univ, New Brunswick, N J* Poults fed a purified diet in which protein was supplied as casein and gelatin and vitamin A as the acetate, required at least 3000 IU of the vitamin per kg of diet (1360 IU/lb) for normal growth. 5000 IU/kg of diet (2270 IU/lb) was required to prevent all external signs of the deficiency, such as ruffled plumage, staggering gait, and cloudiness of the inner eyelid. After 6 weeks on experiment, no liver storage was found even at a level of 6000 IU/kg of diet (2720 IU/lb). In all of the groups receiving the purified diet, the lining of the esophagus and crop presented a white furrowed appearance. Histological studies showed the development of very large cells in the epithelial layer with considerable exfoliation. It is not known whether this condition was the result of inadequate vitamin A or due to the physical character of the purified diet. On a practical mash the furrowed condition did not occur. For normal growth and

external appearance, 5000 IU of vitamin A per kg of diet (2270 IU/lb) are considered adequate.

Metabolism of tryptophan labeled with radioactive carbon DATTAREYA RAO SANADI (by invitation) and DAVID M GREENBERG *Division of Biochemistry, Univ of California Medical School, Berkeley, Calif* DL-tryptophan (200 mg) labeled with C^{14} at the beta-carbon was administered subcutaneously to rats made diabetic with phlorhizin. From the subsequent 24-hour urine collection, glucose was obtained as the phenylglucosazone which was then converted to the phenylglucosotriazole by treatment with cupric sulphate. The ketone bodies were obtained first as the mercury acetone complex and then as 2,4-dinitrophenylhydrazone. The glucose exhibited radioactivity but the acetone was inactive. The activity in the CO_2 , obtained by treating the urine with urease, was too low to explain the activity in the glucose as being entirely due to CO_2 fixation. Upon degrading the glucosotriazole by oxidation with sodium periodate it was found that approximately one-fourth of the activity of the glucose molecule was in carbon 6 and another one-fourth in carbons 4 and 5. From the present knowledge of the products of tryptophan metabolism and from the isotopic distribution in glucose it appears that a two carbon unit with C^{14} in the alpha-position is formed from tryptophan and is incorporated in the tricarboxylic acid cycle directly, but this two carbon unit does not form acetoacetate in the diabetic animal. Oxidative deamination and subsequent oxidative decarboxylation of kynurenine would yield the compound $NH_2 \cdot C_6H_4 \cdot CO \cdot C^{14} \cdot H_2 \cdot COOH$. In the phlorhizinised animal, this compound may break down to anthranilic acid and a two carbon derivative. The formation of such a derivative has been confirmed by giving a normal rat $10 \mu c$ (about 12 mg) of the radioactive tryptophan together with p-amino-benzoic acid. The excreted p-acetylamino-benzoic acid was radioactive.

Vitamin nutriture and tryptophan metabolism in man HERBERT P SARETT and GRACE A GOLD-SMITH *Nutrition Research Lab, Tulane Univ School of Medicine, New Orleans, La* Further studies have been conducted on the excretion of nicotinic acid and tryptophan compounds by subjects maintained on a wheat diet, which provides about 40 gm of protein and 6 mg of nicotinic acid per day (*J Biol Chem* 167 293, 1947). The addition of DL-tryptophan to this diet leads to an increase in excretion of N'-methylnicotinamide which is not affected by supplementing the diet with 50 mg of nicotinamide per day or with a combination of the other B vitamins. The high level of nicotinamide does not decrease the conversion of tryptophan to nicotinic acid compounds. The addition of DL-tryptophan to the diet leads to an increased excretion of two other compounds, one has tryptophan-like activity for *Lactobacillus*

containing ethanol extracted casein (55-60°C for 72 hours) in place of the soybean protein and methionine but otherwise the same components. These rats were bred and their young were fed the soybean ration. On the soybean ration the 6 weeks post weaning weight gains were for the parent generation, males (12) 172 gm \pm 15.4 (SD), females (26) 123 gm \pm 8.4, for the F₁ generation males (31) 175 gm \pm 13.6, females (32) 112 gm \pm 12.5, not significantly lower than those on a casein containing ration. To date from 46 pregnancies there were 3 probable resorptions and 43 litters with 14 dead and 263 live young. Of the latter 14 whole litters and 101 young failed to survive the third day after birth. Of 162 rats surviving the third day, 153 (94.4%) were weaned at 21 days with a mean weight of 35.6 gm. The cause of the high early mortality is not known but in no case could it be ascribed to failure of the young to obtain sufficient milk since lactation performance in all cases was excellent.

Nutrition of women during normal and abnormal pregnancy in Panama and the Canal Zone. NEVIN S. SCRIMSHAW (by invitation), MARTHA J. THOMASON (by invitation), ROBERT P. BAYS (by invitation) and ESTELLE E. HAWLEY. *Dept. of Obstetrics and Gynecology, Univ. of Rochester, School of Medicine and Dentistry, Rochester, N. Y.* The dietary intake of 308 pregnant women representing 5 distinct economic or racial groups in Panama and the Canal Zone was studied by diet interviews. The incidence of pre-eclampsia for each of these groups in over 12,000 deliveries did not indicate a true tendency for this condition to occur more frequently in groups with a lower average nutritional intake. Furthermore, when the diet histories of 75 women with pre-eclampsia were analyzed, there was no reasonable statistical possibility that their diets were poorer than those of their own racial or economic group in general. Blood samples from 242 pregnant women representing the above 5 groups and 2 interior villages were analyzed for total protein, vitamin A, carotene, free and total riboflavin and alkaline phosphatase. Alkaline phosphatase showed little variation with diet and economic status, increased with gestation, and appeared to be lower in the white Americans than in the groups with greater skin pigmentation. Of the 5 groups studied in detail, the one with the lowest estimated protein intake had significantly higher serum total protein values than did the others. Women in the villages of La Gartarita and Parita had serum total protein values considerably above those reported for the United States or the Canal Zone, despite a lower protein intake. However, the women from these villages average much lower in serum carotene values. These and other biochemical data are discussed in relation to the diet histories and the clinical states represented.

Vitamin C deficiency in the ringtail monkey

JAMES H. SHAW. *School of Dental Medicine, Harvard Univ., Boston, Mass.* The ringtail or brown capuchin monkey (*Cebus fatuellus*) is of interest in dental investigations because of its relatively small adult size and its tractability. Investigations of the ringtail monkey's need for various nutrients have been conducted in this laboratory to determine how similar its nutritional requirements were to those of man and the rhesus monkey. In a study of whether vitamin C was required in the diet, 10 young adult ringtails were offered a purified diet which was believed to be adequate in all known nutritional essentials but deficient in ascorbic acid. These monkeys maintained their original body weight, normal appearance and activity reasonably well for periods of 12 to 20 weeks when the classical manifestations of a chronic state of scurvy began to appear. The deficiency was characterized by rapidly increasing debility, moderate decreases in body weight, extreme tenderness of the joints and extensive hemorrhages over large areas of the head, arms and legs. Oral lesions were particularly severe and rapidly progressing, with widespread hyperemia, hypertrophy and necrosis of the gums accompanied by extensive destruction of alveolar bone and sloughing of the teeth. The injection or oral administration of crystalline ascorbic acid soon after gross manifestations of scurvy appeared resulted in extremely slow but complete recovery. Administration of ascorbic acid at later stages was ineffective. Comparable ringtail monkeys offered the same diet supplemented with adequate ascorbic acid were maintained in normal health throughout the experimental period.

Nutritional evaluation of the iso-oleic acids Elaidic, petroselinic and petroselinelaidic acids. HENRY SHERMAN (by invitation), LAURA M. CAMPBELL (by invitation), WILLY LANGE (by invitation) and ROBERT S. HARRIS. *Nutritional Biochemistry Lab., Massachusetts Inst. of Technology, Cambridge, Mass.* Male weanling rats were fed diets consisting of 20% casein (Labco) 61.5% corn starch, 14% fat, 4% salts and liberal quantities of 14 vitamins. The fat in each of four diets varied as follows: olive oil randomized with an equal quantity of a) trielaidin (Δ^9_{10} trans), b) tripetroselinin ($\Delta^{6,7}$ cis), c) tripetroselinelaidin ($\Delta^{6,7}$ trans) or d) pure olive oil. Olive oil was used because it contains 80-85% Δ^9_{10} cis oleic acid. Distilled water and diets were supplied *ad libitum* for 42 days and rats were weighed biweekly. Before sacrifice data were taken for body surface calculations. The fatty acids extracted from the various groups of pooled carcasses were analyzed for iodine value, neutralization value and arachidonic acid content. The results indicate that rats on a) grew less rapidly, had smaller body surface and the carcass fat contained less arachidonic acid than rats fed d). Rats fed b) grew better than those fed c) and their carcass fat contained more arachidonic acid. Rats fed c) grew less and de-

posited less arachidonic acid than those fed *d*. Rats fed *b* grew as well as those fed *d*, but their carcass fat contained less arachidonic acid. *Cis* isomers of $\Delta^{6,7}$ and Δ^{11} oleic acids appear to be more nutritious than their respective *trans* isomers, and the $\Delta^{6,7}$ *trans* isomer more nutritious than the Δ^{10} *trans* isomer.

Influence of dietary protein and fat on the toxicities of trinitrotoluene (TNT) and 2,4-dinitrotoluene (DNT) for the rat MAURICE E. SHILS and LEONARD J. GOLDWATER (introduced by C. G. KING) *Division of Industrial Hygiene, School of Public Health, Columbia Univ., New York, N. Y.* Conflicting reports have been published on the effect of diet on the closely related compounds TNT and DNT. A study was made of the comparative effects of protein, fat and carbohydrate on both TNT and DNT toxicity by feeding to rats diets which contained low and high levels of each of these nutrients. The intake of each toxic compound was kept equal for each group. The growth-inhibiting effect of DNT (fed initially at 300 mg % level in low fat diets and 600 mg % level in high fat diets) was less pronounced when the fat content of the diet was increased from 6.0% of the dietary calories to 60.0%. The beneficial effect of fat was noted on both the high and low protein diets. Variations in fat intake had little effect on the growth of rats on low protein diets receiving TNT (fed initially at 150 mg % level in low fat diets and 300 mg % level in high fat diets). The DNT and TNT-treated rats and their controls on the diets containing protein equivalent to 25% of the calories all grew at better rates than similarly treated animals on diets containing protein at half this level. The treated groups grew less than the controls but since the absolute increases in weight of each of these three groups in response to the higher protein intake was of the same order of magnitude, it appears that protein has little or no specific effect in overcoming the growth inhibition of the toxic compounds. However, at the higher protein intake there were no deaths in the DNT-poisoned rats as compared to a high mortality on the low protein diets.

Radioactive phosphorus in the study of caries-resistant and susceptible albino rats RAY L. SHIRLEY (by invitation) and CARL A. HOPPERS *Kedzie Chemical Laboratory, Michigan State College, East Lansing, Mich.* In 1931-32 Hoppers et al. demonstrated that dental caries could be developed in rats by feeding diets containing coarsely ground corn or rice. Subsequently, a genetic study was undertaken with Hunt in which two distinct strains of albino rats were developed that vary markedly in their susceptibility to dental caries. The present study was undertaken to compare some chemical constituents of the teeth of these developed strains. The caries-resistant and susceptible rats have been demonstrated in this investigation not to differ significantly in the following respects: a) weight of

teeth, b) % ash, c) % phosphorus on the basis of dry weight, and in the ash, d) rates of deposition and removal of radioactive phosphorus in the teeth when the isotope was injected into the peritoneal cavity, e) occurrence of radioactive phosphorus in the teeth of offspring of females that were injected intraperitoneally with the isotope during pregnancy, f) the *in vitro* adsorption of the isotope by the teeth from aqueous solution. The data obtained indicates that the differences in susceptibility to dental caries of the rats used in this study are not due to differences in the phosphorus metabolism of the teeth.

Determination of vitamin-D activity in the rat by means of radioactive phosphorus R. H. SNYDER (by invitation), HAROLD J. EISNER (by invitation) and HARRY STEENBOCK *Dept. of Biochemistry, Univ. of Wisconsin, Madison, Wis.* In the course of an investigation of the mechanism of vitamin-D activity it was found that the response of the rachitic rat to the administration of radiophosphorus could be directly quantitated to small amounts of vitamin D. The vitamin was administered orally in a single dose a short time before the tracer was given intraperitoneally. An external 'count' of the forepaw of the anesthetized rat was chosen as the simplest technique of measurement. A relatively simple apparatus was designed for placing a rat in a reproducible position under a mica end-window G. M. tube for counting. Though a difference in the external count due to D developed almost immediately after administration of the P^{32} it was found that the best time to take the counts was from 8 to 10 days later. The counts of groups with different doses were compared with a control group which received no vitamin D, or with a group receiving a known dose, and/or with previously established reference curves. Although no attempt was made to develop the method exhaustively, the collected data indicate quite definitely that it is possible to determine vitamin D accurately over a range of from one-half to 40 units with groups of 10 animals each. Good results were obtained using a preparatory period as short as 14 days on ration 2965.

An unidentified dietary factor concerned in human growth SELMA E. SNYDERMAN (by invitation), ROSA LEE NEMIR (by invitation) and L. EMMETT HOLT, JR. *Dept. of Pediatrics, New York Univ. and the Children's Medical Service, Bellevue Hospital, New York City.* In the course of studies designed to measure the requirements for B vitamins we have had occasion to feed a number of infants on purified diets. The diets were constructed from vitamin-free casein, crisco and a special de-vitaminized dextrimaltose; they provided 15% of the calories as protein, 35% as fat and 50% as carbohydrate. This was supplemented by a mineral mixture, a fish oil concentrate to supply fat-soluble vitamins and a mixture of water-soluble vitamins containing ascorbic acid, thiamine, riboflavin, nico-

tinamide, pyridoxine, pantothenic acid, inositol, choline and p-amino benzoic acid. Biotin and pteroyl glutamic acid were added in more recent studies. Only one component of the vitamin mixture, that which was being studied, was varied at a time. Barring a few intercurrent infections, the infants remained in good health and gained weight normally. It was observed, however, that if the experimental diet was continued for more than 5 to 6 months a cessation of weight gain would occur regularly, although the infants still gave every appearance of health. Attempts to supplement the experimental diet with amino acids or by increasing the quantities of B vitamins failed to restore weight gain. The addition of Brewer's yeast caused resumption of weight gain in from 5 to 23 days, but the weight gain was in some instances subnormal. Liver preparations have given inconclusive results. Return to natural food caused resumption of normal weight gain at once.

Influence of dietary proteins on the urinary excretion of amino acids by human subjects. BETTY F STEELE (by invitation), MAY S REYNOLDS (by invitation) and C A BAUMANN *Dept of Biochemistry and Home Economics, College of Agriculture, Univ of Wisconsin, Madison, Wis*. Two separate controlled diet studies were made. In the first study 3 adults ingested diets containing 25, 50 and 100 gm of protein/day for periods of 6 days. The principal sources of protein in this study were eggs and cottage cheese. In the second experiment 4 subjects consumed diets containing 25, 100 and 200 gm of protein/day for 6 days. The main sources of protein were ground beef, white bread and milk. Twenty-four hour samples of urine were collected and analyzed microbiologically for 18 amino acids. Both 'free' and 'total' amino acids were determined in the urines, and the amino acid contents of the diets ingested were also determined. Large amounts of 'free' histidine and cystine were present in the urine samples. Glutamic acid, glycine, histidine, aspartic acid, serine, proline, threonine, tryptophan and methionine were present in fairly large quantities in samples of hydrolyzed urine. Increasing the protein intake four fold caused roughly a 2-fold increase in the excretion of alpha amino nitrogen. Of those amino acids present in large amounts, histidine and threonine excretion appeared to bear a positive relationship to intake. Glutamic acid, aspartic acid, tryptophan and methionine excretion seemed to be independent of the amount of the amino acid ingested. Many of the other amino acids were present in urine in only low concentrations.

Ascorbic acid metabolism of older adolescents. CLARA A STORVICK, BESSIE L DAVEY (by invitation), RUTH M NITCHALS (by invitation), RUTH E COFFEY (by invitation) and MARGARET L FINCKE *Nutrition Research Laboratory, Agr Exp Sta,*

Oregon State College, Corvallis, Ore. In 1946-47, 4 levels of ascorbic acid intake were studied during periods of 7 days each, and in 1947-48, 3 levels were studied during periods of 10 days each. The subjects, 8 girls and 8 boys, were 16 to 19 year-old Freshmen. During the first year, 1946-47, the subjects received the following for periods of 7 days each: 1) their usual diets, 2) a daily supplement of 200 mg of crystalline ascorbic acid in addition to the ascorbic acid in the food, 3) the total daily ascorbic acid intake as recommended by the National Research Council, and 4) 10 mg of ascorbic acid less than they received during the third period. During the second year, 1947-48, the subjects received the following for periods of 10 days each: 1) a daily supplement of 200 mg of crystalline ascorbic acid in addition to the ascorbic acid in the food, 2) the total daily ascorbic acid intake as recommended by the National Research Council, and 3) 10 mg less than they received during the second period. The recommended allowance of the National Research Council did not maintain mean plasma values as high as their respective means during the saturation period when they received 200 mg supplements of crystalline ascorbic acid. A decrease in ascorbic acid intake to 10 mg less than the recommended allowance resulted in a statistically significant decrease in plasma ascorbic acid concentration for only 2 of the 8 girls and for 1 of the 7 boys (values for one boy were not included because of illness).

Role of pyridoxine in economy of food utilization. BARNETT SURE and LESLIE EASTERLING (by invitation) *Univ of Arkansas, Fayetteville, Ark*. Twenty four male and 24 female albino rats were depleted of pyridoxine. In 26 to 59 days 15 pairs developed dermatitis which were used for this study which was conducted in pairs. In each pair the positive control, which received 25 μ g pyridoxine daily, was allowed the same amount of food consumed by its litter mate, which received no pyridoxine. The average results were as follows,—P representing the pathological or pyridoxine-deficient animals, and C the litter mate controls which received pyridoxine. Food consumption P, 325 gm, C, 317 gm, gains in body weight P, 37.5 gm, C, 77.3 gm, gains in weight/100 gm food intake, P, 11.4 gm, C, 25.2 gm, changes in chemical composition of body gains: fat P, -0.24 gm, C, +8.1 gm, protein P, 9.3 gm, C, 17.5 gm, ash P, 2.2 gm, C, 3.4 gm.

Synthesis of amino acids in the rumen. W E THOMAS (by invitation), J K LOOSLI, FENT H FERRIS (by invitation), HAROLD H WILLIAMS and L A MAYNARD *Depts of Animal Husbandry and Biochemistry and Nutrition, Cornell Univ, Ithaca, N Y*. Growing goats and sheep were fed purified diets that contained, at a 10% protein-equivalent level: 1) casein, 2) casein hydrolysate plus trypto-

phan, and 3) urea as the only source of nitrogen. Normal growth was obtained with diets 1) and 2) and $\frac{1}{2}$ normal with diet 3). Biological values, determined from nitrogen balance studies, were higher for diets 1) and 2) than for 3). The content of 10 amino acids (those that have been shown to be essential for rats) of the feeds and of rumen and excreta samples was determined microbiologically. The results showed that the essential amino acid content of the rumen material was similar for the urea and casein diets. Although traces of amino acids were found in the purified diet where urea was given as the sole source of nitrogen, the amounts in the rumen per gram of total nitrogen were 9 to 20 times that ingested. Furthermore the daily loss of each of the essential amino acids in the excreta more than accounted for the traces found in the purified diet. These data together with the surprising growth of the animals indicated a remarkable synthesis of amino acids from urea nitrogen by rumen organisms.

Nutrition of *L. Bifidus*, predominant intestinal organism of the breast fed infant. R. M. TOMARELLI (by invitation), R. F. NORRIS (by invitation), PAUL GYORGY, *Univ. of Pennsylvania Hospital, Philadelphia*, J. B. HASSINEN (by invitation) and F. W. BERNHART (by invitation), *Wyeth, Inc., Mason, Mich.* In the isolation of strains of *bifidus* anaerobically from breast fed infant stools it was observed that better growth was had if milk were added to the conventional liver infusion medium. Skimming did not decrease activity. An ether soluble fraction was isolated whose activity could be replaced by Tween 80, methyl oleate, oleic, linoleic or vaccenic acid. The exact nature of the factor in milk is unknown at present. The various strains of *bifidus* could be grown on a synthetic medium containing acetate buffer if milk or oleate were added. Human milk contains approximately 4 times as much activity as cow's milk. With citrate as the buffer, milk, but not oleate, yielded growth. Citrate proved toxic to the organism, the toxicity being neutralized by the addition of Ca of the milk. The strains varied in their sensitivity to citrate toxicity. Citrate toxicity is the result of the binding of essential minerals. Mn appears to be the only mineral affected, since removal of Mn but not of Fe nor Mg from the acetate medium decreased growth. The utilization of glucose and especially of lactose was influenced by the autoclaving of the carbohydrate with the medium. Unautoclaved lactose was not utilized even after 64 hour incubation. When sterile milk was used as a source of unautoclaved lactose, growth was had in 40 hours indicating the presence of a factor in milk that rendered lactose available. Several strains of *bifidus* were isolated that required unknown growth factors in enzymatic digest of casein.

Relation of factor in a methanol extract of liver and pteroylglutamic acid in mink. S. B. TOVE (by invitation) and C. A. ELVEHJEM, *Dept. of Biochemistry, College of Agriculture, Univ. of Wisconsin, Madison, Wis.* The previously reported (*Proc. Soc. Exp. Biol. & Med.* 62: 169, 1946) pteroylglutamic acid (PGA) deficiency syndrome in mink can be relieved by the administration of a methanol extract of fresh liver (fed at a level of 1 ml/100 gm ration) which contains only 2 γ PGA/ml. If this level of extract is added to the ration from the start, symptoms of a PGA deficiency fail to occur. The addition of 0.3% of a crude antagonist (7-methyl PGA) to a diet containing 0.1 mg PGA/100 gm ration resulted in rapid loss of body weight and hemoglobin. When the inhibitor is added to a ration containing the extract, no deficiency syndromes were seen. Microbiological assay of the PGA content of the blood, urine and feces of mink receiving 0.0, 0.025, 0.1 mg PGA/100 gm ration, and the extract in addition to the low and high levels of PGA indicate that the extract caused an increase in the PGA greater than can be accounted for by the PGA in the extract. Mink receiving the basal diet or this diet supplemented with PGA fail to respond when given vitamin B₁₂ preparations or commercial liver extracts used in pernicious anemia therapy, but when the methanol extract is given a response is obtained. It is thus apparent that the methanol extract factor, which is different from vitamin B₁₂, has a sparing action on pteroylglutamic acid.

Tyrosine excretion in rheumatoid arthritis and normals. E. B. WALLRAFF (by invitation), C. A. STEPHENS (by invitation), A. BORDEN (by invitation), P. HOLBROOK (by invitation), D. HILL (by invitation), L. J. KENT (by invitation) and A. R. KEMMERER, *Dept. of Nutrition, Univ. of Arizona, and Southwestern Clinic and Research Institute, Tucson, Ariz.* Increased tyrosine excretion in pregnancy has been reported (Lawrie, *Biochemical J.* 41: 41, 1947). The relief of the rheumatoid arthritic syndrome during pregnancy has occasioned investigations to determine the possible mechanism of relief. Using a slight modification of the microbiological procedure for urines of Woodson et al (*J. Biol. Chem.* 172: 613, 1948) and the medium of Henderson and Snell (*J. Biol. Chem.* 172: 15, 1948), tyrosine was assayed in 24-hr urine specimens from rheumatoid arthritis and normals as well as pregnant rheumatoid arthritis and pregnant normals. It appears that tyrosine which is bound so as to be microbiologically unavailable without hydrolysis is higher in rheumatoid arthritic urine than in normal urine. Diet analysis for caloric and protein intake indicates no significant differences between the two groups. The difference may in part be due to differences in assimilation of protein, or may possibly be due to metabolic abnormality. Studies on excretion of other amino acids are in progress.

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(For possible corrections in any of the following abstracts see the June issue)

Studies on the enzymatic hydrolysis of the capsular polysaccharide of *Klebsiella pneumoniae* Type B EDGAR E BAKER (by invitation) and WALTHER F GOEBEL *Laboratories of The Rockefeller Institute for Medical Research, New York City* It has been observed that the capsular polysaccharide of *Kl pneumoniae* Type B (FrB), when administered to embryonated eggs is capable of inhibiting the multiplication of mumps virus In order to obtain degradation products of FrB which could be used for studying the mechanism of this inhibitory action, a search has been made for a specific enzyme A motile, gram-negative, spore-forming bacillus has been isolated from soil, which elaborates an enzyme capable of hydrolyzing the polysaccharide Although the microorganism will grow in a medium containing di- or trisaccharides, but not monosaccharides, it can elaborate the enzyme only when FrB is present Ammonium or nitrate ions, casamino acids or peptone may serve as sources of nitrogen Growth factors are not required The enzyme may be obtained from culture filtrates of the microorganism grown in the presence of 0.1% FrB It may be prepared more economically, however, by first culturing the bacilli in a medium containing sucrose and then resuspending the organism in a small volume of medium containing FrB The enzyme specifically hydrolyzes solutions of FrB The former can be determined quantitatively by observing the rate of change of viscosity at 30° and at pH 5.0 The drop in viscosity of the polysaccharide is followed by a loss of serologic activity If the hydrolysis proceeds to completion, the liberation of reducing sugars occurs It is not yet known whether the depolymerization of the carbohydrate and its hydrolysis are caused by a single enzyme

Comparison of methods of determining differences in virulence for mice of strains of *Salmonella typhosa* H C BATSON, MAURICE LANDY and MARTHA BROWN (introduced by MARION C MORRIS) *Dept of Biologic Products, Army Medical Dept Research and Graduate School, Army Medical Center, Washington, D C* Methods employed for determining the virulence for mice of strains of *S typhosa* have been of two main types The first, as utilized almost exclusively by British

workers, is based on the intraperitoneal injection of graded doses of the organisms suspended in saline The second, as employed by most American workers during the past decade, is essentially similar but incorporates the use of 5% hog gastric mucin as a suspending medium A third method which has shown some promise is based on intracerebral injection without the aid of an adjuvant A direct comparison of the efficacy of these methods in detecting differences in virulence of strains of *S typhosa* previously has not been made Such a comparison was the objective of this investigation Strains of *S typhosa* grown under different but defined nutritional conditions were tested for their virulence for mice by the 3 methods described Differences in virulence of the strains could not be determined satisfactorily by the intracerebral method Fatalities resulting from different strains of organisms were comparable but in each case were only poorly related to the size of challenge dose employed Differences between the strains were more distinct when the organisms were administered intraperitoneally as saline suspensions Furthermore, mortality was more clearly dependent upon the size of the challenge dose With the use of mucin as an adjuvant, differences in virulence of strains of organisms were amplified markedly and became readily apparent The effect of dosage on mortality was greatly accentuated It appears that assessment of the virulence of strains is dependent upon the method of testing employed The method incorporating the use of mucin as an adjuvant was the most sensitive in detecting differences in virulence, the intracerebral method was the least sensitive

Further study of toxins and antigens of *Shigella dysenteriae* DANIEL A BOROFF and BEATRICE P MACRI (by invitation) *Camp Detrick, Frederick, Md, Dept of Bacteriology, and George Washington Univ, Washington, D C* In previous studies of serological and immunological properties of toxins and antigens of *Shigella dysenteriae* (Boroff, D A, *Federation Proc* 7: 301, 1948) a suggestion was offered that there may be only 1 dominant antigen in *S dysenteriae* In these studies the data obtained in active protection experiments indicate that it is possible to immunize rabbits with either smooth

or rough whole organisms of *Shigella dysenteriae* or with their respective toxins and thus induce active protection against lethal doses of these substances. Not only whole organisms or solutions of whole organisms afford protection against each other but both chemically and physically purified toxins protected the animals and induced the formation of identical and reciprocally absorbable antibodies. This phenomenon of cross protection is not in accord with the concept that 2 toxins and 2 distinct antigens exist in *S. dysenteriae*. It is, however, explainable by the presence of 1 dominant antigen in the organism. Furthermore, the protection afforded rabbits against the whole organisms of the smooth variant by immunization with toxic lysates of the rough variant lends additional support to the latter hypothesis.

Immunologic relations and comparative invasiveness of strains of poliomyelitis from recent epidemics. GORDON C. BROWN (by invitation), THOMAS FRANCIS, JR. and JOHN D. AINSLIE (by invitation). *Dept. of Epidemiology, School of Public Health, Univ. of Michigan, Ann Arbor, Mich.* The immunologic relationship of strains of poliomyelitis virus was determined by means of virus neutralization tests performed in monkeys. The strains of virus had been obtained from the following epidemics: Cleveland, 1941, Texas, 1943, Buffalo, 1944, Rockford, Illinois, 1945, Tennessee, 1945, Ann Arbor, 1945, and Minnesota, 1946. Several dilutions of each virus were mixed with serum obtained from monkeys convalescent to infection with the various strains under study. Complete reciprocal testing between strains has not been possible but the results clearly demonstrate that all the viruses, with the exception of the Tennessee strain, are closely related immunologically. While studying each of these strains of virus certain characteristic differences in invasiveness were noted. After compiling data from all monkeys infected with each strain it was observed that variation occurred in the percentage showing fever, the height and duration of fever, the percentage, type and degree of paralysis and other clinical signs. Certain of these variations were so characteristic as to partially identify the strain of virus.

Evidence for the controlling influence of protease-inhibitors in allergic phenomena. KENNETH L. BURDON. *Dept. of Bacteriology and Immunology, Baylor Univ. College of Medicine, Houston, Texas.* Traditional theories about allergy, based entirely upon the amount and distribution of antibodies, and upon the supposedly crucial importance of the site of antigen-antibody union in the body, have never been adequate to explain satisfactorily the diverse manifestations of hypersensitivity in man and in different animal species. Yet some universal, underlying mechanism must operate. There is

increasing evidence that such a mechanism is to be found in the system of natural proteases, their inhibitors and activators, present in body fluids. According to this concept, allergic reactions are initiated by the sudden activation of natural proteases, following antigen-antibody combination, and are prevented by the action of protease-inhibitors. New support for this view has been obtained by use on an extended scale of a greatly improved procedure permitting the strictly quantitative titration of the antitrypsin in samples of blood serum and other fluids by a rapid, routine test. Results with several hundred specimens from allergic persons and normal controls, and from sensitized guinea pigs, have shown a sudden drop in antitrypsin in the first seconds of anaphylactic shock, a rise following sub-lethal reactions, and a consistent association of an especially high trypsin-inhibitor titer with the 'desensitized' state, whether this condition was induced by specific or by non-specific means. It was demonstrated that the 'anergy' to tuberculin exhibited at times by some tuberculous men and animals is always accompanied by a markedly increased antitryptic power of the blood.

Prophylactic immunity to experimental enteric cholera. WILLIAM BURROWS and LAWRENCE WARE (by invitation). *Dept. of Bacteriology and Parasitology, Univ. of Chicago, Chicago, Ill.* It was shown earlier that experimental enteric cholera in the guinea pig produced by overwhelming doses (viz., 20 mg.) of the vibrios is sharply modified by prior active immunization, in the immunized animal the percentage vibrios of the total fecal flora is reduced from 50-70% to 1-10% and the period of excretion shortened from 2-3 weeks to 5-7 days. If the inoculum is reduced both of these effects are observed in the normal animal but the ratio of total vibrios excreted to those inoculated becomes very large, many thousand fold, indicating that a true infection is produced. With progressive reduction a minimal dose infecting 50% of the animals (ID_{50}) may be calculated by the usual methods, in this case by inverse probability. The ID_{50} dose for the normal 300-400 gm. guinea pig has been found to be 0.08 mg. (32 million vibrios). In animals immunized with 0 vaccine and infected 4 days after the last of 3 inoculations, the ID_{50} is raised to 1.13 mg., a 14.1 fold increase over the normal. Two weeks later the immunity has fallen off so that the ID_{50} is 0.170 mg., an 8.7 fold increase over the normal. Following passive oral immunization the ID_{50} is immediately raised to 0.36 mg., a 4.05 fold increase, and rises to 0.51 mg., or a 6.4 fold increase 2 weeks later, the latter coinciding with maximum excretion of absorbed antibody. It is concluded that the presence of antibody in the feces is associated with prophylactic immunity to experimental enteric cholera in the guinea pig.

Purification of antibodies by specific methods

DAN H CAMPBELL and LEONARD LERMAN (by invitation) *Gates and Crellin Laby of Chemistry, California Inst of Technology, Pasadena, Calif* Methods have been developed for the efficient removal and purification of antibodies from serums. These methods are based on the removal of antibody with soluble or insoluble antigens and subsequent dissociation with simple specific hapten or dilute acid or both. Recent experiments with adsorption columns or suspensions of R-azo-coagulated egg white and R-azo-phenol-formaldehyde resins indicate that such systems usually remove more antibody than is realized by use of soluble precipitating antigens. Studies have been made chiefly with rabbit antibodies against ovalbumin and p azophenyl arsonate, and include quantitative studies on specific adsorption.

Interference with induction of the anaphylactic state by prior feeding of a hapten-like allergenic chemical MERRILL W CHASE *Rockefeller Inst for Medical Research, New York City* The feeding of allergenic chemicals, such as 2,4-dinitrochlorobenzene and picryl chloride to guinea pigs that have not previously encountered the respective substance, conditions the animals so that the outcome of subsequent attempts to induce hypersensitivity is profoundly modified. The conditioned animals fail to respond, or they respond abortively, to various sensitizing procedures effective in normal animals. This inhibitory effect is observed not only when attempts are made to develop a specific dermal sensitivity of the 'delayed type' but also when anaphylactic sensitization is undertaken. Guinea pigs that have been fed picryl chloride, for example, and have then been treated with an 'artificial antigen' made by coupling the proteins of guinea pig serum with the picryl radical as the serologically determinant grouping show no or scarcely any anaphylactic effects upon challenge with other conjugates having the same determinant grouping. There exists, accordingly, a specific mechanism that can modify the outcome of sensitizing procedures and that results in a certain level of tolerance; the influence of this mechanism is probably encountered also in natural exposures of human beings to sensitizing agents.

Comparative response and persistence of immunity in mice vaccinated intra-abdominally or by gavage with *Shigella sonnei* MERLIN L COOPER, HELEN M KELLER and AGNES M HART (introduced by ALBERT B SABIN) *Children's Hospital Research Foundation and Dept of Pediatrics, College of Medicine, Univ of Cincinnati, Cincinnati, Ohio* One group of mice was vaccinated intra-abdominally with one injection of 100 million killed *Shigella sonnei* as broth vaccine. A control group was given intra-abdominally 1 injection of an equal volume of uninoculated broth medium.

Another group of mice was given by gavage, daily for 15 days, one billion killed *Sh sonnei* as broth vaccine. A control group was given by gavage, daily for 15 days, an equal volume of uninoculated broth medium. One week, 6 weeks and 12 weeks following completion of vaccination, mice from the 2 vaccinated groups and the 2 control groups were challenged intra-abdominally with the homologous *Sh sonnei* suspended in 3% sterile mucin. The mice vaccinated intra-abdominally withstood after 1 week more than 24,000,000 LD₅₀ doses of culture, after 6 weeks 5,000,000 LD₅₀ doses and after 12 weeks 1,700,000 LD₅₀ doses. The mice vaccinated by gavage withstood after 1 week 263,000 LD₅₀ doses, after 6 weeks 5,600 LD₅₀ doses and after 12 weeks 85 LD₅₀ doses. The range of LD₅₀ doses of culture withstood by the groups of control mice was from less than 10 to 30. Data will also be presented regarding the passive mouse protective powers and agglutinative titers of sera obtained from each group of mice 1, 6 and 12 weeks following completion of vaccination.

Antisheep agglutinins in infectious mononucleosis, further studies I DAVIDSOHN and CHIYO KASHIWAGI (by invitation) *Mount Sinai Medical Research Foundation and Chicago Medical School, Chicago, Ill* In over 200 patients with infectious mononucleosis antisheep agglutinins were studied in the serum. Earlier observations on the ability of certain antigens to remove the agglutinins were confirmed: beef erythrocytes completely absorbed the agglutinins present in infectious mononucleosis, whereas guinea pig kidney was unable to do so. In more than $\frac{1}{2}$ of the tests, the guinea pig antigen removed from 50-75% of the originally present agglutinins, less than $\frac{1}{10}$ of the tests showed a maximum absorption of 87.5%, and in no instance of infectious mononucleosis was complete removal of the agglutinins observed. According to recent reports bovine albumin as diluent raises the titer of antisheep agglutinins in infectious mononucleosis. This problem was reinvestigated by studying the effect of several diluents on the titers of the agglutinins. Under standard conditions, bovine albumin and human plasma used instead of saline as diluent lowered the titers in both the absorbed and unabsorbed serums, or left them unchanged. Investigation of the effect of time and temperature of incubation showed that prolonged incubation at ice-box temperature increased the titers, and hence the sensitivity of the test. This effect was independent of the diluent. Studies are also reported on the preparation of the guinea pig kidney and beef red cell antigens used in the differential test.

Polysaccharides isolated from tissues of normal, immune, and immunologically paralyzed mice LLOYD D FELTON, BENJAMIN PRESCOTT (by invitation), GLADYS KAUFFMANN (by invita-

tion) and BARBARA OTTINGER (by invitation) *Laby of Infectious Diseases, Microbiological Inst., National Inst of Health, Bethesda, Md*. A preliminary observation has been reported that, when mice were inoculated with a large dose (0.5 mg) of pneumococcus antigenic polysaccharide, their immunological system was 'paralyzed' so that they failed to respond to a subsequent immunizing dose. From the tissues of such mice it was possible to separate a soluble antigen which was antigenic in both precipitin and active immunity tests. In continuing this study, a comparison has been made of the polysaccharide which has been obtained from the tissues of a) normal mice of C₃H strain, b) mice immunized with 0.002 mg of antigenic polysaccharide of each type, I, II, and III respectively, and c) mice immunologically paralyzed with type I, II, and III. The tissues studied included blood, heart, lung, liver, kidney, spleen, lymph, thymus, stomach, bone marrow, muscle, and skin. Like tissues from 10 mice of each of these groups were pooled, ground, and a polysaccharide obtained by methods employed for isolating antigenic polysaccharide from pneumococci. The product, a dry white powder, was soluble in 7.5% trichloroacetic acid and at neutral pH, and insoluble in alcohol. In all alike, tests for glycogen and protein were negative. The polysaccharide so obtained from tissues of normal mice failed to demonstrate antigenic activity. The immunized mice gave low yields of polysaccharide which showed a comparatively low titer immunity in 9 of the 12 tissues of the type I mice, in 10 of the type II tissues, and in 6 of the type III tissues. The immunologically paralyzed mice, on the other hand, yielded greater amounts of polysaccharide. Furthermore, the immunizing titer was appreciably higher in the polysaccharides from the tissues of these 'paralyzed' mice.

Procaine penicillin and sulfonamide antagonism. H. FISHBACK (by invitation), H. WELCH, E. Q. KING (by invitation), J. LEVINE (by invitation), C. W. PRICE and W. A. RANDALL *Federal Security Agency, Food and Drug Administration, Washington, D. C.* Procaine has long been known as an antagonist of the sulfonamides. This antagonism may result through direct action of the procaine as such or through conversion of the procaine to p-aminobenzoic acid. The accepted ratio of procaine-antisulfonamide action is much lower than the PABA-antisulfonamide action. The introduction of procaine-penicillin as a chemotherapeutic agent raised the question as to whether sufficient procaine or procaine derivatives would be formed in the body to act antagonistically toward sulfonamides during combined therapy. Accordingly, 1,500,000 units of procaine penicillin for aqueous injection was administered to 37 individuals and blood was drawn for examination 3 hours

after the injection. The penicillin levels were determined and the serum examined for procaine, p-aminobenzoic acid and an acylated derivative of p-aminobenzoic acid. The concentration of penicillin varied from 0.5-8.0 u/ml, averaging 2.5 u/ml. The blood concentration of procaine was approximately 0.1 µg/ml in 68% of the cases. None could be detected in the remainder. A level of 0.1 µg/ml, for PABA was found in only 18% of the cases. However, previous reports indicate that this concentration of PABA is sufficient to antagonize the therapeutic action of sulfanilamide but insufficient to interfere significantly with the therapeutic action of sulfathiazole or sulfadiazine. An acylated derivative of PABA was found in each of the 37 individuals in quantities ranging from 0.2-1.0 µg/ml. *In vivo* experiments are now under way to determine the actual antagonism when experimental infections are treated with combined therapy.

Neutralization technique in *Tsutsugamushi* disease (scrub typhus) and the antigenic differentiation of Rickettsial strains. JOHN P. FOX (introduced by P. K. OLITSKY) *International Health Board, Rockefeller Foundation, New York City*. Neutralization of *R. orientalis* by homologous antiserum was most effectively demonstrated when mice were inoculated by the intraperitoneal route with mixtures of undiluted antiserum and serial dilutions of infective material. Satisfactory results could not be obtained by employing a constant infective dose and serial dilutions of antiserum nor by using the subcutaneous or intracerebral routes of inoculation. Furthermore, neutralization was independent of complement. Evidence was obtained to suggest that neutralization depends principally upon a union of antigen and antibody *in vitro*, however, passive immunity may best explain the zone phenomenon often observed. The satisfactory expression of the neutralizing capacity of an antiserum was rendered difficult by the above mentioned zone phenomenon and by the otherwise frequently irregular pattern of mouse survival observed in neutralization tests. In spite of this difficulty, it was possible on the basis of a large series of cross-neutralization tests to distinguish 4 clearly differentiated antigenic types and a possible 5th type among the 8 strains of *R. orientalis* included in the study. The strains and the grouping observed were as follows: 1) *Karp* (from New Guinea), 2) *Queensland* (from Australia), *Raub* (from Malaya), *Seerangayee* (from Malaya), 3) *Calcutta* (from India), *Imphal* (from India), 4) *Gilliam* (from Assam-Burma border), 5) *Pescadores* (from Pescadores Islands). These results augment evidence previously reported by others as to the antigenic heterogeneity of strains of *R. orientalis*, a fact of importance epidemiologically.

and from the standpoint of developing vaccines of proper antigenic composition

Streptococcal anti-hyaluronidase in rheumatic fever SUSANNA HARRIS (by invitation) and T N HARRIS *Children's Hospital of Philadelphia, Dept of Pediatrics, School of Medicine, Univ of Pennsylvania, Philadelphia, Penna* The occurrence of antibodies to streptococcal hyaluronidase, as measured by the mucin-clot-prevention test, was studied in groups of convalescents from streptococcal infection, in patients with acute rheumatic fever and in the normal population. In preliminary studies the optimal relations of hyaluronidase, the substrate (hyaluronic acid) and neutralizing antibody were determined, the potency of the enzyme as measured by mucin-clot-prevention (*in vitro*) was compared with spreading activity (*in vivo*) in the rabbit's skin, and the serologic specificity of neutralizing antibodies was established by comparative studies against hyaluronidase of testicular, clostridial and pneumococcal origin. It was found that antibodies to streptococcal hyaluronidase occurred with increasing frequency in the normal population from infancy up to the adult age group. Following streptococcal infection there was an increase in the titer of antibodies to streptococcal hyaluronidase and in acute rheumatic fever the titer was found to be still higher. In a comparative study of the antibody response to 4 streptococcal antigens, hyaluronidase, hemolysin, and 2 somatic fractions, the cytoplasmic particles and supernate proteins, it was found that the mean titers to all of these antigens were elevated in both rheumatic and streptococcal infection. The mean titer was somewhat higher in rheumatic than in streptococcal infection, in the case of 3 of these antibodies. In the case of the 4th, anti-hyaluronidase, this difference was considerably greater. The anti-hyaluronidase titer showed better correlation with changes in the activity of the rheumatic infection than did the other tests. There was, however, no striking correlation between this titer and the severity of the illness. The possible implications of these data, both diagnostic and etiologic, are discussed.

Histochemical evidence for the synthesis of protein by lymphocytes following parenteral injection of antigen T N HARRIS and SUSANNA HARRIS (by invitation) *Children's Hospital of Philadelphia, Dept of Pediatrics, School of Medicine, Univ of Pennsylvania, Philadelphia, Penna* Recent developments in histochemistry have indicated that cells which are actively forming new protein are characterized by large amounts of ribonucleic acid in their cytoplasm. Since there is considerable immunologic evidence of the production of antibody by the popliteal lymph node of the rabbit following the injection of antigens in the foot pad, and since a tissue in which antibody is being

formed could be expected to show evidences of protein synthesis, it seemed feasible to attempt to identify the cells involved by histochemical methods. A study was therefore undertaken of the localization and concentration of ribonucleic acid in the cells of the popliteal lymph node in correlation with the production of antibody by that node. Antigens were injected into the foot pads of rabbits, and at various intervals thereafter the draining lymph nodes were examined histologically and chemically. Diffuse hyperplasia was found in the cortex of the lymph node, involving transitional forms between reticulum cells and lymphocytes, as well as young (large) lymphocytes. Large numbers of these cells showed pyronine staining granules (ribonucleic acid) in the cytoplasm, which were abolished by previous incubation of the sections with protease-free ribonuclease or with hot trichloroacetic acid solution. Pyronine-staining nucleoli and crescents of cytoplasm were also seen in these cells. Chemical analysis of the nodes showed an increase in ribonucleoprotein but not in desoxyribonucleoprotein. These changes reached a maximum intensity just before the time of maximal antibody content of the node.

Dissociation of antigen-antibody complexes and their bond energy FELIX HAUROWITZ and LALE ETILI (by invitation) *Dept of Chemistry, Indiana Univ, Bloomington, Indiana, and Dept of Medical Chemistry, Istanbul, Turkey* Experiments 4.5 ml rabbit immune serum against anthranil-azo-beef serum globulin, mixed with 10 mg antigen, kept at 37° for 30 minutes and at -12°C for 24 hours, gave 27.8 mg precipitate, containing 3.0 mg antigen. No precipitate resulted with 150 mg antigen. When the precipitate produced by 10 mg antigen was kept at 37° for 30 minutes with 140 mg antigen, a considerable part was dissolved. The weight of the undissolved precipitates (in bracket antigen) was 9.5 (3.7), 8.8 (3.3) and 12.1 (4.6) mg in 3 experiments. The precipitates were isolated and analyzed as described previously (J Immunol 43:331, 1942). —Results 6 of the 8 antibody molecules bound per antigen molecule in the original precipitate are split off by the antigen excess. No antigen is split off. —It is assumed that the irreversibly bound antibody molecules are attached to antigen molecules by the whole area of the determinant site. If an area of 50 Å² is assumed for the bis-azophenylcarboxylic group and 100 Å² for the species-specific group of the globulin, a total bonding area of 150 Å² results, corresponding to 14 water molecules per layer. The bond energy between antigen and antibody must be higher than that of the water displaced from this area. If it is assumed that only 1 layer of water molecules is displaced, a bond energy of ~70 kcal 1 antigen-antibody bond or ~0.47 kcal 1 Å² results. The loose linkage of those antibody molecules

which are split off by the antigen excess is attributed to steric hindrance by the firmly bound antibody molecules and/or to imperfect complementarity of the specific sites. Both factors cause increase of the distance between bonding groups. If this distance increases from 3 to 4, 5 or 6 Å, the bond energy will decrease from ~ 0.47 to approximately 0.25, 0.15 and 0.10 kcal/Å² respectively.

Indirect complement-fixation test for psittacosis-lymphogranuloma venereum group antibodies in chicken antisera. M. R. HILLEMANN and, R. J. HELMOLD (introduced by ANNABEL WALTER), *Dept. of Virus and Rickettsial Diseases, Army Medical Dept. Research and Graduate School, Army Medical Center, Washington, D. C.* It is well established that antisera prepared in certain mammals and birds are incapable of fixing complement in the presence of homologous antigen even though antibody can be demonstrated in them by other techniques. Such sera may contain blocking antibodies which, in the presence of homologous antigen, prevent fixation of complement by ordinary antibodies that are capable of binding complement. Sera from chickens immunized against viruses of the psittacosis-lymphogranuloma venereum group do not fix complement in the usual type of test. Such sera have now been shown to possess blocking antibodies which interfere with the binding of complement by mammalian antibodies in the presence of homologous antigen. The technique of the indirect complement-fixation test has been used to determine whether under these conditions chicken antisera provide the broad group reaction displayed by mammalian antisera in complement-fixation tests with this group of agents, or whether they follow the highly strain specific reaction found when chicken antisera are used in neutralization tests. It would appear that when the indirect complement-fixation technique is employed, the chicken sera approach the broad type of group reaction characteristic of mammalian sera in direct fixation tests with this group of viruses.

Tissue reaction to foreign particulate matter altered by presence of adsorbed antigen. HOWARD C. HOPPS and ALFRED M. SHIDELER (by invitation), *Dept. of Pathology, School of Medicine, Univ. of Oklahoma, Oklahoma City, Okla.* Rabbits were given intradermal injections of finely particulate aluminum hydroxide, activated carbon, magnesium silicate (talc) and silicon dioxide (flint). Tissue reactions were studied at 7, 14, 28 and 56 days. A second series of intradermal injections was made, similar to the first, with the exception that horse serum was absorbed upon the particulate matter prior to its administration. Tissue reactions in this group of lesions were compared with those of the former group with special attention to the character of inflammatory exudate, ratio between macro-

phages and fibroblasts, occurrence of focal necrosis and cellular degenerative changes.

Effect of previous experience with homologous and heterologous types of poliomyelitis virus upon the alimentary carriage of virus by the chimpanzee. HOWARD A. HOWE (by invitation), DAVID BODIAN (by invitation) and ISABEL M. MORGAN, *Poliomyelitis Research Center, Dept. of Epidemiology, The Johns Hopkins Univ., Baltimore, Md.* Eighty oral inoculations have been carried out in chimpanzees with 4 strains of poliomyelitis virus representing 2 major immunologic types, the Brunhilde, Kotter and Frederick strains comprising 1 type, and the Lansing strain the other. The first experience with virus was provided by oral inoculation in the majority of the animals but toward the end of the experiment 14 animals were vaccinated with active Lansing virus given intramuscularly. Evidence of response to the immunization was obtained by testing for virus in stools or for rises in serum antibody titer. The control group consisted of 27 animals of which all responded positively, 2 with paralysis. Challenge was carried out at 1 month or later following first virus contact by the oral administration of titrated pools of rhesus monkey cords. In many instances 2 or 3 homotypic viruses were given to the same animal. Animals which became paralyzed or carried virus in the stools after the first post inoculation week were considered to have shown no evidence of immunity. No animal was considered negative unless no evidence of activity was found in at least 4 stool specimens collected during the 2-4th weeks and tested in 2 monkeys each by intranasal inoculation. The results were as follows. Of 9 animals receiving homologous challenge, 1 subsequently produced virus in the stools. This result differs in no essential respect from the results of 22 homotypic challenges in which 1 animal had virus in the stools on the second week. Twenty-two animals were immunized with Lansing virus either by mouth (8) or by intramuscular vaccination (14). Subsequent oral challenge was carried out with the heterotypic Brunhilde strain, following which 3 animals became paralyzed while 13 showed virus in the stools. Although the tests are not quite complete the infection rate appears to be 15/22 or 68%.

Universal serologic reaction with lipid antigen in tuberculosis. REUBEN L. KAHN, *Serology Lab., Univ. Hospital, Ann Arbor, Mich.* It was previously shown that it is possible to demonstrate positive reactions with the serums of normal human beings by special precipitation techniques with tissue extract antigens (Kahn, Kolmer, cardiopipin). A standardized precipitation technique based on different NaCl concentrations, serum dilutions and incubations was then developed with Kahn antigen, known as universal serologic technique. With this technique normal human beings

(giving negative Kahn reactions for syphilis) give precipitation reactions of characteristic serologic patterns. It was observed that, in Kahn-negative patients with acute tuberculosis, precipitation in the universal reaction is increased above the normal level and, as the patients gradually improve, precipitation is correspondingly decreased. This finding indicates that the universal serologic technique might prove helpful as an indicator of tuberculous activity. No increase in precipitation above the normal has been noted in far advanced and terminal cases of tuberculosis.

Development of the specific antibody for a distinctive constituent of Brown-Pearce carcinoma cells. BERNARD KALFAYAN (by invitation) and JOHN G. KIDD, *Dept. of Pathology, New York Hospital, Cornell Medical Center, New York City*. Certain rabbits implanted with Brown-Pearce tumor cells develop an antibody that reacts specifically with a distinctive constituent of them, as previous work has shown (J. Exper. Med. 71: 335, 351, 1940). In ordinary market-bought hybrid rabbits the specific Brown-Pearce antibody was frequently masked by the presence of induced tissue antibodies, which react with extracts of a variety of normal and neoplastic rabbit tissues (J. Exper. Med. 82: 21, 1945), though about half of the inbred 'blue-cross' rabbits implanted with Brown-Pearce tumor cells developed the specific antibody in high titer, usually without the concomitant development of the induced tissue antibodies (J. Exper. Med. 82: 41, 1945). In recent experiments the specific Brown-Pearce antibody appeared in the blood of every one of 17 Havana and 23 Chocolate-Dutch (Havana x Dutch hybrids) rabbits implanted with the tumor cells, often reaching titers of 1:512 or higher. The induced tissue antibodies, by contrast, were present in titers of 1:8 or less in a few specimens but were entirely absent from most of them. Normal rabbits of both breeds provided sera that were devoid of the specific Brown-Pearce antibody and of the induced tissue antibodies.

Quantitative measurement of antibody to a whole bacterial antigen. MARIAN KOSHLAND (introduced by WILLIAM BURROWS), *Dept. of Bacteriology and Parasitology, Univ. of Chicago, Chicago, Ill.* A quantitative method for the measurement of the antibody response to a whole bacterial antigen, the cholera vibrio, has been developed. The method depends upon the colorimetric determination of specific complement fixation using the 50% hemolytic unit. The excess complement procedure, proposed by Heidelberger and his co-workers, was employed in this study, and was adapted to the requirements of a whole bacterial antigen. The results obtained using a whole bacterial antigen have been compared from a theoretical and practical point of view with those involving viral or bacterial extract antigens. The method has been used to

determine the antibody levels produced in the serum and the feces of guinea pigs, either actively immunized with the cholera vibrio or passively immunized with anti-cholera rabbit serum.

Equilibrium studies on the reaction of antibody with hapten. LEONARD LERMAN (introduced by DAN H. CAMPBELL), *Gates and Crellin Lab. of Chemistry, California Inst. of Technology, Pasadena, Calif.* Quantitative studies have been made on the combination of antibody with a simple monohaptenic substance. Antibody was specifically purified from rabbit antiserum homologous to the p-azophenylarsonic acid hapten by precipitation with a simple trihaptenic antigen, followed by dissociation of the washed precipitate with a monohaptenic substance. A cellophane bag containing the purified antibody solution was immersed in a buffered solution of normal rabbit gamma-globulin (at the same protein concentration) together with a dialysable monohaptenic dye. After continuous agitation at constant temperature for about 24 hours the dye concentrations in the 2 solutions were measured colorimetrically to obtain the equilibrium composition in terms of bound and unbound hapten. From measurements at varying ratios of hapten to antibody the free energy of combination, the maximum binding capacity, and related quantities have been determined. Under various conditions the free energy change is of the order of -7.5 to -8.0 kcal per M of hapten bound.

Some properties of purified diphtheria toxoid. LEO LEVINE and LOUISE WYMAN (introduced by GEOFFREY EDSALL), *Antitoxin and Vaccine Lab., Massachusetts Dept. of Public Health, Boston, Mass.* Crude toxoid, obtained as the detoxified filtrate of diphtheria bacillus culture grown on Mueller's semi-synthetic medium, modified in this laboratory, has been purified by a simple 2-stage salt fractionation, and has been evaluated with respect to purity, antigenicity, and stability. Preparations have been obtained assaying 2100 Lf/mg of protein nitrogen. Preliminary laboratory studies have indicated that stability of the toxoid protein is impaired by addition of glycine to the diluent in the usual quantities, whereas gelatine appears to increase stability. Injections of 11 Lf have protected guinea pigs against the standard challenge dose of 10 M.L.D. Potency tests with varying amounts of antigen indicate that the antigenicity per Lf unit increases as the purity is increased. A linear relationship has been observed between the logarithm of the flocculation time (K_f) and that of the dilution (or of the Lf) at the equivalence point. These observations support the evidence of previous workers that a relationship may be established between antigenic efficiency and flocculation time. This product is now on clinical trial; the practical significance of the findings presented will be discussed.

Allergenic fraction of ragweed pollen prepared by electrophoresis-convection (Nielsen-Kirkwood cell) MARY HEWITT LOVELESS and ROBERT BEST (by invitation) *New York Hospital and the Dept of Medicine, Cornell Univ Medical College, New York City* The principle of electrophoresis-convection has been applied to the problem of fractionating ragweed extract, with the eventual aim of obtaining a single allergen from pollen. The Nielsen-Kirkwood apparatus affords a gentle means of separation and larger yields of fraction than are available from the Tiselius cell used by Abramson. Preliminary results with products obtained from one or more runs in the Nielsen-Kirkwood apparatus reveal that the electrophoretically immobile material (called 'top' fraction) is simplified over the original extract. This is evidenced by a) the almost complete removal of color from this fraction, b) reduction in precipitable nitrogen as well as in carbohydrates, c) a decrease in reagin-binding activity roughly related to its loss of precipitable nitrogen, d) immunological crosstests which showed removal of some antigenic constituents. Despite the purification of 'top' fraction, it uniformly elicited the characteristic immediate responses in allergic skin and conjunctiva, as well as producing the typical Prausnitz-Kustner reaction with pollen-allergic sera. Occasional sera were encountered which contained reagins for only those antigens which remained in the 'top' fraction. Patients who were given a course of therapeutic injections with 'top' fraction produced 'protective,' or thermostable, antibodies in their blood streams and showed increased tolerance toward the fraction when tested intracutaneously and by conjunctival test. Furthermore, a significant proportion of them proved to be resistant clinically when exposed to natural pollination. This clinical immunity tended to be selective since some individuals responded much better to the fraction than they had to whole extract in prior years, while for others the reverse was true.

New observations on an autoagglutinin H H LUBINSKI and D MENDEL (by invitation) *Pathological Lab and Dept of Medicine, Jewish General Hospital, Montreal, Canada* The serum of a patient suffering from hemolytic anaemia and belonging to Blood Group A contained an autoagglutinin which had the following characteristics. When serum dilutions and red cell suspensions were made with normal saline, clumping of red cells was observed at room temperature, but not at 37°C, clumps dissolved if warmed to incubator temperature. When, instead of saline, 30% beef albumin solution was used, agglutination also occurred at incubator temperature. Using human serum or plasma, clumping occurred only at room temperature. The autoantibodies could be adsorbed on human cells of Group A or O and transferred into normal saline with the

technique described by Landsteiner and Miller, even when the whole process was perfected in an incubator room at 37°C. These purified agglutinin solutions reacted with human red cells, suspended in saline, at room temperature, but not at 37°C; red cells suspended in albumin were clumped at 37°C as well. The agglutinin solution, furthermore, agglutinated and hemolyzed rabbit red cells suspended in saline at both temperatures.

Relation of complement to thermolabile hyaluronidase inhibitor of serum STANLEY MARCUS and JOHN K FULTON (by invitation) *Rackham Arthritis Research Unit and Dept of Medicine, Univ of Michigan, Ann Arbor, Mich* A modification of a biological technique (streptococcal decapsulation) for the quantitative determination of hyaluronidase using testicular or streptococcal enzyme has been used. By this method and turbidimetric assay also, it was found that sera heated at 56°C for 20 minutes lost their enzyme inhibiting capacity. Activity of the enzyme inhibitor decreased during refrigeration. These findings suggested an analogy to complement (C') function. Loss of inhibition following heating was replaced by addition of lyophilized guinea pig C'. While fresh guinea pig serum contained inhibitor, reconstituted lyophilized serum did not. It was concluded that inhibition depends upon a thermostable component lost upon lyophilization and a thermolabile component surviving this process. Pooled guinea pig serum was fractionated to yield C'3 and C'4 deficient C'. Both C'3 and C'4 deficient fractions lacked enzyme inhibitory capacity. Although heated guinea pig serum (C'3, C'4) plus ammonia or zymine-treated serum did not show inhibitory capacity, heated serum plus ammonia and zymine-treated fractions of C' had fully restored inhibitory ability. It has been assumed, therefore, that serum inhibitor of hyaluronidase depends upon the presence of the 4 characterized C' components plus still another thermostable component. Although it is demonstrable by turbidimetric procedure that heated serum does not inhibit enzyme action, preliminary data indicate that C' will not reactivate the thermolabile inhibitor measured by this technique. This is believed to be further evidence that different procedures may measure different stages in the hydrolysis of hyaluronic acid. There was no fall in inhibitor concentration of guinea pig serum following anaphylaxis, whereas C' concentration was decreased. C' concentrations of human sera could not be correlated with inhibitor titers. The implications of these findings will be discussed.

Effects of ghotoxin and other sulfur-containing compounds on tumor cells *in vitro* JOHN W MASON (by invitation) and JOHN G KIDD *Dept of Pathology, New York Hospital, Cornell Medical Center, New York City* Ghotoxin, held in brief contact with tumor cells *in vitro*, inhibits their subsequent

proliferation upon implantation in susceptible hosts, as previous work has shown (Science 105 511, 1947) Two other compounds having unusual sulfur linkages (4-acetamido-5,5-dimethyl-1,2-dithiolane-3-one and 4-methyl-5-methylimino-1,2-dithiazolidone-3—synthesized and made available by Professor John R. Johnson) have likewise proved highly effective against lymphosarcoma cells in mice, inhibiting their growth after 1 hour's contact *in vitro* in concentrations of 1–5 $\mu\text{g}/\text{cc}$, the effective level of gliotoxin in concurrent tests being 1 $\mu\text{g}/\text{cc}$ or slightly less Two additional compounds of similar sort (4-benzamido-5,5-dimethyl-1,2-dithiolane-3-one and 4-methyl-5-methylimino-3-thio-1,2-dithiazolidone-3), were somewhat less soluble, but were also inhibitory in low concentrations Dethiogliotoxin had no effect on tumor cells in concentrations of 800 $\mu\text{g}/\text{cc}$ Simple monothiol compounds (cysteine, glutathione, and monothioglycerol) had no inhibitory effect on the lymphosarcoma cells in concentrations up to 800 $\mu\text{g}/\text{cc}$, while two dithiols, BAL and dithiobiuret, were inhibitory at 100–200 $\mu\text{g}/\text{cc}$ When mixtures containing low concentrations of gliotoxin and high concentrations of the mono- and dithiol compounds were allowed to interact for a few minutes and then tested against tumor cells, the inhibitory effect of the gliotoxin was diminished or abolished This effect of gliotoxin was not modified, however, when very large amounts of cysteine, glutathione, monothioglycerol and BAL were brought into contact with tumor cells that had previously been exposed for 1 hour to gliotoxin in a concentration of 10 $\mu\text{g}/\text{ml}$

Immunogenic difference between two related strains of poliomyelitis virus JOSEPH L. MELNICK
Section of Preventive Medicine, Yale Univ School of Medicine, New Haven, Conn By means of vaccination and neutralization tests the 2 strains of poliomyelitis virus—Lansing and Y-SK—are immunologically related The Lansing strain, isolated in the monkey from the CNS of a fatal case in Lansing, Mich, in 1937, was successfully established by Armstrong in cotton rats and mice When used in the present experiments, the strain had been through 260 serial passages in mice and still retained its pathogenicity for monkeys The Y-SK strain, isolated by Trask and Paul from the stools of an abortive case in New Haven, Conn, in 1937, was subsequently found to produce in mice, cotton rats, and hamsters a myelitic disease The virus transferable in these rodents was shown to be immunologically the same as the parent simian strain At the time it was used for these experiments, this strain had been passed through 20 transfers in monkeys and 15 in cotton rats and mice It too remained pathogenic for primates Previous work had established that single or multiple feedings of the Y-SK virus to cynomolgus monkeys usually result in the production of antibodies either by the first day of

paralysis or by the 4th week in the asymptomatic animals When the Lansing virus was fed repeatedly to a group of cynomolgus monkeys, none of them responded either by apparent disease or by the development of neutralizing antibodies When these same monkeys were then fed Y-SK virus, they again remained in apparent good health, but they were now found to have developed neutralizing antibodies Subsequent challenge by the intranasal route indicated an appreciable degree of resistance among the group Thus in contrast to the Lansing strain of poliomyelitis virus, the homotypic Y-SK strain is capable of engendering antibodies when it is administered orally to cynomolgus monkeys and such monkeys may have an increased resistance to virus instilled intranasally

Resistance to endotoxins of enteric bacilli in patients convalescent from typhoid and paratyphoid fever HERBERT R. MORGAN and FRANKLIN A. NEVA (introduced by THOMAS FRANCIS, JR.)
Dept of Epidemiology, School of Public Health, Univ of Michigan, Ann Arbor, Mich Patients convalescent from enteric fevers due to infection with *Salmonella typhosa* or *S. paratyphi* were shown to be more resistant than normal individuals to the pyrogenic and general systemic effects of the toxic, somatic antigens (endotoxins) of *S. typhosa* or *Shigella dysenteriae* following their intravenous administration The significance of these findings in regard to the clinical course of the disease and the process of recovery will be discussed

Precipitin tests for the diagnosis of Chagas' disease, with special reference to the specificity of polysaccharides A. PACKCHIANIAN and L. TURCK (by invitation) Dept of Bacteriology and Lab of Microbiology, Univ of Texas, School of Medicine, Galveston, Texas *Trypanosoma cruzi* antigens for diagnosing Chagas' disease by the precipitin test were prepared from washed, packed *Tr. cruzi* grown *in vitro* The cytolysis of the trypanosomes was accomplished by various means, and the active principle for the precipitin test was extracted by several methods The first antigen, a crude aqueous extract, was found to be specific and gave positive precipitin reactions with both *Tr. cruzi* antiserum and sera from known human and animal cases of Chagas' disease Inasmuch as the precipitin titer of this aqueous antigen was low, further studies were conducted to isolate from the cellular substance of *Tr. cruzi* the active component, which proved to be the polysaccharide complex Several well known immunochemical methods of isolating polysaccharides from microorganisms, such as Avery's, Felton's, Fuller's and Sevag's methods, were employed in the preparation of *Tr. cruzi* 'polysaccharide antigens' The specificity and potency of these antigens were determined and compared These antigens gave much higher precipitin titers than the crude aqueous extract Quantitative studies of serological

cross reactions between the *Tr cruzi* antigens and several antisera and serum samples from other trypanosomiasis, leishmaniasis and bacterial infections were made. Likewise, polysaccharides isolated from several species of trypanosomes, leishmania and bacteria were tested with *Tr cruzi* and homologous antisera. The relative merits of the *Tr cruzi* antigens for the precipitin test in the diagnosis of Chagas' disease have been evaluated.

Amino acids, analogues and the propagation of Theiler's GD VII virus in mouse brain tissue culture HAROLD E. PEARSON and RICHARD J. WINZLER (by invitation) *Depts of Bacteriology and Biochemistry, Univ South Calif School of Medicine and the Laby Div, Los Angeles County Hospital, Los Angeles, Calif*. The effects of various amino acids and certain analogues on the propagation of the GD VII strain of Theiler's mouse encephalomyelitis virus in cultures of minced, one day mouse brain were studied. Tissue suspended in Simm's solution at pH 8-9 and contained in 50 ml Erlenmeyer flasks was incubated for 2-3 days at 35°C. The titer of virus attained in cultures as tested by intracerebral injection of mice was reduced slightly by the presences of 1 mg/ml L-lysine monohydrochloride but not by L-arginine monohydrochloride, L-cystine, L-cysteine hydrochloride, glycine, L-histidine monohydrochloride, L-leucine, L-proline, DL-alanine or DL-phenylalanine. The titer was markedly reduced by the presence of 3-5 mg/ml DL-ethionine, beta-2-furyl-DL-alanine or beta-2-thienyl-DL-alanine, the addition of DL-phenylalanine in the same concentration did not reverse the actions of the last 2 compounds. Glutathione 5 mg/ml but not 1 mg/ml also reduced the titer of virus in cultures.

Effect of lithium periodate on crystalline bovine serum albumin GERTRUDE E. PERLMANN (by invitation) and WALTHER F. GOEBEL *Labys of The Rockefeller Inst for Medical Research, New York City*. When certain biologically active proteins are subjected to the action of dilute lithium periodate at physiological values of pH, their activities are rapidly altered and finally destroyed. In order to gain an insight into the nature of the chemical changes which occur, a study has been made of the action of 0.03 M lithium periodate on crystalline bovine plasma albumin. It has been found that after contact with the reagent the ultraviolet absorption spectrum as well as the electrophoretic behavior of the protein has been radically altered. In addition, the solubility of the protein in salts has been changed, as has its solubility in the acid range of pH values. At a pH of approximately 3.5 the protein is entirely insoluble, but dissolves as the solution becomes more acid. The cystine, cysteine and tyrosine content of the protein has been greatly lowered, and the tryptophane appears to be completely destroyed following treatment with 0.03 M

lithium periodate at room temperature over a period of 72 hours. These chemical and physico-chemical changes are accompanied by a complete loss in the ability of the oxidized protein to incite antibodies in experimental animals, yet the changes are not so profound as to cause loss in precipitability of the oxidized albumin in an antiserum prepared against the native protein.

'Nonspecific' complement-fixing activity of cloudy avian sera CHRISTINE E. RICE *Div of Animal Pathology, Science Service, Dominion Dept of Agriculture, Animal Diseases Research Inst, Hull, Quebec, Canada*. Whereas inactivated chicken and turkey sera do not ordinarily fix complement with homologous bacterial or viral antigens, an atypical type of complement-fixing activity has been observed with higher dilutions of sera collected from birds during periods of active egg-production. Since fixation of complement was recorded with normal as well as immune sera and was noted with various unrelated antigens, it appeared to be 'nonspecific' in nature. The fact that fixation was most pronounced with cloudy sera suggested that serum lipids may have played some rôle in the reaction. The results of chemical analyses seemed to support this supposition. The possible relationship of such activities to certain 'nonspecific' reactions encountered in the serologic diagnosis of human infection will be discussed.

Relationship between catalase activity and virulence in *Pasteurella pestis* MORRIS ROCKENMACHER (introduced by A. P. KRUEGER) *Office of Naval Research Task V, Dept of Bacteriology and U S Naval Medical Research Unit No 1, Univ of California, Berkeley, Calif*. The catalase activity of virulent and avirulent strains of *Pasteurella pestis* was measured by an adaptation of the procedure of Huddleson (*Univ of Michigan Technical Bulletin* 182 January 1942). Optimal conditions for enzyme activity in these organisms, as shown by a study of the kinetics of the reaction, were used. Catalase activity was expressed as ml of 0.1 N hydrogen peroxide decomposed in 60 minutes by a standardized suspension of organisms. A minimum of 2 separate measurements was made for each strain. Enzyme activity appeared to follow the curve for a mono-molecular reaction. The catalase activity of virulent strains was greater than that of avirulent strains. In the 14 virulent strains studied, enzyme activity ranged from 12.9 to 19.9 ml, with a mean value of 16.7 ± 0.4 . For 11 avirulent strains, values of 2.1 to 8.6 ml were found, with a mean value of 5.1 ± 0.5 . The difference between the mean values of the virulent and avirulent organisms was significant. Virulence titrations were simultaneously performed by the subcutaneous inoculation of the suspension of organisms used for catalase testing into 7- to 12-week old mice. The resultant LD₅₀ values ranged from 6 to

5000 virulent organisms. Avirulent strains caused no deaths when 20,000 organisms were injected subcutaneously.

Intradermal antibody-antigen and antigen-antibody reactions in persons having poliomyelitis, contacts and non-contacts in relation to poliomyelitis EDWARD C. ROSENOW *Bacteriological Research, Longview Hospital, Cincinnati, Ohio*. The solutions of thermal antibody and of antigen injected intradermally consisted of the bacteria-free supernatants of saline suspensions of streptococci isolated in studies of poliomyelitis. The former after autoclaving suspensions containing twenty billion streptococci/ml for 96 hours and adding an equal volume of saline containing 0.4% phenol, the latter after heating suspensions containing ten billion streptococci/ml at 70°C for one hour and adding 0.2% phenol. Three hundredths ml of each and of control solutions were injected into the skin of the anterior aspect of the forearm of 432 persons having poliomyelitis, of 434 contacts and 2193 non-contacts. Reactions of 5 sq cm or more to 'poliomyelitis' streptococcal thermal antibody indicating specific antigen and a corresponding streptococcal infection occurred in all persons having poliomyelitis, in nearly all contacts and non-contacts during September and in almost no one in spring and winter remote from poliomyelitis. Reactions indicating antigen were greatest, alike in males and females, and reactions indicating antibody were least at the onset of poliomyelitis. Antigen diminished and antibody increased during recovery far more rapidly in persons having mild attacks than in persons severely paralyzed and antibody titers were uniformly higher in females than in males corresponding to the lower incidence of and mortality from poliomyelitis in females. 'Poliomyelitic' streptococcal antibody titers in September were strikingly greater in persons recovering, in persons that had recovered from mild poliomyelitis and in well persons that had previously been exposed to epidemic poliomyelitis than in corresponding persons not previously exposed.

Antigenic relationship of dengue and yellow fever viruses with those of west-Nile and Japanese B encephalitis ALBERT B. SABIN *The Children's Hospital Research Foundation, Univ of Cincinnati College of Medicine, Cincinnati, Ohio*. Rhesus monkeys inoculated intracerebrally with human serum containing various immunological types of dengue virus (Hawai, New Guinea 'B,' 'C' or 'D') developed complement-fixing antibodies not only for the Hawai dengue virus (benzene extracted 20% mouse brain antigen) in titers of 1:64 to 1:128 but also for the Japanese B and West Nile viruses in titers of 1:16 to 1:64 and to a lesser degree for the virus of yellow fever, but not for the St. Louis, Western equine or Rift Valley fever viruses. The serum of human volunteers residing in the U. S. A.

who recovered from infection with the Hawai type of dengue virus by mosquito bites or inoculation of human serum, also had C-F antibodies for the Japanese B, West Nile and yellow fever viruses, but not for the St. Louis, Western equine, or Rift Valley fever viruses. Rhesus monkeys inoculated with yellow fever virus (French neurotropic or Asiatic viscerotropic strains or both) which developed C-F antibodies for the yellow fever virus in titers of 1:64 to 1:256 also regularly developed C-F antibodies for the Japanese B and West Nile viruses in titers of 1:4 to 1:32 and occasionally also for the Hawai dengue virus, but not for the St. Louis, Western equine or Rift Valley fever viruses. A Japanese B antiserum prepared in mice with a homologous C-F titer of 1:256, had a titer of 1:128 with West Nile, 1:32 with yellow fever, 1:4 with Hawai dengue, 1:2 with St. Louis encephalitis virus, and yielded negative results with similar antigens prepared from the Western equine and Rift Valley fever viruses. The common antigen (or antigenic groups) possessed by the dengue, yellow fever, Japanese B encephalitis and West Nile viruses is present in low concentration and can be demonstrated only in the most potent preparations, although it is not necessarily proportional to the number of homologous antigenic units. Thus, while a Japanese B antigen with 128 homologous units had only 4 units when tested with a dengue antiserum, the West Nile antigen with 16 homologous units was as active as the Japanese B antigen in its reaction with the antisera for the related viruses.

Differences in antigenic capacity among influenza virus strains JONAS E. SALK, ANGELA M. LAURENT (by invitation) and RACHEL C. MCGINNIS (by invitation) *Virus Research Lab., Dept of Bacteriology, Univ of Pittsburgh School of Medicine, Pittsburgh, Penna.* In the course of studies carried out in 1947-48 employing influenza virus vaccines containing the FM1 strain (Type A-prime), it was observed that the antibody response in man to the FM1 component of the vaccine was noticeably less than was the response to the other two components, PR8 (Type A) and Lee (Type B). For this reason studies were undertaken to investigate the hypothesis that strains of influenza virus differ in their antigenic capacity. Because of the importance of this question, in relation to continuing field studies of vaccination, a study was made, in man as well as in experimental animals, of the antigenic capacity of selected strains. The studies in man were carried out by inoculating groups of individuals with univalent vaccines containing comparable amounts of virus hemagglutinin. Determinations were then made of the titer, in the pre- and two-week post-vaccination sera, of hemagglutination-inhibition antibody, measured with homologous and heterologous antigens. Similarly, groups of mice were inoculated intraperitoneally

with the univalent vaccines, diluted serially, to determine antigenic extinction titer. The latter is an index of the highest dilution of vaccine that is capable of inducing, in the mouse, the formation of neutralizing antibody measured *in ovo*. Both in man and in the mouse, marked differences were found in the effectiveness of different strains in calling forth antibody. It was of further interest that different lines of the same strain differed in this respect. The implication is evident of the significance of these findings in relation to the problem of selecting strains of virus to be included in vaccines for use in man.

Loss of hemolytic complement and of granulocytes following reinjection of an antigen into the rabbit. A. B. STAVITSKY (by invitation), R. STAVITSKY (by invitation) and E. E. ECKER, *Inst of Pathology, Western Reserve Univ., Cleveland, Ohio*. The decrease of complement observed in anaphylaxis, serum sickness and certain infectious diseases has been attributed to its fixation by an antigen-antibody complex *in vivo*. To determine whether such fixation occurs, the following experiments were performed. Various antigens were injected into rabbits and their serum complement estimated immediately before and after the intravenous reinjection of each antigen. Thirty and 60 minutes after the reinjection of the antigens, such as ovalbumin, etc., a great reduction of hemolytic complement activity was noted. This reduction occurred both in the presence and absence of anaphylaxis. Since it did not occur in the absence of extracellular antibody, it is most likely that complement was bound by an extra-cellular antigen-antibody complex *in vivo*. A similar mechanism may be assumed to mediate the loss of complement in allergic and infectious diseases. The 'Complement Reduction Reaction' may prove to be useful for the detection of extracellular antigens and antibodies. It is highly specific and sensitive, apparently unaffected by excess antigen or antibody, and may require relatively little antigen for its production, but is not as yet quantitative. A granulocytopenia generally accompanies the drop in complement. This response appears to be related to anaphylactic and anaphylactoid reactions as well as to an *in vivo* antigen-antibody combination. This response may be employed for the early detection of the anaphylactic type of hypersensitivity to a given antigen. The significance of the loss of antibody, complement and granulocytes during the early part of the 'negative phase' in immunization deserves further clarification.

Antigen-antibody reaction of intact leukocytes. 1. Plasma cell, 2. Variable specificity of maturing cells. BERNHARD STEINBERG and RUTH A. MARTIN (by invitation), *Toledo Hospital Inst of Medical Research, Toledo, Ohio*. We indicated previously (*Proc Soc Exp Biol & Med* 56: 50-52, 1944, J

Immun 51: 421-426, 1945, 52: 71-75, 1946, 53: 137-141, 1946) that various types of mature and immature INTACT leukocytes possess specific antigenic substances and that each cell type can be demonstrated by agglutination. Normal and leukemic leukocytes were found to be antigenically similar, whereas cells from lymphosarcoma were antigenically dissimilar from those of lymphatic leukemia. These findings suggest an immunochemical difference between normal and leukemic cells on one hand and malignant cells on the other. In the present study, the antigenic similarity of the plasma cell in relation to other leukocytes was investigated. In addition, leukocytes of various stages of immaturity were tested for antigenic specificity. Antisera were developed for granulocytes, lymphocytes, monocytes and plasmocytes of mature and of several phases of immaturity. Agglutinations of intact leukocytes indicated individual variations but with a group specificity for all immature forms of a given type of cell. Plasmocytes were found to be antigenically dissimilar from granulocytes, lymphocytes and monocytes.

Metabolism of Brown-Pearce rabbit carcinoma cells in normal and immune serum. W. H. SUMMERSON (by invitation) and JOHN G. KIDD, *Depts of Biochemistry and Pathology, Cornell Univ Medical College, New York City*. The metabolism of viable Brown-Pearce rabbit carcinoma cells was studied in the presence of normal rabbit serum and of rabbit serum that contained high titers of the antibody that reacts with a distinctive constituent of the Brown-Pearce cell. For the immune sera, the relation between the number of cells and the volume and titer of serum used was such that an excess of the specific antibody was present. Measurements were made of oxygen consumption, R.Q., aerobic and anaerobic glycolysis, glucose utilization, and lactic acid production, over a 3 to 4-hour period, under physiological conditions of temperature and pH. No significant effect of the immune sera on these metabolic characteristics of the Brown-Pearce cell was evident, even though control bioassays demonstrated that incubation of the cells with immune sera under conditions similar to those used in the metabolism studies rendered the cells incapable of giving rise to new tumors when implanted in susceptible host rabbits.

Antigenicity of staphylocoagulase. MORRIS TAGER and HARRIET B. HALES (by invitation), *Dept of Bacteriology, Yale Univ School of Medicine, New Haven, Conn.* Although the antigenicity of coagulase has been a matter of controversy, interest in this problem has been reopened by studies indicating that many human sera inhibit coagulase activity, that similarly most plasmas show varying degrees of resistance, and that changes in inhibitory properties of sera may follow staphylococcal infection. Using standardized and

concentrated coagulase purified 300-fold (activity to nitrogen ratios) and showing 2 mobile components on electrophoresis, rabbits were inoculated with a) coagulase alone, b) coagulase plus Wood 46 alpha lysin as adjuvant, and c) the lysin only. Five of 6 test rabbits developed an increase in serum neutralization, increased plasma resistance to coagulase action, complement fixing bodies, and agglutination of coagulase-coated collodion particles. The 3 controls and 1 test animal failed to respond. The antigenicity of the coagulase, and the specificity of the responses observed, is further supported by such considerations as the high degree of purity of the injected coagulase, the consistently better response of the animals treated with the adjuvant toxin as well as the coagulase, by the sharp anamnestic reaction following a single booster dose, and by the specific removal of neutralizing bodies by adsorption on coagulase-coated collodion particles. Although the findings warrant the conclusion that antibodies were produced to coagulase in 5 or 6 rabbits, there are data indicating that coagulase activity may be blocked by other mechanisms as well.

X-irradiation on antibody decline WILLIAM H. TALIAFERRO and LUCY G. TALIAFERRO (by invitation) *Dept of Bacteriology and Parasitology, Univ of Chicago, Chicago, Ill.* Changes in hemolysin titer have been followed by photometric methods in 3 series of rabbits after receiving per kg: 1) 2×10^7 sheep red cells in one injection, 2) 2×10^8 cells in one injection, and 3) 17×10^9 cells in 12 injections. After the antibodies began to decline, half of each series was irradiated twice (1 or 2 weeks apart) with 400 r hard X-rays (0.5 mm copper and 1 mm aluminum filters). In no animals was there a significant rise in hemolysin titer after irradiation. At this level of irradiation there is marked destruction of lymphocytes but the macrophages are highly phagocytic and show no evidence of injury. In view of the accumulated evidence indicating that lymphocytes contain large amounts of antibody, these negative results raise questions as to 1) the relative importance of the macrophages and lymphocytes in producing and storing antibodies at this stage of immunization, and 2) the possibility that the particular method used to destroy lymphocytes may lead to the destruction of antibody.

Tuberculostatic activity of the blood and urine of animals given gliotoxin RALPH TOMPSETT (by invitation), WALSH McDERMOTT (by invitation), and JOHN G. KIDD *Depts of Medicine and Pathology, The New York Hospital, Cornell Medical Center, New York City*. Two samples of purified gliotoxin, generously provided by Professor Johnson, have regularly inhibited the growth of virulent tubercle bacilli of human origin in concentrations of 0.01-0.04 $\mu\text{g/ml}$ in various liquid media. These

samples were likewise effective against mouse lymphosarcoma cells *in vitro* in concentrations of 1.0 $\mu\text{g/ml}$ or slightly less, as indicated in the preceding abstract. The purified gliotoxin was much less active against a number of other bacterial species, the inhibitory concentrations ranging from 250 $\mu\text{g/ml}$ for *Ps. aeruginosa* to 8.0 and 4.0 $\mu\text{g/ml}$ for *Str. viridans* and *Pneumococcus* respectively. Concentrations within this range were inhibitory to strains of *Enterococcus*, *Streptococcus* (Group A), *Staphylococcus*, *B. subtilis*, *A. aerogenes*, and *E. coli*. From five to fifteen minutes after the oral or parenteral administration of gliotoxin to mice, guinea pigs and rabbits, the plasma of the animals inhibited growth of tubercle bacilli in dilutions as high as 1:320, and significant degrees of activity persisted for at least two hours thereafter in most instances. Urine procured from animals of all three species two to three hours after they have been given gliotoxin proved inhibitory to the growth of tubercle bacilli in dilutions from 1:320 to 1:1280. Despite prolonged administration of gliotoxin in doses calculated to provide inhibitory concentrations in the blood for a large part of each day, no favorable influence on the course of tuberculosis in mice was demonstrable.

Inactivation of influenza virus by oxidizing agents ROBERT R. WAGNER and IRVEN B. STACY, JR. (introduced by LAVERNE A. BARNES) *Naval Medical Research Inst., Bethesda, Md.* The PR8 strain of influenza A virus is inactivated *in vitro* by dilution in various oxidizing agents. Infectivity was determined by inoculation of chick embryos and the hemagglutinating capacity of harvested allantoic fluid. P-benzoquinone, potassium permanganate and sodium periodate were effective in inactivating up to 50,000 I.D. of virus. Oxygen, ammonium iodoxybenzoate and cytochrome C were ineffective and it appears that this inactivation is associated with dehydrogenation. Glucose counteracts the effect of periodate but not of quinone, permanganate or hydrogen peroxide. L-ascorbic acid, in equimolar concentration, is antagonistic to the action of all 4 compounds, L-cysteine protects only against quinone and peroxide. The degree of activity of these agents is roughly associated with the redox potentials, except for quinone which appears to exhibit greater specificity. These compounds, at 0.001 M concentration, destroy the capacity of the virus to be adsorbed onto chicken erythrocytes, this also correlates with loss of infectivity. However, virus treated with quinone at lower concentrations, although rendered non-infective, will retain its ability to be adsorbed. Furthermore, inactivation by low concentrations of quinone can be reversed by cysteine and glutathione but not by ascorbic acid. These preliminary results indicate that at 0.001 M concentrations these oxidizing agents act as denaturants of PR8.

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virus, but the site of action of quinone in lower concentrations may be on a sulfhydryl moiety of the virus and that this reaction involves no loss of adsorbability and is reversible

Antibody against encephalomyocarditis virus in the sera of American wild rats JOEL WARREN, SUDIE B. RUSS and HELEN JEFFRIES (introduced by JOSEPH E. SMADEL) *Dept of Virus and Rickettsial Diseases, Army Medical Dept Research and Graduate School Army Medical Center, Washington, D C* In the search for natural reservoirs of the viruses of the encephalomyocarditis group (Columbia-SK, M.M., E.M.C. and Mengo viruses) sera from various species of wild-caught rodents have been examined for the presence of neutralizing antibody for E.M.C. virus. Seventy-nine of 440 sera from *Rattus norvegicus* and *Rattus alexandrinus* possessed considerable amounts of neutralizing substances for E.M.C. virus. Tests performed intraperitoneally in mice showed that such sera were capable of neutralizing 1,000 to 100,000 MLD's of the agent. The geographic distribution of immune rats varied considerably. For example, of 40 rat sera collected in rural areas of the state of Mississippi 39 were positive, whereas only 9 of 75 from Florida and 9 of 64 from Georgia contained this antibody. In the state of California 11 of 32 rats from Orange county were positive while only 2 of 49 from San Diego county had antibody. In general, the incidence of rats with E.M.C. antibody was greatest in southern areas and least in northern regions. Indeed, none of the 38 sera from rats caught in the states of Michigan or Washington or in British Columbia possessed immune substances. None of the sera from a group of 61 rodents made up of jack rabbits, squirrels, marmots and chipmunks contained neutralizing antibody, even though they were trapped in regions where there were a number of E.M.C. immune rats. It appears that wild rats constitute one of the natural reservoirs of the E.M.C. group of viruses.

Role of cathepsin II in the mechanism of caseation in experimental tuberculosis of rabbits CHARLES WEISS and JULIUS SCHULTZ (by invitation) *Labys of the Jewish Hospital, Philadelphia, Penna.* One series of rabbits was infected intratracheally with 0.001 mg of a virulent (Ravenel) culture of *M. tuberculosis* and killed at intervals of from 5 to 20 weeks. A second series was sensitized with 2 successive weekly intravenous injections of 1 mg of a non-virulent R_1 strain, reinfected intratracheally 2 weeks later with a Ravenel culture, and treated similarly. Sections of lung and kidney showed histologically varying amounts of caseation, usually surrounded by granulomatous tissue. Weighed organs, preserved in a dry ice refrigerator, were homogenized in a Waring blender and cathepsin II estimated (Method of estimating hydrolysis of BAA was described by

Schultz, J., *Jour Biol Chem* 1948, in press). After primary infection with virulent tubercle bacilli, the catheptic activity of certain organs was altered. While there were individual variations, there were statistically significant decreases in the hydrolysis of BAA by the livers and kidneys of rabbits killed 5 weeks after primary infection. The lungs, now weighing 2 to 10 times their normal values, had 2 to 10 times the normal enzymatic activity based on total organ content. The animals which were first sensitized with R_1 cultures and then reinfected with a virulent strain, showed decreases in the liver and marked increases in the kidneys and lungs, if killed 4 to 5 months after reinfection. Thus, the cathepsin II activity expressed as the first order reaction constants or $K/gm N$, were for normal liver, kidney, and lung 28 ± 2.1 , 24 ± 1.9 , and 8.6 ± 0.8 while those for the immunized and reinfected animals were 17 ± 2.1 , 38 ± 6.0 , and 2.1 ± 2.9 , respectively. In explanation the following is suggested. The liver is an organ of high natural immunity to infection with tubercle bacilli, hence, the bacilli of reinfection do not multiply actively there and do not call forth an intense mononuclear infiltration. The kidneys and lungs, on the other hand, possess much less natural immunity, therefore permit a greater multiplication of tubercle bacilli and a much more rapid and intense mobilization of inflammatory cells which are probably the source of much of this added enzyme.

Color reactions with malignant sera EMIL WEISS *Tumor Clinic, Michael Reese Hospital, Chicago, Ill.* A number of aniline dyes in dilutions 1:10,000 do not show any color changes on addition of malignant sera. Sera of normal individuals, those afflicted with various diseases or benign tumors turn the original blue color of the same dyes green. The glassware must be clean and free of any acid or dye. The sera must be fresh and thoroughly centrifuged. Hemolyzed or icteric sera can not be used. The dyes are kept in 0.1% stock solutions of rubbing alcohol (70% isopropyl alcohol). The stock solution is diluted with distilled water 1:10 before use. To 0.5 cc of the diluted dye 0.5 cc of serum is added, the tube is shaken for a few seconds and the color changes are noted. A positive control containing a known malignant serum and a negative control containing a known normal serum are handled in the same manner. A dye control correspondingly diluted with water also is used. The tubes are placed overnight or for 12 hours in the refrigerator and then the final reading is made. Eventual color changes become more distinct after standing several hours. Over 300 sera were examined with the above technique. Positive (blue) reactions were obtained in 89.1% of malignant sera. Azur II, toluidine blue, trypan blue and victoria blue were found to be equally

useful This method applies to all types of malignancy Hodgkin's disease and various forms of leucemia give consistently positive results

Immunochemical studies on egg white proteins
L R WETTER, M COHN and H F DEUTSCH (introduced by A M PAPPENHEIMER, JR) *Dept of Physiological Chem, Univ of Wisc Medical School and Dept Microbiology, New York Univ, College of Medicine, New York City* Although chicken egg white (EW) possesses at least seven electrophoretic components, only ovalbumin has been extensively characterized Immunochemical methods were used to furnish information concerning the minor and poorly understood components of chicken EW The curve describing the quantitative precipitin reaction between EW and rabbit antisera is extremely complex Nevertheless, when purified preparations of ovalbumin, conalbumin, ovomucoid and lysozyme were used as precipitinogens, various portions of the curve could be ascribed to reactions between these proteins and their respective antibodies Although ovalbumin comprises 60% of EW, when the latter was used for immunization only a small proportion of the total antibody was directed against it Conalbumin induced greater antibody production than ovalbumin although it comprises only 16% of the total protein A very small proportion of the total antibody was directed against lysozyme and ovomucoid The major portion of the antibody was not directed against the above four components, which comprise approximately 95% of the total protein, but rather against the remaining 5% Rabbit anti EW sera were used to study the cross reactions of the antigens in chicken EW with the corresponding antigens in egg whites of turkey, guinea hen, goose and duck It was demonstrated that several other components cross react in addition to ovalbumin, especially those comprising the minor portion of chicken EW

Anaphylactic studies in mice A H WHEELER, E BRANDON, and H PETRENCO (introduced by REUBEN L KAHN) *Serology Lab, Univ Hospital, Ann Arbor, Mich* As is well known, mice do not respond typically to anaphylactic shock Experiments were carried out in this laboratory showing that, if mice are injected with egg albumin plus Freund's adjuvant and then challenged with the egg albumin, fatal anaphylactic shock occurs in about 60% of the animals Generally only about 10% fatality occurs in the control animals injected with the protein alone It was also observed that, following a challenge, abortion occurs more frequently in sensitized pregnant mice than in non-sensitized ones This finding suggests that in mice, abortion may prove a more sensitive indicator of anaphylaxis than anaphylactic death

Demonstration, occurrence and significance of iso-immunization against the A and B factors

during pregnancy ERNEST WITEBSKY *Dept of Bacteriology and Immunology, Medical School, Buffalo, N Y* Fifty cases of heterospecific pregnancies were examined for immune anti-A and immune anti-B isoantibodies Their presence was demonstrated by 2 methods One consisted in the replacement of saline solution by undiluted human serum and the second method by neutralization of the isoantibodies by means of the purified blood group specific substances followed by titration in saline solution as well as in undiluted human serum More than half of the cases examined did not reveal any trace of isoimmunization, others in varying degrees from slight to strong antibody formation Even in those few instances in which a potent immune antibody was found, the baby upon delivery proved to be normal In a series of investigations carried out independently from the 50 cases mentioned, 2 umbilical cord bloods were found whose cells proved to be sensitized when suspended in normal adult serum (9-drop method) The only clue which could be found for the cause of this phenomenon was isoimmunization against the A and B factor respectively It was interesting to note that in both instances the Coombs test was negative in our hands Only in one baby was there slight evidence of hemolysis and jaundice The only case of fatal erythroblastosis presumably caused by isoimmunization against the A factor so far experienced by us was that of a baby of blood group A born to a mother belonging to the blood group B (5th child) A potent immune anti-A antibody was found in the mother's circulation The baby's umbilical cord serum also contained an immune anti-A antibody whose strength roughly compared with the immune anti-A antibody present in the mother's serum following *in vitro* neutralization with A substance It is apparently much more difficult to neutralize immune anti-A antibodies than normal isoantibodies By comparing the cord serum with the mother's serum the impression was gained that the baby's body acted *in vivo* similar to the addition of A substance to the mother's serum in the test-tube The titer of both sera was somewhat alike The conclusion is therefore drawn that isoimmunization against the A and B factors is only a rare cause of erythroblastosis The demonstration of increasing antibody titer or even the presence of immune antibodies in the mother's serum is insufficient evidence to predict erythroblastotic changes in the newborn

Relationship of blood sugar and hypoproteinaemia to antibody response in diabetics MICHAEL G WOHL, S O WAIFE, STANLEY GREEN, and GEORGE B CLOUGH (introduced by MAX B LURIE) *Philadelphia General Hospital, Philadelphia, Penna* Diabetic patients have a lessened capacity to form antibodies The low antibody response to antigenic stimulation appears to bear



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